POSSIBLE LONG-TERM HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO CHEMICAL AGENTS

Volume 1 Anticholinesterases and Anticholinergics

prepared by the
Panel on Anticholinesterase Chemicals
Panel on Anticholinergic Chemicals
Committee on Toxicology
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences

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PREFACE

The Department of the Army asked the Committee on Toxicology (COT) of the National Research Council (NRC) Assembly of Life Sciences to conduct a study of the possible chronic adverse health effects on servicemen of experimental exposure to various chemicals at U.S. Army Laboratories at Edgewood, Maryland, in about 1958–1975. The Edgewood tests were conducted to learn how specific chemicals may affect humans. Some 6,720 soldiers took part in the program; 254 chemicals were administered by various routes. Chemicals were tested for various military applications. Among them were acutely toxic anticholinesterase chemicals; incapacitating agents, which included the glycolates, atropine-like anticholinergic compounds of which BZ (3-quinuclidinyl benzilate) is a prototype; the indoles, represented by EA 1729 (LSD-25); the cannabinols, or marijuana-like compounds; and the sedative, or "tranquilizer," group.

Review of the Edgewood tests included interviews with key administrators, investigators, nurses, and technicians. The account of practices and procedures undertaken at Edgewood comes from a wide spectrum of sources, both documents and interviews. They represent a wide spectrum of attitudes, but are essentially in agreement. Committees were formed at Edgewood to review classified chemicals and reports for declassification and use by NRC panels. Extensive extracts were prepared of preclinical animal and human protocols and technical reports at Edgewood Libraries and other Edgewood facilities where records of subjects and details of exposure conditions and clinical findings are maintained. A repository was established at Edgewood Arsenal (August 1980) for storing the reports and information obtained from other sources.

The NRC staff organized the test chemicals into several pharmacologic classes; the first two classes consisted of 15 anticholinesterase (Appendix A) and 24 anticholinergic (Appendix B) chemicals. Two expert panels were established (on anticholinesterases and on anticholinergics) with chairmen selected from the COT and members drawn from the scientific community on the basis of familiarity with some aspect of the pharmacologic class involved or expertise in a discipline needed for proper evaluation of potential adverse health effects.

Research and experimental case files on volunteers were extracted and summarized. Digests of the literature were prepared.

The charge of the two panels was to determine whether, on the basis of the data available, it is possible to demonstrate the likelihood of long-term health effects or delayed sequelae and, if so, whether the involved chemicals, as tested, are likely to produce long-term health effects or delayed sequelae. The charge did not include policy recommendations regarding human testing. This report is the first of two on the Edgewood tests. It presents the two panels' tentative conclusions which are based on a review of data obtained from Edgewood in nine documents provided by NRC staff (listed in Appendixes C and D), on mortality data organized by the NRC Medical Follow-Up Agency, and on separate discussions and papers generated by the panel members. Specific issues addressed were based on this information and relevant items from the published literature.

A second report will contain an evaluation of most of the remaining chemicals tested at Edgewood and an evaluation of the effects on morbidity state of the test subjects. The information on morbidity may permit firmer conclusions than are presented here.

EXECUTIVE SUMMARY

In response to a request from the Department of the Army, the Committee on Toxicology (COT) of the National Research Council Assembly of Life Sciences conducted a study to evaluate the possibility of long-term or delayed adverse health effects of chemical agents tested on military volunteers during the 1960s and 1970s.

The task was begun about 2 years ago, with interviews of key people who had been associated with the soldier-volunteer test program of the Army Chemical Center (Edgewood Arsenal), Maryland. Initial efforts included a thorough review of the Army's laboratory and clinical records and of reports in the scientific literature. Some 6,720 soldiers took part in the Edgewood program as test subjects in about 1958–1975, and 254 chemicals were administered in an experimental setting.

The chemicals were divided into eight major pharmacologic classes and organized within each class according to structure. The most extensively studied classes are the anticholinergic and the anticholinesterase chemicals, and these are the subjects of this report; the other classes will be reported on later. Panels were then formed to study these two main classes. The chairmen were selected from the COT, and the members were selected for expertise in some aspect of the review of the pharmacologic class in question.

The anticholinesterases are generally organophosphates; these are nerve agents resembling parathion. Major symptoms of low-level anticholinesterase exposure include salivation, increased sweating, contracted pupils, and bronchospasm. The anticholinergics are generally “glycolates”, (substituted glycolic and tropic acid esters) of which the representative and best-known member is atropine. Major symptoms of low-level atropinization include dry mouth, dilated pupils, and tachycardia. There were 24 anticholinergics tested on about 1,800 subjects. There were 15 anticholinesterases tested on about 1,400 subjects. These two classes are readily paired, in that members of each are used as treatment for overexposure to members of the other.

The next step involved organization of Edgewood data and reports. Some of this material had to be declassified before use by the panels. Digests of the entire available literature, classified and unclassified, were prepared by consultant pharmacologists, and various documents were made available to the panels for their use in investigating the possibility that the Edgewood test experience resulted in persistent adverse effects. The panels met several times, beginning in June 1980.

The specific charge to the two panels was to determine:

- Whether the data available are sufficient to estimate the likelihood that the test chemicals have long-term health effects or delayed sequelae.
- Whether the involved chemicals, as tested, are likely to produce long-term adverse health effects or delayed sequelae in the test subjects.

ANTICHOLINESTERASE CHEMICALS

The panel concludes that although no evidence has been developed (to date) that any of the anticholinesterase test compounds surveyed carries long-range adverse human health effects in the doses used, the results of an ongoing NAS/NRC morbidity study may shed further light on this issue. The panel therefore is unable to rule out the possibility that some anti-ChE agents produced long-term adverse health effects in some individuals. Exposures to low doses of OP compounds have been reported (but not confirmed) to produce subtle changes in EEG, sleep pattern, and behavior that persist for at least a year. Whether the subjects at Edgewood incurred these changes and to what extent they might now show these effects are not known. If such changes occurred and persisted, they would be difficult to detect now. They could be determined scientifically only by a new study in which EEG, sleep state, and psychologic-test scores were compared with those from nonexposed control subjects. This might be considered, if reasonable suspicion develops, based on responses obtained in the referenced morbidity study, that selected subjects experienced behavioral changes traceable in onset to experimental exposure to the anti-ChE agents.

ANTICHOLINERGIC CHEMICALS

No firm evidence has been seen that any of the anticholinergic test compounds surveyed produced long-range adverse human health effects in the doses used at Edgewood Arsenal. More intensive study is required to confirm this conclusion. The high frequency of uncontrolled variables makes evaluation of behavioral effects difficult.

On the basis of available data, in the judgment of the panel, it is unlikely that administration of these anticholinergic compounds will have long-term toxicity effects or delayed sequellae. An ongoing morbidity study should provide more definitive information once it is completed.

MORTALITY

Standardized mortality ratios were derived from mortality data for the soldiers (all males) who participated in the Edgewood Tests and from U.S. mortality rates. For each class of chemicals, the mortality rates among the soldiers were not significantly higher than the rates of the U.S. population, categorized by age and calendar year.

MORBIDITY

An ongoing morbidity study among the test subjects is expected to provide a more complete understanding of the long-term consequences of exposure to anticholinergic and anticholinesterase chemicals.

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INTRODUCTION

HISTORY OF THE EDGEWOOD TESTING PROGRAM

Human experimentation appears to have been an Integral part of the history of the U.S. Army chemical warfare (CW) research efforts until its suspension in 1975. On June 28, 1918, the President directed the establishment of the Chemical Warfare Service (CWS). Four years later, in October 1922, the CWS created a Medical Research Division to conduct research directed at providing a defense against chemical agents. No matter how exhaustively an agent was tested in animals, it was felt that its efficacy in humans also had to be studied.

In early 1941, the threat of war increased the urgency of the development of protection against CW agents and, consequently, engendered a need for a larger source of volunteers. Formal authority to recruit and use volunteer subjects in CW experiments was initiated in 1942. The Secretary of War was asked to rule on the permissibility of using enlisted men for testing agents of the mustard-gas type. In July 1943, the CWS was assigned responsibility for all medical research related to CW. This extension of the CWS mission included toxicologic research and the study of hazards to the health of personnel in the CWS.

The issue of the use of human volunteers was considered by the Armed Forces Medical Policy Council during the early 1950's. The Council concluded that essential data could not be obtained unless human volunteers were used, and the use of humans in medical research was authorized. By 1954, the Chemical Corps (formerly CWS) had established a framework within which to conduct human experimentation, but it lacked an adequate pool of volunteers. In 1955, it was decided that the most practical source of volunteers would be enlisted men stationed at Army installations in the vicinity of Edgewood Arsenal. It was emphasized that voluntary consent of each human subject was absolutely essential. It was also stated that, in all experiments involving volunteer subjects, the subjects would be thoroughly informed of all procedures and of what might be expected as a result of each test. Furthermore, each volunteer would be free to determine whether he desired to participate in a given experiment. In October 1959, approval was granted for the conduct of research on volunteers to investigate defense against incapacitating CW agents.

The search for incapacitating agents intensified when the Kennedy administration took office. The involvement with incapacitating agents represented a departure from an earlier period, begun in 1946, when interest in highly toxic (acute) anticholinesterase chemicals resulted from their development in Germany during World War II. The basic purpose of a military incapacitating agent is to produce temporary ineffectiveness without permanent injury or death. Incapacitating agents (anticholinergic chemicals) and highly toxic (acute) anticholinesterase chemicals produce functional and structural effects on the nervous system which cause rapid or delayed effects on an individual's performance and behavior.

PROCEDURES USED AT THE EDGEWOOD CHEMICAL TESTING PROGARM

A fairly extensive discussion of the procedures used is provided in the Inspector General's report, Use of Volunteers in chemical Agent Research, prepared by Colenel James R.Taylor and Major William H. Johnson and dated March 1976 (listed in Appendixes C,D).

RECRUITMENT OF VOLUNTEERS

Recruiting teams (initially administrative officers, but later often including military physicians from the Edgewood laboratory) visited Army installations where a briefing, usually with a film and handouts, was presented to a large number of enlisted men. Generally 10 to 20 percent of the audience expressed interest and these men were asked to complete a personal history, which included medical and psychologic items and the Minnesota Multiphasic Personality Inventory (MMPI). It was not unusual for 400–600 men to request assignment in the course of a tour of seven to ten installations. Of these, no more than 100 were selected and eventually assigned for a 1- to 2- month period of temporary duty at Edgewood Arsenal.

The “incentives” for volunteering consisted of a small monetary allowance (approximately $1.50 a day for temporary duty), the assignment of only light duties while at Edgewood, and almost every weekend free. Some volunteers were genuinely interested in the scientific and experimental aspects; however, if curiosity or the desire to “test one's self” seemed too strong, the applicant was usually <u>not</u> accepted.

As a group, the volunteers were above average in physical and mental qualifications, with a mean IQ near 110, good behavior records, and “normal” MMIPs with profiles generally within two standard deviations of the population mean on all scales.

GUIDELINES FOLLOWED IN THE PROTECTION OF SUBJECTS

The Nuremberg and Helsinki guidelines were regarded by the investigators and their supervisors as appropriate constraints in studies performed on volunteers, although this was not clearly articulated in official memoranda until the mid-1960s. The provision of accurate, informative explanations of what was planned and what might be expected was regarded as essential to the continuance of the program. Written consents, witnessed by medical staff members, were required from the outset and became more elaborate with time. However, minutes of hearings conducted by the U.S. Senate Subcommittee on Health and Subcommittee on Administrative Practice and Procedure, September 10–12, 1975, stated that the consent information was inadequate by current standards.

INVESTIGATORS

When BZ studies were begun in 1960, the need for a psychiatrist with biologic training and interest was recognized, and one was assigned to the program in January 1961. Physicians trained in internal medicine, anesthesiology, cardiology, surgery, dermatology, ophthalmology, neurology, and other specialties were assigned as the program proceeded. Many were research-oriented and have since gained excellent reputations in academic medicine at leading universities.

SELECTION OF DOSES FOR HUMAN TESTS

Subthreshold doses based on estimates from animal potency studies were used in the first few subjects. For example, the earliest exposures to BZ, one of the anticholinergic test compounds, were at doses between 0.1 and 0.5 µg/kg, which was less than one-tenth the incapacitating dose (ID) ultimately established at approximately 5.5. µg/kg. The intravenous route was preferred initially, but other routes of administration were also used. Inhalation studies were sometimes undertaken after a compound had been thoroughly studied by one of these parenteral routes. Oral and percutaneous studies were performed when effectiveness via these routes was of interest.

As the program developed, it became customary to test agents at dose increments of 40 percent, once the approximate effects of the lower doses were known. Placebos were used in some studies, but the cost with respect to subject confinement time, staff workload, and delay in achieving estimates of potency made this impractical except in special cases (e.g., evaluation of antagonists). Instead, low and high doses were assigned in a randomized manner by someone not involved in an experiment. Placebo responses were minimal. Signs of drug effects at all but the lowest doses were significant and made the value of placebo or “no treatment” inconsequential.

RANGE OF DOSES

Rarely did the intramuscular or intravenous doses exceed 1.5 times the incapacitating dose. Inhalation doses were higher, but potencies were lower by this route (usually about 60 percent of that by the intravenous or intramuscular route). Compared with doses described in the scientific literature on atropine coma therapy (18–23) or scopolamine therapy (19), the BZ doses to which volunteers were exposed appear modest. As much as 20 times the ID_{50} of atropine and 30–40 times the ID_{50} of scopolamine have been administered in the past by clinicians—often to older and less robust patients. Many patients received multiple exposures of this magnitude over a period of days or weeks, these therapeutic procedures, reported several decades ago in refereed journals, actually stressed and advocated the benefits of such treatment, despite occasional deaths (most of which appear to have been caused by hyperthermia).

SAFETY MARGIN

The safety margin of a drug is defined as the ratio of the lethal dose (LD) to the effective dose (ED). Sometimes, ratio of the LD_{50} to ED_{50} is used, although a more conservative approach favors the use of the ratio of LD_1 to ED_{99} (standard margin of safety). In the case of incapacitating agents, much reliance is placed on extrapolation from animal experimentation, and estimation of the LD_1 is generally unreliable.

Many other extrapolation techniques have been used in manipulation of animal lethality data in an effort to generate a reasonable human estimate. By taking a conservative approach with data on deaths at low doses, one can derive estimates for man that are modest and in keeping with clinical judgement. Such methods depend on procedures developed and applied in toxicology.

2

ANTICHOLINESTERASES

Anticholinesterases (anti-ChEs) are toxic to humans principally because they interfere with molecular and cellular mechanisms required for the normal functioning of the central nervous system (CNS) and peripheral nervous system (PNS). Their adverse health effects are related mostly to inhibition of acetylcholinesterase (AChE), a critically important CNS and PNS enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Chemical-warfare (CW) agents exploit the acute, life-threatening properties of profound AChE inhibition; some of the anti-ChEs precipitate other clinically significant deleterious effects on sensory and neuromuscular function. All the major anti-ChEs are chemically reactive and potentially capable of alkylating a variety of biologic macromolecules, but the long-term health implications of these reactions are not well known. This chapter therefore focuses on the acute toxic effects of anti-ChEs and the possible long-term effects on CNS and PNS function.

The fundamental cellular component of the nervous system, the neuron, has long, branching, cylindric processes (dendrites and axon) that extend from the cell body. Dendrites are modified for signal reception and transduction and form extensive networks that permit interneuronal communication, coordination, and integration of nervous-system function. The axon typically is a long extension of the neuron specialized for transmission of electric signals and, at its distal end, for chemical communication of information to other neurons or to muscle at sites termed synapses and neuromuscular junctions, respectively.

ACh is the chemical transmitter of information from both somatic and autonomic (PNS) neurons. On the somatic side, the lower motor neuron uses ACh to convey excitatory impulses to voluntary muscle. Cholinergic neurons of the autonomic division of the PNS are grouped in craniosacral (parasympathetic) and thoracolumbar (sympathetic) outflows from the spinal cord. Parasympathetic pathways use ACh at both preganglionic and postganglionic neurons; in the sympathetic system, ACh is restricted to the preganglionic effector. These cholinergic-dependent autonomic neurons play an important role in regulating the function of various vitally important effector organs. Cholinergic pathways are widely distributed in CNS tissue, but their functions are less well understood than those in the PNS.

AChE terminates the transmitter action of ACh. Drugs that inhibit or inactivate AChE (anti-ChE agents) cause ACh to accumulate at cholinoceptive sites and thus produce effects equivalent to continuous stimulation of cholinergic nerve fibers. Before World War II, only “reversible” anti-ChE agents were generally known, of which physostigmine (eserine) is the outstanding example. Shortly before and during World War II, a class of highly toxic chemicals, the organophosphorus compounds (OPs), were developed, chiefly by Schrader of I.G. Farbenindustrie, first as agricultural insecticides and later as CW agents. The high potency of these compounds was found to be due to “irreversible” inhibition of AChE; thus, they produced effects for considerably longer periods than the classical inhibitors. Since the pharmacologic actions of both classes of anti-ChE agents are qualitatively similar, they are discussed as a group, and important features of individual classes or compounds are noted.

CHEMISTRY OF ANTICHOLINESTERASES

A complete list of anti-ChE compounds used in the Edgewood program is contained in the master file (Appendix A). Structure-activity relationships have been reviewed extensively for the "reversible" inhibitors (1,2) the OP agents (3,4) and both classes of compounds (5–7).

"REVERSIBLE" INHIBITORS

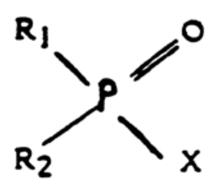
After the structure of physostigmine was established, Stedman (8,9) undertook a systematic investigation of a number of related synthetic compounds. The essential moiety of the physostigmine molecule was found to be the methylcarbamate of a basically substituted, simple o-aminophenol. The quaternary ammonium derivative, neostigmine, is a compound of greater stability and equal or greater potency. Retention of the dimethylcarbamate side chain in the meta position, but with incorporation of the quaternary nitrogen atom into the ring to form a pyridyl nucleus, results in compounds with anti-ChE and other pharmacologic properties similar to those of neostigmine. Pyridostigmine is a drug of this class.

Although the carbamates are the most familiar and the most commonly encountered anti-ChEs, members of other chemical classes are also capable of inhibiting AChE. For example, edrophonium, an analogue of the phenolic residue of neostigmine, is used extensively in clinical medicine. It was not administered to the volunteers at Edgewood, but two other noncarbamates were: 1,2,3,4-tetrahydro-9-acridinamine (Tacrine) and hexafluorenium (Mylaxen). These compounds also are used in clinical medicine but are not as popular as neostigmine, pyridostigmine, or edrophonium.

ORGANOPHOSPHORUS (OP) INHIBITORS

The general formula for this class of cholinesterase inhibitors is shown in Figure 1. A great variety of substituents is possible: R_1 and R_2 may be alkyl, alkoxy, aryloxy, amido, mercapto, or other groups; and X (also called the "leaving group") may be a halide, cyanide, thiocyanate, phenoxy, thiophenoxy, phosphate, alkylthioethylmercaptide, dialkylaminoethylmercaptide, or carboxylate group. It is obviously impossible to discuss here more than a few representative compounds of the more than 50,000 that

Fig. 1: Organophosphorus Compounds
General Formula:



have been prepared; a useful chemical classification of the compounds in this class that are of particular pharmacologic or toxicologic interest has been developed by Holmstedt (3,4). Diisopropyl phosphorofluoridate (DFP) is perhaps the best known and the most extensively studied compound.

Generally, the most acutely toxic compounds are those with one carbon-phosphorus bond (phosphonates). The compounds studied by the Army (Appendix A), with the exception of DFP, contain this feature. In GA (tabun), the leaving group is cyanide. In GB (sarin), GD (soman), and GF, the leaving group is fluoride. In the V agents, VX and EA 3148 (the most potent agent administered to the volunteers), the leaving group is a dialkylaminoalkylmercaptide.

ABSORPTION, FATE, AND EXCRETION OF ANTI-CHES

Physostigmine is readily absorbed from the gastrointestinal tract, subcutaneous tissues, and mucous membranes. It undergoes hydrolytic cleavage at the ester linkage by cholinesterases; renal excretion plays only a minor role in its disposal. In man, a 1-mg dose of physostigmine injected subcutaneously is largely destroyed in 2 h. Neostigmine and related quaternary ammonium drugs are absorbed poorly after oral administration, and much larger doses are needed for effect than when they are administered by injection; both are metabolized by hepatic microsomal enzymes (10) and excreted in the urine.

The commonly encountered OP anti-ChE agents are, with some exceptions (e.g., echothiophate), highly soluble in lipids. Consequently, they are rapidly and effectively absorbed when administered by almost any route, including the gastrointestinal tract, the skin and mucous membranes after contact with the liquid form, and the lungs after inhalation of vapors, finely dispersed dust, or aerosols. Most OP compounds are excreted almost entirely as metabolites in urine. Between the time of absorption and excretion, there are varied periods during which the original compound or its metabolites remain bound to proteins in the blood and tissues.

Both hydrolytic and oxidative enzymes are involved in metabolism of the OP compounds. The OP anti-ChE agents are hydrolyzed in the body by a group of enzymes, the phosphorylphosphatases. These are widely distributed and hydrolyze a large number of OP compounds (e.g., DFP, tabun, sarin, paraoxon, and tetraethyl pyrophosphate, or TEPP) by splitting the anhydride-like P-F (or P-CN) bond. They also hydrolyze several aliphatic esters (e.g., ethyl acetate) and aromatic esters (e.g., phenyl acetate). The enzymes are not irreversibly inhibited by OP compounds, presumably because the phosphorylated active site reacts rapidly with water to regenerate the free form, in contrast with its high stability in the case of the cholinesterases.

Because of the above reactions, the effects of exposure to two OP insecticides may be synergistic. For example, when malathion is administered to animals in combination with O-ethyl (O-p-nitrophenyl phenylphosphonothionate (EPN), the resulting toxicity is as much as 50 times that expected from the sum of their individual toxicities, this results primarily from the inhibition by EPN of enzyme systems that normally metabolize malathion to inactive products. Other combinations of OP insecticides also have shown supra-addition of toxic effects.

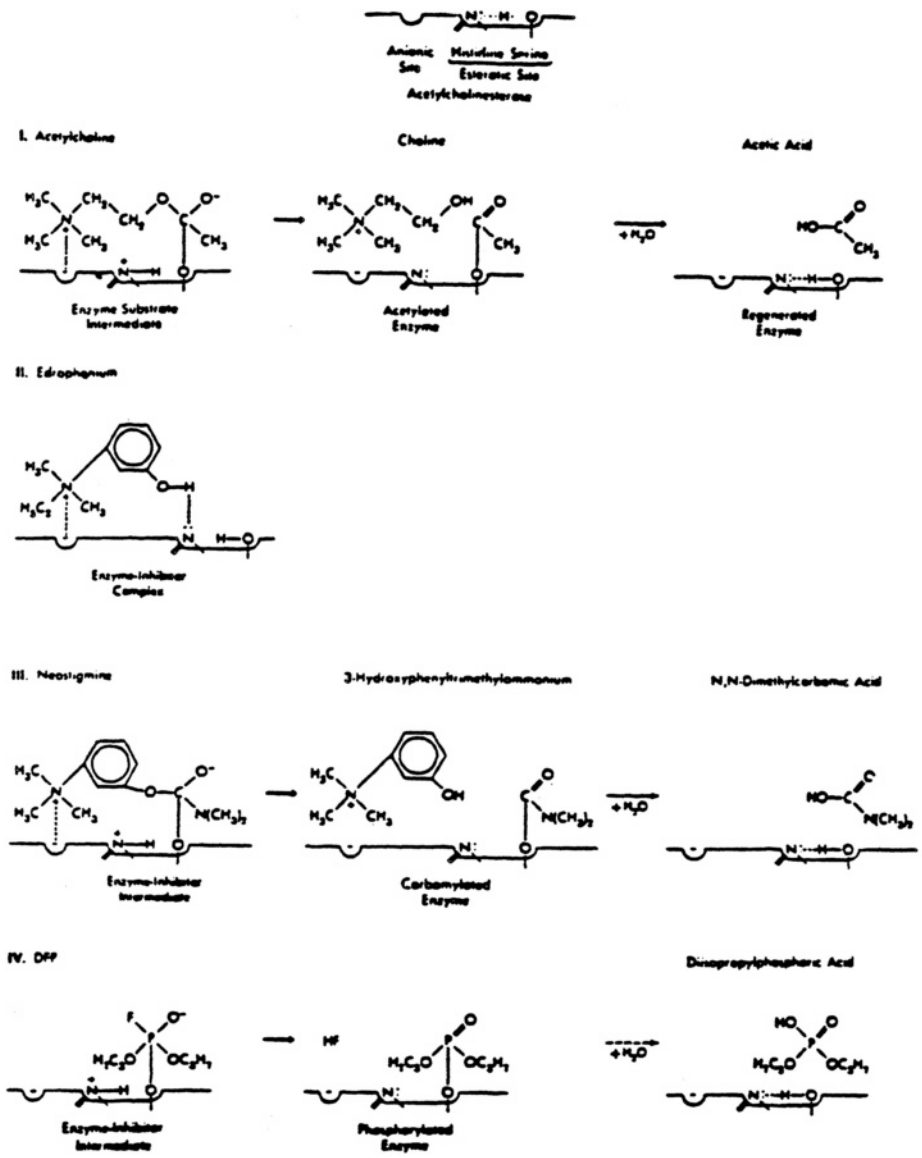


Fig. 2: Steps involved in the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) (I), and in the inhibition of AChE by reversible (II), carbamyl ester (III), and organophosphorus (IV) agents. Heavy, light, and dashed arrows represent extremely rapid, intermediate, and extremely slow or insignificant reactions, respectively. Reproduced from Koelle, G.B., In: The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 448.

MECHANISMS OF ACTION

INHIBITION OF ACETYLCHOLINESTERASE BY ANTICHOLINESTERASES

The mechanisms of action of compounds that typify the three classes of anti-ChE agents are shown in Figure 2. They differ primarily in quantitative respects from the reaction between AChE and its normal substrate, ACh.

Simple quaternary compounds, such as edrophonium, form electrostatic bonds with the anionic site of the enzyme and hydrogen bonds with the imidazole nitrogen atom of the esteratic site. In all such cases, inhibition is rapidly reversible, and such drugs have a very short duration of inhibitory action.

It was at one time generally assumed that physostigmine, neostigmine, and related inhibitors that possess a carbamyl ester linkage or urethane structure, in addition to a tertiary amino or quaternary ammonium group, inhibit the enzyme in the same reversible fashion. However, careful kinetic studies showed that physostigmine and neostigmine are hydrolyzed by cholinesterase (11–14). Initially, inhibitors of this class form complexes in which the inhibitor is attached to the enzyme at both anionic and esteratic sites; subsequently, hydrolysis proceeds in a manner analogous to that of ACh, and the alcoholic moiety is split off, leaving a carbamylated and inhibited enzyme. Later, this enzyme reacts with water to release a substituted carbamic acid and the regenerated enzyme. The main difference between the reaction of the natural substrate, ACh, and that of the carbamate inhibitors is the velocity of the final step; the half-life of dimethylcarbamyl AChE, formed by the reaction with neostigmine, is more than 40 million times that of the acetylated enzyme: 30 min and 42 μs, respectively (15).

The reaction between AChE and most OP inhibitors, such as DFP, occurs only at the esteratic site. It proceeds in a comparable fashion, except that, as a result of the initial hydrolysis, the enzyme becomes phosphorylated. The resulting phosphorylated enzyme is extremely stable: if the attached alkyl groups are methyl or ethyl, substantial regeneration of the enzyme by hydrolytic cleavage requires several hours; with isopropyl groups, as in DFP, virtually no hydrolysis occurs, and the return of AChE activity depends on synthesis of new enzyme, which requires days to months. Some quaternary OP compounds (e.g., echothiophate) combine at both the esteratic and anionic sites, and that probably contributes to their extreme potency and specificity (4,16).

From the foregoing account, it is apparent that the terms “reversible” and “irreversible,” as applied to the carbamyl ester and OP anti-ChE agents, respectively, reflect only quantitative differences and that both classes of drugs react with the enzyme in essentially the same manner as does ACh.

REACTIVATION OF ACHE

Although the phosphorylated esteratic site of AChE undergoes hydrolytic regeneration at a low or negligible rate, Wilson (17) found that the nucleophilic agent hydroxylamine (H_2NOH) can reactivate the enzyme much more rapidly. In the subsequent search for more effective reactivators, a large number of hydroxamic acids

(RCONHOH) and oximes (RCH-NOH) was shown to have this property, e.g., diacetyl monoxime, or DAM. From the data that accrued, it was predicted that highly effective reactivation should be produced by a molecule containing both a quaternary nitrogen atom and an oxime group, spaced at an appropriate distance. This goal was achieved (18) with pryidine-2-aldoxime methyl chloride (2-formyl-1-methylpyridinium chloride oxime, pralidoxime); reactivation with this compound occurs in one millionth the time of that with hydroxylamine (19). Some bisquaternary oximes were later shown to be even more potent as reactivators; an example is obidoxime chloride (Figure 3)—1,1'-(oxydimethylene)-bis(4-formylpyridinium), dichloride dioxime (20).

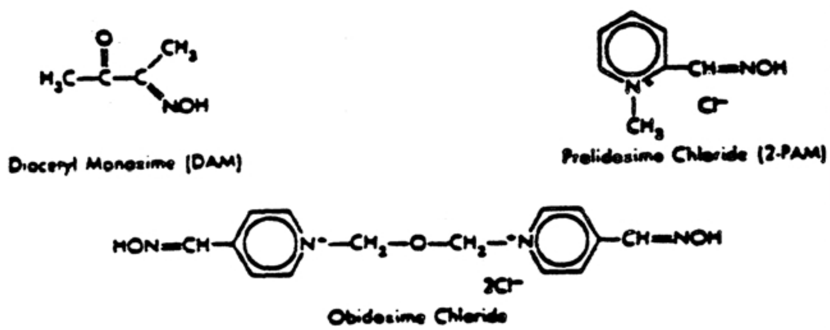


Fig. 3: Cholinesterase reactivators. Reproduced from Koelle, G.B. In: The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 458.

The mechanism of reactivation is sketched in Figure 4. When the quaternary ammonium group of pralidoxime is attracted electrostatically to the anionic site of the enzyme, the oxime group of the former is oriented optimally to exert nucleophilic attack on the electrophilic phosphorus atom of the phosphorylated esteratic site; the oxime-phosphonate is then split off, leaving the enzyme (21,22).

Although the oximes reactivate phosphorylated cholinesterase in vitro and increase recovery from intoxication produced in vivo by some organophosphates, they are not panaceas for the treatment of poisoning by anti-ChEs. Pralidoxime and obidoxime are quaternary ammonium compounds, and their capacity to reactivate brain enzymes is inhibited by the blood-brain barrier. It is also debatable whether pralidoxime and related agents can effectively antagonize the manifestations of intoxication by neostigmine and other carbamyl ester inhibitors. Moreover, most phosphorylated AChEs undergo a fairly rapid process termed “aging” and within the course of minutes or hours, thereby become completely resistant to reactivators.

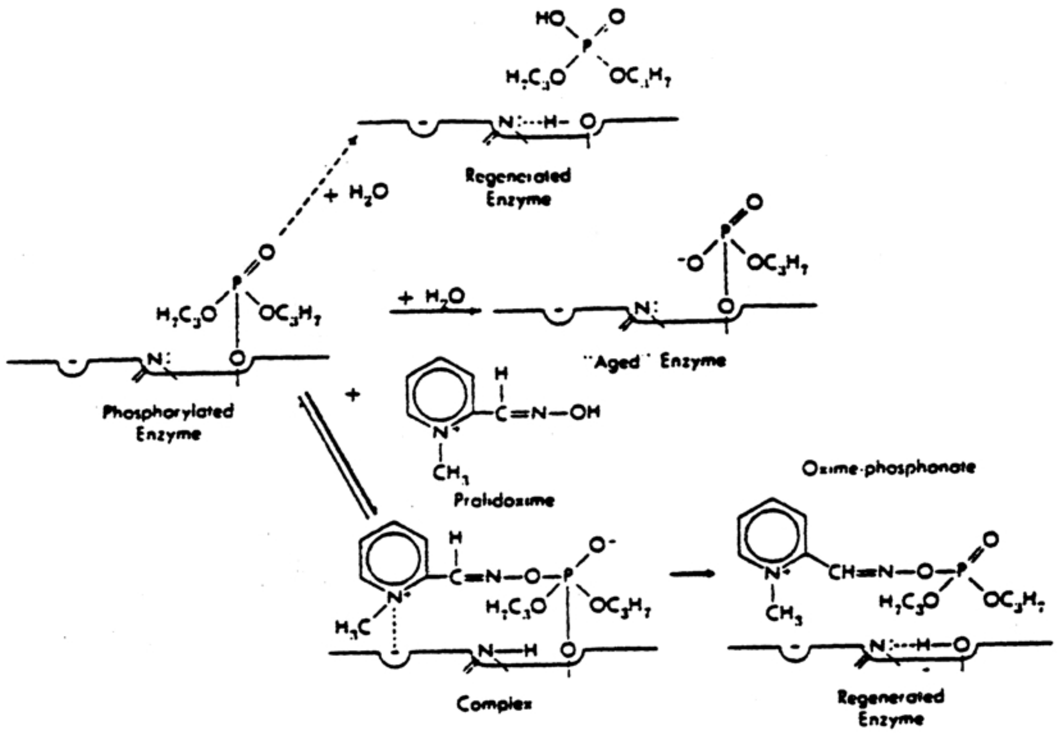


Fig. 4. Reactivation of alkylphosphorylated acetylcholinesterase (AChE). After alkylphosphorylation of AChE by DFP (left), spontaneous hydrolytic reactivation occurs at an insignificant rate (upper reaction), as indicated by the dashed arrow. "Aging" is the loss of one of the isopropoxy residues that occurs more rapidly than spontaneous hydrolysis; the product is very resistant to regeneration by pralidoxime. Pralidoxime (lower reaction) combines with the anionic site by electrostatic attraction of its quaternary nitrogen atom, which orients the nucleophilic oxime group to react with the electrophilic phosphorus atom; the oxime-phosphonate is split off, leaving the regenerated enzyme. Reproduced from Koelle, G.B.: In The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 457.

AGING OF INHIBITED ACHE

Aging causes inhibited enzymes to become refractory to reactivation. The phenomenon was first reported in 1955 by Hobbiger (23) and later observed with OP-inhibited AChEs (24,25) and with an OP-inhibited atropinesterase (26). An extensive discussion of aging may be found in Berend's thesis (27).

The process determines the time during which reactivators can be expected to be beneficial for those exposed to OP anti-ChEs. In the case of OP anti-ChEs, an inhibited (e.g., phosphorylated) enzyme, which initially can be reactivated by oximes, is changed to a form that cannot be reactivated by these compounds (19). The term "aging" has been applied because the amount of inhibited enzyme refractory to reactivation increases with time.

For example, Berry et al. (28) observed that, although pretreatment of animals with a combination of pralidoxime (2-PAM) and atropine increased the LD_{50} values of several compounds (e.g., TEPP and DFP), it had little effect on the lethality of sarin and none on the lethality of soman; they concluded that this was the result of the rapid aging of the ChEs inhibited by sarin and soman. More direct evidence of in vivo aging was obtained by Harris et al. (29), who injected ^{32}P-labeled sarin and soman into rats and observed that the rate of aging of the inhibited rat-brain AChE was the same in vivo as in parallel in vitro experiments.

Aging is probably due to the splitting-off of one alkyl or alkoxy group from the inhibited enzyme, leaving a more stable monoalkyl- or monoalkoxy-phosphoryl AChE (30,31). Wilson (32) pointed out that the difference in reactivatability may be associated with the relative reactivity of secondary and tertiary phosphate esters. Whereas the original inhibited enzyme is a tertiary phosphate ester, the dealkoxylated derivative is a secondary phosphate ester. Thus, the rate of aging depends on the phosphoryl group (33) and not on the group hydrolyzed from the OP by cholinesterase (ChE). Berry and Davies (28) observed, as have many others, that soman yields the most rapidly aged-inhibited ChE obtained from any available anti-ChE; the half-life of the pinacolyl phosphonylated enzyme was determined to be less than 1.5 min. The next most rapidly aged-inhibited enzyme also contained a branched-chain secondary group; $(CH_3)_2$-CH-CH(CH_3)-OH-) Some straight-chain secondary groups, such as CH_3-CH_2-CH (CH_3)-OH-, were also associated with relatively rapidly aged-inhibited enzymes, the phosphonylated enzyme half-life being about 0.5 h. Berry and Davies (28) noted that aging "is slow when the alkyl group is a primary alcohol, whether or not the carbon chain is branched, but is much more rapid if the alkyl group is a secondary or cyclic alcohol."

CHOLINERGIC RECEPTOR AND ACH CHANNEL

In addition to reacting with ChEs, OPs and other anti-ChEs also can react with other critical molecules in nerves or in effector organs. OP drugs may exert direct effects on the cholinergic receptor or on its phospholipid environment, at both CNS and PNS synapses (34–38). OPs also react with other neural and metabolic enzymes, and some of them are capable of alkylating DNA. The biologic consequences of these reactions are not as well understood as are those inhibiting ChE.

White and Stedman (39) suggested that, in addition to inhibiting AChE, OP compounds have an effect on the site where the ACh molecule reacts at the neuromuscular junction. Riker and Wescoe (40) showed a direct agonist action of neostigmine at the neuromuscular junction, and many others have found that neostigmine and some other anti-ChE agents have anticurare effects not apparently related to inhibition of ChE (41). Additional observations indicated that preparations that had been denervated for over 20 d responded with a contracture when exposed to sarin, and findings described below indicate that anti-ChE agents like neostigmine cause marked destruction of denervated muscle (42,43); this phenomenon is most likely correlated with the partial agonist effect of neostigmine.

Similarly, Miquel (44) suggested that OP compounds react with other sites on the muscle, in addition to the enzyme itself. Studies by Xavier and Valle (45) disclosed that Phosdrin^R, an OP insecticide, was able to affect both the ACh receptor and the ion channel associated with it, but without affecting AChE itself. They also found, using two different methods, that physostigmine and neostigmine, in addition to producing blockade of AChE, potentiated the muscle response to ACh when applied in the presence of complete AChE blockade. Albuquerque's studies with OP compounds (46) have suggested an additional effect on the ionic channel that is unrelated to the sites of reaction of ACh on AChE or on the ACh receptor. The simple presence of OP compounds makes the reaction of many agonists, particularly ACh and anatoxin (AnTX), more intensive and speeds the effects of some compounds that block the ion channel. Several agents that react both with the ACh receptor and the channel appear to antagonize the action of the OP compounds in this manner. Conversely, the binding rate of OPs is increased when increasing concentrations of the agonist are present (46), and the rate of binding induced by the presence of large quantities of agonist can occur whether ACh is the agonist or other agents—such as AnTX, subaryldicholine, and succinylcholine—are used in place of ACh.

PRESYNAPTIC ACTION

Anti-ChE agents have presynaptic effects that are related mostly to a reaction with the presynaptic nerve terminal (47–55).

A number of workers reported that compounds such as neostigmine and physostigmine augment and prolong spontaneous release of ACh (miniature endplate potentials or MEPPs) and increase the size of endplate potentials (EPPs) (48,56–59). Boyd and Martin (48) reported a biphasic effect of ChE inhibitors: an increase at low concentrations and a decrease at high concentrations. In a detailed study of the action of neostigmine, ambenonium, edrophonium, and methoxyambenonium on cat tenuissimus muscle, Blaber and Christ (60) reported that MEPP frequency was increased by several ChE inhibitors, and concluded that the effect is probably not related to ChE inhibition but might be related to excitation-secretion coupling, the process by which an action potential releases ACh.

The only published study on presynaptic effects of the more potent ChE inhibitors is that of Abraham and Edery (49), who examined the effect of soman on synaptic transmission in rat diaphragm. In vitro, soman increased the frequency of MEPPs (an effect blocked by Mg^{2+}), caused muscle depolarization that reversed spontaneously, and increased quantal content. It was suggested that the observed changes in transmitter release resulted from an effect on the action potential invading the nerve terminal, although no direct evidence was offered.

In addition, several ChE inhibitors generate antidromic action potentials in motor nerves; these may occur spontaneously (57,61, 62) or after an orthodromic nerve volley (47,50,62–64). The potentials are apparently not caused by action potentials originating in muscle, inasmuch as the antidromic repetitive firing has been observed in nerves from muscle incapable of twitching (62). Riker et al. (50) and Werner (51,52) concluded that the antidromic discharges were produced by direct actions on the motor nerve terminal. Although ChE inhibition, with later accumulation of ACh and an increase in extracellular potassium concentration, may be the cause of the observed effects, these possibilities are unlikely.

NERVE MEMBRANE

Another example of an effect apparently unrelated to ChE inhibition is found in studies of the actions of ChE inhibitors on ionic conductances of electrically excitable membranes. When single frog nerve fibers were used, physostigmine (1–10 mM) attenuated action potential and current, markedly prolonged the duration of the current, and slowed conduction. This mechanism might be involved in functional sensory deficit and—if selective for inhibitory fibers, as is the case with local anesthetics (65)—might play a role in generation of facilitation before depression.

CENTRAL NERVOUS SYSTEM

The situation is still more complex in the CNS. Even if the action of anti-ChEs were limited to the inhibition of postsynaptic AChE, the complex circuitry of the brain provides ample opportunity for effects at other sites. Because brain cholinergic pathways are diffuse and connect with many other systems, overactivity or blockade of cholinergic synapses can lead to abnormal activity in many other neurons. Apparently, there is no end to the list of transmitters and bioactive substances that can be affected indirectly or directly by cholinergic agonists (66). Among the effects in question are those on the γ -aminobutyric acid (GABA) system which are important in brain excitability and epileptogenesis (67), as well as those involving peptide transmitters and bioactive peptides (68). It is unknown whether these effects are brief or long-lasting. For example, it is unlikely that a perturbation in GABA content would be long-lived after the initial effect of the anti-ChE on the GABA system. However, the circuits are complex, and even a temporary perturbation might lead to reverberations that persist for a long time. The CNS has only a limited capacity to regenerate, and recovery after tissue damage might be slow or incomplete. It is also known that a brief presence of excess transmitter in a synapse can lead to compensatory changes in the number of postsynaptic receptors.

“NEUROTOXIC” ESTERASE (NTE)

Some OPs react with a poorly characterized enzyme—“neurotoxic esterase” (NTE)—of unknown function resident in CNS, PNS, and some other tissues and precipitate a delayed CNS-PNS distal axonal degeneration, which is expressed clinically as a sensorimotor neuropathy (69). Although a causal association between NTE inhibition and delayed neurotoxicity has not been demonstrated, they are strongly correlated.

NTE is operationally defined as the esteratic activity against phenyl phenylvalerate (the preferred substrate), phenyl valerate, or closely related esters that is "resistant" to paraoxon and sensitive to DFP and mipafox (N,N'-diisopropylphosphorodiamidic fluoride). Two groups of compounds inhibit NTE (Figure 5): one group consists of various phosphates, phosphoramidates, and phosphonates, which induce neuropathy; and the second contains sulfonates, phosphinates, and carbamates, which do not. The latter can react covalently at the phosphorylation site involved in delayed neurotoxicity and thereby protect against later doses of OP compounds that would otherwise induce neuropathy. In contrast, neuropathic OP compounds induce irreversibly (aged) inhibited NTE. Aging involves transformation of the phosphorylated enzyme to a further modified form in which one R group has been cleaved from the phosphorus and a negatively charged residue remains attached to the enzyme. Once this process occurs, regeneration of the active site of the enzyme is no longer possible, and neuropathy will ensue if the NTE-inhibition threshold has been reached or exceeded within a required period. The relationship between this phenomenon and the onset of axonal degeneration is unknown, but it seems likely from recent experimental studies with DFP (70,71) that the target site in nervous tissue is in the nerve fiber itself.

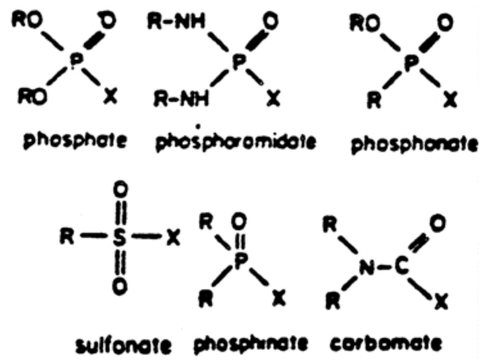


Figure 5. Two groups of NTE inhibitors; Group A (upper) induce delayed neuropathy, Group B (lower) protect against the neuropathic potency of the upper group. R and R' may be alkyl, aryl or heterocyclic substituents; X is the leaving group which is ejected when the inhibitor reacts covalently with the enzyme. Redrawn from Johnson, M.K., 1974: A comparison of the structures of inhibitors of "neurotoxic esterase". J. Neurochemistry 23:786, Fig. 1, Raven Press, N.Y.

BIOCHEMICAL AND METABOLIC CHANGES

OP and related drugs have effects on dehydrogenases and on gluconeogenesis and oxygen uptake (6,72). Failure to gain weight normally accompanies chronic intoxication with some OP agents, and weight loss accompanies OP-induced neuropathy. Some of these biochemical changes may not be specifically related to the anti-ChEs; e.g., changes in blood lactate and acid-base relationships could be related to severe hypoxia, and the hyperglycemia could result from discharge of catecholamines via cholinergic control of the adrenal medulla.

OP ALKYLATION

Some organophosphates are strong alkylating agents in vitro (73,74). Examples are tetramethyl pyrophosphate, dichlorvos, methyl paraoxon, tetrachlorfenvinphos, mevinphos, crotoxyphos, and methyl parathion. Review of several studies on the alkylation of DNA with dichlorvos indicated that dichlorvos induced methylation in isolated DNA or in DNA of bacterial and animal cells treated in vitro. The extent to which OP esters alkylate DNA in vivo is not clear. On the one hand, the existence in mammals of considerable extrahepatic esterase activity provides an important detoxifying capability that diminishes the chances of deleterious alkylation (74,75). On the other hand, it was reported (76) that [^{14}C]parathion administered in the diet or by intraperitoneal injection resulted in binding of metabolites to liver DNA. It was also assumed that the esterase detoxification pathways may operate only slowly, or not at all, for simple alkyl phosphates like trimethyl phosphate (74,75).

BIOLOGIC AND CLINICAL EFFECTS

ACUTE EFFECTS: PHARMACOLOGY

The characteristic acute pharmacologic effects of the anti-ChE agents classically are ascribed to the inhibition or inactivation of AChE at sites of cholinergic transmission, with the consequent accumulation and action of endogenous ACh liberated both by stimulated cholinergic nerve and (in much smaller amounts) by continual leakage during the resting stage. AChE is present in most tissues in a quantity in excess of that required for normal function; to exert a marked effect in vivo, an anti-ChE agent must generally inhibit 50–90% of the functional AChE at a given site. This can be achieved readily, because most of the anti-ChEs produce 50% inhibition of the enzyme at concentrations of 10^{-7} M or lower.

In principle, it should be possible to predict the pharmacologic properties of anti-ChE agents merely by knowing the loci at which ACh is physiologically released by nerve impulses, and the responses of the corresponding effector organs to the chemical mediator. Potentially, the anti-ChE agents can produce all the following effects: stimulation at autonomic parasympathetic effector organs; stimulation, followed by depression or paralysis, of skeletal muscle and of all autonomic ganglia (nicotinic actions); and stimulation, followed by depression, of cholinoceptive sites in the CNS. Because cholinergic stimulation of ganglia increases activity in the sympathetic, as well as the parasympathetic, postganglionic nerves, all the autonomic effectors are activated.

These assumptions are only broadly correct, inasmuch as, with smaller doses of anti-ChEs, particularly those used therapeutically, several modifying factors are present. Most importantly, an enormous number of compounds can inhibit cholinesterase, and no two are identical in either biochemical properties or pharmacokinetics. Thus, although they all share some general characteristics, the detailed effects vary considerably from compound to compound. For example, compounds containing a quaternary ammonium group do not penetrate cell membranes readily; hence, some anti-ChE agents are excluded by the blood-brain barrier from exerting substantial action on the CNS. But quaternary ammonium compounds act relatively strongly at the neuromuscular junctions of skeletal muscle through both their anti-ChE and their direct cholinomimetic mechanisms and have comparatively less effect at autonomic effector sites. Their ganglionic actions are generally intermediate. The more lipid-soluble agents, such as tertiary amines and most OP compounds, have ubiquitous effects at both PNS and CNS cholinoceptive sites.

The main acute pharmacologic actions of anti-ChE agents that are of concern here are those on the eye, the intestine and other organs innervated by the autonomic division of the PNS, the skeletal neuromuscular junction, and the brain. Effects of cholinergic and adrenergic stimulation on effector organs are summarised in Table 1.

Eye

When applied to the conjunctiva, anti-ChE agents cause conjunctival hyperemia and constriction of the iris sphincter (miosis) and ciliary muscle (spasm of accommodation). Miosis is apparent in a few minutes and becomes maximal in 0.5 h. The pupil may be pinpoint sized, but it generally contracts even further when exposed to light. It returns to its normal size in a few hours to several days, depending on the drug and its concentration. The spasm of accommodation is more transient and generally wanes considerably before termination of the miosis. Intraocular pressure usually decreases concomitantly, but in some cases anti-ChE agents may cause an initial increase in intraocular pressure owing to dilatation of the finer blood vessels and increased permeability of the blood-aqueous humor barrier; this is generally followed by a decrease to below initial pressure.

Systemically administered anti-ChEs similarly affect the cholinergic terminals supplying the circular muscles of the eye to produce miosis, but they also affect sympathetic ganglia that operate the apposing radial muscles, and they act on the brain in ways that may reduce activity in cholinergic nerves to the eye. Thus, the effects of systemic administration are not as predictable as those of local administration, and either constriction or dilatation of the pupils may be seen.

Gastrointestinal Tract

Although the actions of various anti-ChE agents on the gastrointestinal tract are nearly identical, neostigmine has been studied most extensively in this regard. In man, neostigmine increases gastric contractions and increases the secretion of acidic gastric juice. The drug tends to counteract the inhibition of gastric tone and motility induced by atropine and increases the stimulatory effect of morphine.

Neostigmine augments the motor activity of the small and large bowel; the colon is particularly stimulated. Atony is overcome or prevented, propulsive waves are increased in amplitude and frequency, and transport is thus promoted. Atropine inhibits, but does not abolish, the intestinal effects of neostigmine. The total effect of anti-ChE agents on intestinal motility probably represents a combination of actions at the ganglion cells of Auerbach's plexus and at the muscle fibers, as a result of the preservation of ACh released by the cholinergic preganglionic and postganglionic fibers, respectively.

Actions at Other Autonomic Sites

Secretory glands that are innervated by postganglionic cholinergic fibers include the bronchial, lacrimal, sweat, salivary, gastric, intestinal, and acinar pancreatic glands; low doses of anti-ChE agents cause, in general, an augmentation of their secretory responses to nerve stimulation, and higher doses increase the resting rate of secretion.

Smooth muscle fibers of the bronchioles and ureters are contracted by these drugs, and the ureters may show increased peristaltic activity.

The cardiovascular actions of anti-ChE agents are extremely complex, in that they reflect at any given moment the sum of the excitatory and inhibitory actions of accumulated endogenous ACh at several levels. The predominant cardiac effect of the peripheral action of accumulated ACh is bradycardia, which results in a decrease in cardiac output and in hypotension. The effective refractory period of cardiac muscle fibers is shortened, and the refractory period and conduction time of the conducting tissue are prolonged. The blood vessels are in general dilated, although the coronary and pulmonary circulation may show the opposite response. The sum of the foregoing effects should result in hypotension, but at the ganglionic level ACh has first an excitatory and, at higher concentrations, an inhibitory action. Hence, the excitatory action on parasympathetic ganglion cells tends to reinforce the above effects, whereas the opposite sequence results from the action of ACh on sympathetic ganglion cells. Excitation followed by inhibition is also produced by ACh at the medullary vasomotor and cardiac centers. All these effects are further complicated by the hypoxia resulting from bronchoconstriction and other actions on the respiratory system. The hypoxia reinforces both sympathetic tone and ACh-induced discharge of epinephrine from the adrenal medulla. It is not surprising, therefore, that a wide variety of hemodynamic effects of anti-ChE agents has been reported, depending on drug, dose, route of administration, species, and other factors.

Neuromuscular Junction

The actions of anti-ChEs are thought to be due to inhibition of ChE at the motor endplate and retention of ACh at the junctional region. This combination culminates in a marked reaction of the transmitter with the receptor and a great activation of the entire receptor-ion-channel complex. If this process continues, a number of undesirable reactions can occur, among them paralysis, the desensitization of the junctional receptor, and structural changes in the muscle and nerve ending.

Normally, a single nerve impulse in a terminal motor axon liberates enough ACh to produce a localized depolarization (the endplate potential) that initiates a propagated muscle action potential. The liberated ACh is rapidly hydrolyzed by AChE, and the muscle relaxes. Therefore, each motor-nerve impulse initiates only one muscle contraction. After partial inhibition of AChE, however, the ACh liberated by a single nerve impulse may persist long enough to set up repetitive muscle action potentials, with a resulting increase in strength of contraction. Furthermore, sufficient ACh may diffuse to neighboring muscle fibers and excite them as well, causing asynchronous contractions (fibrillation). In addition, the action of anti-ChE agents on the axon terminal can initiate antidromic firing, which results in activation of the motoneuron and leads in turn to the synchronous contraction of an entire motor unit (fasciculation). In the presence of a sufficiently high dose of an anti-ChE agent, the local concentration of ACh may produce a depolarizing blockade of the neuromuscular junction and paralysis. Thus, a small dose of anti-ChE may increase the skeletal muscle contraction produced by a single maximal nerve stimulus, but larger doses, or repetitive nerve stimulation at a high physiologic rate, may result in depression or block of neuromuscular transmission.

Brain

The mechanisms by which anti-ChEs perturb brain function are more complex, harder to study, and consequently less well understood. The brain is an extraordinarily complex network of neurocellular pathways which uses electrochemical mechanisms to conduct signals needed to perform and integrate cognition, awareness, memory, language, sleep and wakefulness, locomotion, sensation, and hormonal and autonomic functions. Cholinergic neurons are probably involved in many intraregional and interregional pathways of the brain, although their identity and specific functions are poorly understood. Several sets of presumptive central cholinergic pathways have been proposed: medial septal nucleus to dentate gyrus, and subiculum of hippocampus habenula to interpeduncular nucleus; cortical internurons to cortical pyramidal neurons; and thalamus, putamen, and caudate to neurons in the caudate (77). The presence of cholinergic synapses in central motor pathways (pyramidal and extrapyramidal) and of afferent systems involving both the reticular formation and the thalamus, hippocampus, and limbic system, suggests the possibility of their participation (with other types of chemical synapses) in initiation and control of movement, in sleep, arousal, and wakefulness, in memory, and in emotional regulation. This, in turn, implies susceptibility of these functions to anti-ChEs and cholinomimetic drugs.

The electroencephalogram (EEG), a record of changes in the voltage-field distribution over the head as a function of time, is a tool for studying some aspects of brain function. Adults have characteristic EEG patterns under standard conditions, but these vary according to the state of consciousness (alert, startled, drowsy, dreaming, or deeply sleeping). Awake subjects display a high-voltage (50 μV), low-frequency (8–14 Hz) alpha rhythm. This is most prominent in the occipital region of the scalp and when the eyes are closed. The resting alpha rhythm is replaced during periods of attention and problem-solving by a low-voltage (5–10 μV), high-frequency (15–30 Hz) beta rhythm, most prominent in frontal and parietal regions. The changeover from alpha to beta rhythm, termed “desynchronization”, can be induced by mental concentration or external stimuli (including anti-ChEs). Other frequencies of electric activity commonly observed in the EEG are theta (4–7 Hz) and delta (≤3 Hz) waves.

Among brain functions, sleep is particularly well studied with the EEG. When sleep occurs, the alpha pattern disappears and, over a period of 4–5 min, the EEG changes from a low-voltage to a higher-amplitude, 4- to 6-Hz pattern with intermittent 14- to 16-Hz "spindle" activity. Later, over the course of 1–2 h, the voltage increases, the frequency decreases (to 1–3 Hz), and spindle activity becomes less frequent. The EEG then becomes desynchronized, rapid eye movement (REM) occurs, and the person dreams. REM sleep lasts approximately 15–20 min and occurs three to five times during a normal sleep cycle of 7–8 h. These characteristics are constant from night to night if the person is healthy, is well adapted to the environment, and has an habitual, normal, 24-h sleep-wake cycle.

The control of the sleep-wake cycle appears to be a complex phenomenon involving several groups of neurons (nuclei) in the brainstem that use different synaptic transmitter chemicals: the locus ceruleus (norepinephrine), the dorsal raphe nuclei (serotonin), and the gigantocellularis nuclei (ACh) of the pontine reticular formation (78). Cholinergic neurons in the gigantocellularis nuclei concentrate their electric discharges during REM sleep: activity begins minutes before the onset of REM sleep, continues at a high rate during the REM period, and abruptly ceases as REM sleep terminates. An attractive postulate of the control of the sleep-wake cycle is that, during non-REM sleep (and waking), an inhibitory system composed of neurons in the locus ceruleus or the dorsal Raphe nuclei tonically prevents cholinergic neurons in the gigantocellularis nuclei from firing. At a critical point, the latter escape this inhibition and fire at very high rates, thereby initiating the REM period. The inhibitory neurons then increase their activity and inhibit the cholinergic discharge, and REM sleep ceases. Dependence on ACh as the excitatory transmitter implies the sensitivity of this system to anti-ChEs, which, a priori, tend to increase neuronal activity. This postulate is consistent with empirical evidence from humans and animals treated with cholinergic agonists, which tend to decrease the latency of REM sleep and increase the number of REM-sleep episodes. Atropine exerts opposite effects.

Table 2 lists some of the effects of anti-ChEs and cholinomimetic drugs on brain function (79–84). Doses of OP compounds that are toxic, but too small to threaten life, produce a variety of clinical manifestations, including miosis, muscular fasciculation, and apprehension (85,86). The acute behavioral alterations are usually accompanied by marked desynchronization of the EEG (87). Larger doses of OP compounds—which may induce convulsions, muscular paralysis, and death—cause slowing of the EEG pattern followed by the appearance of spike waves that herald the onset of seizures. Symptomatic recovery is normally complete within 2–9 wk, at which time the erythrocyte cholinesterase content usually has returned to normal (88,89).

Repeated low-dose administration of OP compounds can produce symptoms and signs that are not seen after single exposures to the same doses. For example, subjects given daily injections of DFP reported the additional symptoms of insomnia, excessive dreaming, emotional lability, increased libido, paresthesias, visual hallucinations, and tremor (90); and prolonged administration in animals induces sensorimotor neuropathy.

The subjective manifestations of brain dysfunction usually disappear shortly after cessation of exposure; in most instances, EEG abnormalities reportedly disappear within 2 wk of acute exposure or termination of chronic exposure (90–92).

ACUTE TOXIC EFFECTS

Toxicity of particular OP compounds does not vary greatly among mammals. Signs and symptoms differ mainly in sequence and in individual prominence (93–96). Rapidity of appearance, intensity, and timecourse depend on dose and route of administration.

The effects of acute intoxication with anti-ChE agents are manifest by muscarinic and nicotinic signs and symptoms and, except for compounds of extremely low solubility in lipids, signs referable to the CNS. Effects may be local or general. Local effects are due to the action of vapors or aerosols at their site of contact with the eyes or respiratory tract or to the local absorption after liquid contamination of the skin or mucous membranes, including those of the gastrointestinal tract. General effects rapidly follow systemic absorption by any route; they appear most rapidly after inhalation of vapors or aerosols, in which case severe effects may appear within a few minutes. In contrast, the onset of symptoms after gastrointestinal and percutaneous absorption is delayed. The duration of effects is determined largely by the nature of the compound; it may vary from minutes, as after an overdose of edrophonium, to several days or even weeks after irreversible alkylphosphorylation of AChE, as by DFP or sarin.

After exposure to vapors or aerosols or after inhalation, ocular and respiratory effects generally appear first. Ocular effects include marked miosis, conjunctival congestion, ciliary spasm, and browache, along with watery nasal discharge; respiratory effects consist of “tightness” in the chest and wheezing due to the combination of bronchoconstriction and increased bronchial secretion. After ingestion, gastrointestinal effects appear first, including anorexia, nausea and vomiting, abdominal cramps, and diarrhea. After percutaneous absorption of liquid, localized sweating and muscular fasciculation in the immediate vicinity are generally the earliest manifestations. Severe intoxication is manifest by extreme salivation, involuntary defecation and urination, sweating, lacrimation, bradycardia, and hypotension.

Nicotinic actions at the neuromuscular junctions of skeletal muscle usually consist of fatigability and generalized weakness, involuntary twitching, scattered fasciculation, and eventually severe weakness and paralysis. The most serious consequence of the neuromuscular actions is paralysis of the respiratory muscles.

The effects on the CNS include confusion, ataxia, slurred speech, loss of reflexes, coma, and central respiratory paralysis. Actions on the vasomotor and other cardiovascular centers add to the peripheral actions to complicate the hemodynamic pattern. After large doses or inhalation of high concentrations, the time course may be telescoped into a few minutes and many of the above signs overshadowed by dyspnea, apnea, and collapse. A case of severe accidental poisoning in man is illustrative (97). Development of signs after smaller doses has been described by Grob (98,99) and others (100–104).

After a single exposure, death may come within 5 min or not for some 24 h, depending on dose, route of administration, drug, and other factors. The cause of death is primarily respiratory failure, usually accompanied by a cardiovascular component. Muscarinic, nicotinic, and central effects all contribute to respiratory embarassment; they include laryngospasm, bronchoconstriction, increased tracheobronchial and salivary secretion, and peripheral and central respiratory paralysis. Although the blood pressure may fall alarmingly and cardiac irregularities may intervene, these effects probably result as much from hypoxia as from the specific actions mentioned, inasmuch as they can often be reversed by the establishment of adequate pulmonary ventilation.

Differences in hazard among members of the OP group arise from differences in inherent potency (EA 3148 is the most potent), vapor pressure (GB is hazardous by inhalation), and ability to penetrate the skin (VX and GD) (102,105,106). GB as a liquid administered cutaneously has also been reported to cause severe poisoning (107, 108). Absorption of some of these compounds from the respiratory tract has been estimated to approach absorption after intravenous administration in completeness (105,109,110). Incapacitating concentrations of GB and others by inhalation have been estimated in animal tests, (111) and extrapolations to man have been attempted (105,112,113).

Two antidotes are currently used for acute poisoning by anti-ChEs. One is atropine, which acts as a muscarinic receptor antagonist and reduces the excessive stimulation of parasympathetic functions, thus reversing the effects on the eye, lung, gastrointestinal muscles, and, most important, the heart. Atropine also relieves effects of poisoning at central muscarinic synapses, but not at central nicotinic synapses. The other antidote is an oxime (such as 2-PAM or obidoxime) that relieves poisoning at skeletal muscle endplates (20). It acts as an activator of phosphorylated AChE; through nucleophilic attack, it removes the phosphate group, thus restoring enzyme function at motor endplates. The oximes are inefficient if the phosphorylated enzyme has aged. These oximes contain quaternary nitrogen and therefore do not penetrate the blood-brain barrier and have no effect at cholinergic synapses in the brain or spinal cord.

The acute effects of the anti-ChEs are short-lived and do not outlast the inhibition of the enzyme. Indeed, some systems develop tolerance rapidly, so function returns to normal even before there is substantial regeneration of measurable enzyme activity. It has been amply documented that, even with over 99% inhibition of all ChEs, animals (and presumably humans) can survive without oxime or atropine treatment, if they are supported for a couple of hours by pharmacologic or nonpharmacologic means, such as artificial respiration, (72). This recovery from the "irreversible" effects of inhibitors may depend on rapid regeneration of ChEs, particularly some AChE isoenzymes (114); desensitization of the postsynaptic membrane, a phenomenon that limits the response to accumulated ACh; or compensatory changes in presynaptic and postsynaptic receptors (115).

DELAYED NEUROPATHY (CNS-PNS DISTAL AXONOPATHY)

Clinical Features

Degeneration of particular regions of the nervous system is a well-characterized adverse health effect of human and animal exposure to many OP esters (phosphates, phosphoroamidates, and phosphonates) that may or may not also display anti-AChE properties. Some neuropathic OP esters can precipitate prominent neurologic abnormalities after a single exposure (as well as after multiple exposures), the clinical disease usually beginning within 2–3 wk. At some time during this clinically quiescent period, a stereotyped sequence of neuropathologic changes takes place that leads to the appearance of sensorimotor neuropathy. The degree of clinical impairment and the prognosis for functional recovery depend directly on the extent of nervous system damage, which in turn depends on the neuropathic potency of the responsible OP compound, as well as the dose and duration of exposure.

The first recorded cases of paralysis from OP intoxication occurred at the end of the nineteenth century, when patients with tuberculosis were treated with phosphocreosote, an uncharacterized mixture of esters derived from phosphoric acid and coal-tar phenols (116). Several thousand cases appeared in the southern states in 1930 when alcoholic extracts of Jamaica ginger, widely consumed during Prohibition, were adulterated (117) with 2% tri<u>ortho</u>cresylphosphate (TOCP). Adulteration of cooking oil contaminated with lubricating oil containing cresyl phosphates has proved responsible for several outbreaks of OP neurotoxicity, including a major epidemic in Morocco in which more than 10,000 people reportedly were affected (118). More recently, the OP pesticide leptophos has been associated with an outbreak of occupational neurotoxicity among workers at a plant in Texas; the victims displayed pronounced clinical features of spinal-cord damage, and some had psychologic manifestations (119).

Approximately 2 wk after ingesting cresyl phosphates, during which gastrointestinal disturbances may be manifest, affected persons experience pain, aches, and tingling in the feet and calves, followed within days by progressive weakening of leg and foot muscles that leads to paralysis. The thighs and then the hands and arms may become weak during succeeding days. Weakness is always more severe in the legs than in the arms and in both limbs is greatest in distal muscles. During the progressive phase of the illness, which lasts 1–2 wk (depending on dose), the weakness spreads steadily, but usually stops short of complete quadriplegia. Neurologic examination reveals signs of damage to the spinal cord (hyperactive knee jerks) and peripheral nerves (hypoactive ankle jerks). Foot drop is pronounced, and victims adopt a high-stepping gait. Muscle denervation is evident from electromyography, and atrophy in the lower legs and hands may become severe. Recovery in mild cases takes months or years, but severely affected persons who recover some muscle strength may have ataxia and spasticity permanently (120).

Many experimental species are vulnerable to the delayed neurotoxic effects of OP compounds, such as TOCP, although it is accepted that neurotoxic doses vary markedly from one species to another. Rodents are relatively resistant and fowl very susceptible; hens are widely used to assay AChE compounds for ability to induce paralysis (121). In all sensitive species, there is a period of 1–2 wk before the onset of neurologic signs, during which body weight changes and nerve fibers degenerate. Hens given TOCP develop a steadily increasing flaccid paresis of the hindlimbs, with an ataxic, broad-based gait. Paresis spreads over the course of several days and, if respiratory muscles become involved, may lead to death.

Neuropathologic Features

The limited neuropathologic information available from studies of affected persons demonstrates that OP poisoning induces degeneration of nerve fibers in spinal cord and peripheral nerves (122).

Neuropathologic examination of experimentally poisoned animals reveals a characteristic pattern of distal, retrograde degeneration of peripheral nerves. Long nerve fibers of large diameter seem to be affected before shorter and smaller fibers, so sensory and motor manifestations of nerve damage generally affect the legs before the arms. A similar principle holds for involvement of the spinal cord: long ascending (gracile and spinocerebellar) and long descending (e.g., corticospinal) tracts are symmetrically involved in the degeneration process. Neuropathologic changes initially appear distally and multifocally in affected pathways, leading to degeneration of distal axons and structural and functional disconnection of sensory and motor terminals. Axonal degeneration progresses steadily toward, but stops short of, the nerve cell bodies (71). Loss of axons precipitates a secondary loss of the normal myelin sheath in affected regions of spinal cord and peripheral nerves; this phenomenon has been erroneously described as "demyelination"—a term reserved to describe the neurotoxic properties of substances, such as hexachlorophene, that damage myelin without causing axonal degeneration. In sum, the clinical and pathologic features of delayed OP neuropathy are classified as a central-peripheral distal axonopathy (123).

Neuropathic Potency

Neuropathic potency can be assayed by determining the response of a vulnerable species (the hen, Gallus gallus domesticus) to OP intoxication or predicted from the degree of inhibition of the nervous-system enzyme NTE. It is important to note that chemical reactivity of an OP compound with NTE is unrelated to its ability to inhibit AChE (69). Some OP agents designed for chemical warfare can inhibit both NTE and AChE. However, doses needed to inhibit NTE and induce neuropathy may be much higher than those which would prove fatal to animals or humans without protection against the acute anti-ChE effects. Alternatively, the degree of NTE inhibition may be insufficient to induce clinical neuropathy. Phosphorofluoridates induced delayed neuropathy in chickens after 9–15 d at doses of 0.3–2.5 mg/kg; the dimethyl compound required doses of 30 mg/kg. Five alkylphosphorofluoridates were active at 1–5 mg/kg given in divided daily doses. Diethyl phosphofluoridothionate induced neuropathy at 0.75 mg/kg, but not at 0.5 mg/kg. Various dialkylphosphinic fluorides and dialkylpyrophosphonates were negative (124). Neuropathologic studies designed to detect subclinical damage to spinal cord or peripheral nerves in animals treated with CW agents are not available.

LONG-TERM BRAIN DYSFUNCTION

Several studies have suggested that some subjects experience long-term sequelae from a single OP exposure or a period of low-level exposure. Minor disorders of affect, emotion, and memory were reported by Tabershaw and Cooper (125) in 38% of 114 subjects after acute OP poisoning. Rowntree et al. (126) suggested that OP exposure might exacerbate psychiatric problems. Metcalfe and Holmes (82) claimed that OP exposure may lead to persistent EEG changes; they also reported that workers with histories of both OP and chlorinated-hydrocarbon exposure, but with no recent exposures, had EEG patterns that showed excessive slowing during drowsiness and after hyperventilation. Moreover, all-night-sleep EEGs reportedly displayed patterns commonly associated with narcolepsy. Psychologic dysfunction in this group included disturbed memory and difficulty in maintaining alertness and appropriate focusing of attention.

Duffy and co-workers (127) examined the brain electrical activity of workers occupationally exposed to sarin and with documented single or repeated accidental exposure to toxic concentrations of it at least a year before EEG recording. Standard clinical EEG measurement, computer-derived EEG spectral analysis, and standard overnight-sleep EEGs, were examined in 77 exposed workers, and the results were compared with those from a control group of 38 nonexposed industrial workers. Statistically significant group differences in sarin workers included increased beta activity, delta and theta slowing, decreased alpha activity, and increased REM sleep.

The results of Duffy et al. were consistent with those from a previous study that examined EEG changes in rhesus monkeys exposed to sarin or dieldrin (87). Two dose schedules were used: a single "large dose" (sarin at 5 μg/kg or dieldrin at 4 mg/kg, administered intravenously), which produced overt signs of toxicity, and a series of 10 weekly "small doses" (sarin at 1 μg/kg or dieldrin at 1 mg/kg, administered intramuscularly). The effects of anoxia in the first group were precluded by pretreating animals with gallamine triethiodide and providing artificial respiration. Animals treated with single doses of either sarin or dieldrin displayed significant increases in the relative amount of beta voltage (15–50 Hz) in the EEG that persisted for a year. For sarin, the predominant effect was in the EEG derivation from the temporal cortex, and for dieldrin, from the frontal cortex. For both drugs, the increase in beta activity was most prominent when subjects were awake in darkness or drowsy.

In summary, research results have indicated that a single symptomatic exposure or a series of subclinical exposures to sarin can alter the frequency spectrum of the spontaneous EEG for up to a year (83).

The effect of anti-ChEs on the structural integrity of brain tissue has rarely been investigated, although the destructive effects of some of these compounds on spinal cord and peripheral nerves are well known. There is a clear need to study, with current ultrastructural techniques, the possibility of selective brain damage underlying the reported long-term changes in EEG patterns of animals and humans exposed to anti-ChEs, especially in light of a recent provocative report of widespread axonal and terminal degeneration in the brains of rats treated with soman. As an incidental finding in an unrelated study of the effects of this agent on behavior of rats, Petras (128) described nerve-terminal degeneration in the limbic system, corticofugal system, and central motor system-areas associated with mood, affect, judgment, emotion, posture, and locomotion. The author pointed out that damaged regions would be unlikely to regenerate and that long-term psychiatric and motor deficits might be anticipated. Only 16 animals were studied by Petras, and only seven brains had observable damage. The seven had significant acute toxicity at the time of exposure, including muscle fasciculations, tremors, and seizures. Only an abstract of this work has been published, and the research has not been pursued systematically. It cannot now be concluded that the tissue damage was a direct effect of the OP, rather than an indirect effect (e.g., related to brain hypoxia). Nor can it be ascertained whether the response was specific for soman.

JUNCTIONAL NEUROMYOPATHY

Pathologic changes develop subacutely and reversibly in some motor-nerve terminals, neuromuscular junctions, and associated muscle fibers after administration of anti-ChE drugs to laboratory animals. ChE inhibitors with long-lasting effects-such as paraoxon, DFP, tabun, sarin, soman, and parathion-and “reversible” inhibitors, such as physostigmine and neostigmine, induce these neuromyopathic changes (129–131). The effect may result not only from AChE inactivation, but also from an increase in the rate of spontaneously released ACh secondary to a prejunctional action of anti-ChE drugs (54,132). Thus, guanidine, which increases ACh release, also causes a subacute myopathy similar to that produced by anti-ChE agents (133).

The diaphragm is most severely affected in treated animals (134), but abnormalities are also prominent in the soleus, gastrocnemius, and quadriceps muscles. Ultrastructural changes may appear within hours of drug administration, progress over a period of a few days, and resolve within a couple of weeks. Pathologic changes in motor-nerve terminals, neuromuscular junctions, and muscle fibers are associated with an initial decrease in contractile strength after a few days and then a return to nearly normal strength after several more days (135). These pathophysiologic events may be clearly delineated from the degeneration of motor-nerve terminals and atrophy of muscles that follow administration of agents that induce delayed neuropathy (131), in that subacute neuromyopathic changes not only resolve before the onset of delayed retrograde axonal degeneration, but also fail to develop with TOCP, an O-P compound that inhibits NTE but has little AChE activity. They may also be prevented by protecting the neuromuscular junction with curare or a reactivator of phosphorylated ChE, such as 2-PAM. The myopathic process seems to depend on the degree and duration of ChE inhibition (136); this suggests that skeletal-muscle hyperactivity is causally associated with the phenomenon.

The significance of these observations for humans exposed to OP agents is unknown, but it seems likely from animal experiments that myopathy does not develop in the absence of muscle hyperactivity induced by anti-ChEs. There is some evidence from human autopsy material of focal necrosis of diaphragmatic and intercostal muscles after accidental exposure to a single large dose of OP insecticide (137).

Motor-nerve terminals show various degrees of subcellular changes within 30 min to 2 h after injection of soman or paraoxon (138); soman induces the more severe changes. Nerve terminal alterations include the appearance of intra-axonal myelin figures, membrane enclosures, and an increased number of large-coated vesicles. Three days after DFP injection, soleus motor-nerve terminals are reduced in number and naked endplates are common. Pathologic changes also appear in the subneural apparatus and in the immediate subjacent muscle; the latter displays swollen mitochondria, myelin figures, enlarged nucleoli, dilatation of the sarcoplasmic reticulum, loss of myofibrillar striation, and, later, myofilament loss and fragmentation of Z bands (132). Focal muscle necrosis then ensues. Prejunctional and postjunctional subacute changes are resolved within 2 wk after administration of DFP; however, because this compound also inhibits NTE and induces delayed neuropathy, a week after recovery from the subacute neuromyopathic changes motor-nerve terminals undergo a second phase of degeneration and regeneration, with reinnervation of damaged endplates 6–8 wk later (131).

OTHER ADVERSE HEALTH EFFECTS

Mutagenic or carcinogenic action is not a general feature of the OP compounds, although some may have these properties. There appears to be a lack of information on the mutagenicity or carcinogenicity of carbamate anti-ChEs.

Some agents on the list of compounds under scrutiny belong to chemical classes other than OP compounds and carbamates. Edrophonium is a quaternary ammonium inhibitor of ChE. Methacholine is a stable choline ester and, although urecholine is a carbamate, it is not hydrolyzed by ChE. These two compounds are direct-acting cholinomimetic agents. They are approved for use in clinical medicine, but have few indications. Hexafluorenium is a bisquaternary ammonium distantly related to tubocurarine, and it has both anti-ChE and curariform actions. None of these quaternary ammonium compounds is known to have genotoxic action. Another compound is tacrine, 9-amino-1,2,3,4-tetrahydroacridine; as a class, acridines are notorious for mutagenicity and carcinogenicity, but this particular chemical has been used in clinical practice for more than 20 yr without known sequelae.

Mutagenicity

The OP malathion has been investigated extensively in a number of mutagenesis test systems. Some studies were done with a metabolic activating system, some without (malathion requires metabolic activation for its AChE-inhibiting effect). Malathion also has been tested in somatic cells; tests for chromosomal aberrations in human hematopoietic cell lines, sister chromatid exchange (SCE) in human fetal fibroblasts, and the micronucleus test in mice have been performed. A mouse dominant-lethal mutation assay has also been carried out with malathion, although the study was compromised, in that the dose (300 mg/kg) was well below that which is maximally tolerated. Among these studies, only one (reported in an abstract) claimed positive findings (139). These occurred in the mouse micronucleus and SCE tests and in a host-mediated Salmonella.

assay. The findings concerning the mutagenicity of malathion are therefore equivocal.

A 1975 review (140) concluded that the OPs bidrin, dichlorvos, dimethoate, methyl parathion, and oxydemeton methyl, were mutagens in a single microbial mutation assay. In addition, trimethyl phosphate is a clear-cut mutagen in male mouse germ cells; six other OP compounds (bromophos, diazinon, fenitrothion, malathion, parathion, trichlorphon) apparently are not. Results of assays in four microbial test systems were inconclusive for several OPs (141). Further study of these compounds has not been undertaken.

Carcinogenicity

Malathion given in the diet for 103 wk was not carcinogenic in male and female rats, although the dose used may have been less than that which is maximally tolerated. No increased tumor incidence could be found in mice given malathion for 80 wk.

An International Agency for Research in Cancer (IARC) monograph reported strong evidence of the carcinogenicity of trimethyl phosphate (142) in one species and evidence of carcinogenicy in another. There is equivocal evidence of the carcinogenicity of parathion and dichlorvos in animals. A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes (143).

With the exception of urethane (ethyl carbamate), a potent mutagen and established carcinogen in laboratory animals (144,145), carbamate compounds have been largely unstudied.

Fetal and Teratogenic Effects

Parathion reportedly increases resorption and lowers the weight of rat fetuses (146). Results for malathion are questionable (147). No data appear to be available on the potential mammalian teratogenicity of military nerve agents.

Effects on Male Reproduction

A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes severely (143).

Cataractogenic Effects

The introduction of DFP and other long-acting anti-ChE agents in the late 1940s appeared to represent an important advance in the treatment of open-angle glaucoma. These drugs maintained control of intraocular tension when administered frequently and were effective in cases that were no longer controllable with the shorter-acting compounds. Then, in 1966, independent reports simultaneously implicated the long-acting anti-ChE agents in the causation of lenticular opacities (148, 149). The incidence of this effect appeared to be as high as 50%; its development varied directly with the strength of the solution, frequency of instillation, duration of therapy, and age of the patient. With physostigmine and other short-acting anti-ChE agents, the occurrence of lenticular opacities appeared to be no greater than that in nontreated subjects in the same age groups.

The mechanism of the cataractogenic action of the long-acting anti-ChE agents has remained obscure (150). It has been shown that vervet monkeys can serve as a model for the production of an identical picture with the daily, long-term instillation of echothiophate (151). In an extensive series in which children were treated for accommodative esotropia (strabismus) with the instillation of short- and long-acting anti-ChE agents, no lenticular opacities or other serious effects were detected (152).

Hemocoagulation

Aberrations of the clotting mechanism attend exposure to OP anti-ChEs (153). Study of 31 persons exposed to sarin or parathion revealed a biphasic reaction consisting of rapid coagulation immediately after exposure, followed by a prolongation of clotting time (91,154). Anti-ChEs also increase fibrinolysis. Fibrinolysis and hypercoagulability were noted in two patients with mevinphos poisoning (155). Increased coagulation was associated with increased prothrombin activity (secondary to increased Factor VII activity) and with increased prothrombin consumption. Coagulation abnormalities normalize several weeks after exposure, and there is no overt liver damage.

IMMEDIATE AND DELAYED EFFECTS IN VOLUNTEERS AFTER SINGLE OR REPEATED EXPOSURE TO ANTI-CHES AT EDGEWOOD

According to the available information, 1,406 subjects were tested with 16 agents of unstated purity (Table 3–3). Some of the subjects also were treated with protective or reactivating agents (case file data of Edgewood subjects, Appendix E).

For this review, approximately 15% (219) of the medical records were selected on the basis of high dosage, repetitive exposure, or the presence of additional physiologic stress. Case records were also selected randomly on the terminal digit of the case number (i.e., ending in 3). Brief summaries were made available to the Panel. In addition, complete records of 32 persons given EA 3148 intravenously were examined. These were chosen because this agent is considered the most potent of the anti-ChEs tested (156). In all cases, subjects were identified by pharmacologic class, by agent, and by letter code; names were withheld.

The case summaries are brief and anecdotal. With the exception of one case, they deal only with the period immediately after the test dose. There are no reports of neurologic or psychologic examinations (with the exception of subject A5J), and only four reports of EEGs—three made before treatment with the anti-ChE agent, and one after. The medical records of subjects tested with EA 3148 comprise doctors' orders, observations of the patient recorded largely by nurses, a clinical master log recording only blood pressure and pulse before and after exposure, results of a test of numerical facility, and ChE concentrations in blood, plasma, and red cells, before and at intervals after exposure. Physician's workup, progress, and discharge notes are absent. Descriptions of the subjects' reactions are rather vague and are certainly not sufficient for careful analysis of long-term effects of these agents. The various subjective complaints listed in the case-file data of Appendix E are not further documented by examination of findings.

The major focus of this Panel's investigation is the possibility of long-term or delayed effects. Essentially, anecdotal information is provided in the case summaries and some of this information seems to suggest that immediate psychological effects can follow the administration of both reversible and irreversible cholinesterases. However, the summaries do not provide hard data that would allow the panel to address, in a definitive manner, the question of whether or not there is a possibility of long-term or delayed effect. As noted above, the case summaries were concerned with the period immediately after administration of the agent and therefore give no indication of the possibility of long-term adverse effects. The paucity of data in the medical records prevents further study in relation to the goal of this report. However, there are published papers which appear to demonstrate long lasting EEG changes from single or repeated exposure to OPs (83).

In reviewing the detailed case report of the single subject who experienced long-lasting psychologic symptoms, the Panel noted that he had had both physical and psychologic evaluations before acceptance in the volunteer program. Evidently, this included examinations and psychologic testing (the MMPI was mentioned). It is possible that these data, if available, can be used as a basis for comparison with a long-term followup study, if such is undertaken.

MORTALITY DATA

A preliminary review of the standardized mortality ratios (SMRs) suggests that mortality was not significantly increased by exposure to anti-ChE chemicals, as tested at Edgewood (Table 4). Whether one looks at subjects exposed to sarin only or sarin plus another chemical (sarin total), VX only or VX plus another chemical (VX total), anti-ChE only or anti-ChE plus another chemical, the SMRs are roughly 80% (or less) of the rates expected for the U.S. population as a whole. This presumably reflects the fact that those who enter the military service do not have chronic diseases. These findings provide a first approximation of mortality and were intended to reveal trends. A more thorough evaluation of mortality findings is contained in Chapter 4.

EVALUATION OF THE LIKELIHOOD OF LONG-TERM ADVERSE HEALTH EFFECTS

The following commentary is based on evaluation of the known adverse health effects of anti-ChEs on humans and animals, the type, number, amount, and route of administration of agents, and medical records of subjects released by the Army.

The subjects were experimentally given anti-ChE agents in the CW testing program during the 1950s and 1960s. The mortality data, collected during 1981, led to the conclusion that, during the elapsed time since testing, subjects were no more likely to have died than comparable soldiers outside the testing program. Morbidity data are unavailable and should be collected; special focus should be placed on the possible long-term adverse health effects highlighted below.

It must be emphasized that the following opinions represent essentially extrapolations from known data. Although there are published and nonpublished results indicating that EEG changes can occur for one year after exposure to organophosphorus drugs, there is no documentation available as to longer-lasting effects. The statements offered below therefore represent conjecture on the part of Panel members and their consultants, who collectively have broad experience with and expertise on anti-ChEs and their effects in humans and animals.

BRAIN DYSFUNCTION

There are no records to indicate that the soldiers might have experienced subtle changes in brain function that lasted for long periods after discharge from the test environment (except, perhaps, in the one case cited above). But examination of primates and humans exposed to single or repeated doses of sarin has revealed statistically significant changes in the EEG that are apparent for at least a year after exposure (83). These observations have yet to receive laboratory confirmation, and their exact importance is unknown. There are also a number of anecdotal reports of minor disorders of affect, sensation, memory, and sleep in humans accidentally exposed to OP war gases or insecticides. The underlying changes in brain structure and function are unknown. The EEG changes are compatible with abnormalities of sleep and behavior. It is also possible that pre-existing psychopathologic conditions were exacerbated. Some subjects were treated with soman, which has been alleged (in a single, limited experimental study) to induce in laboratory rats profound and irreversible neuropathologic changes in vitally important regions of the brain (128). This laboratory study has not been replicated, and the duration and lifetime significance, if any, of the above-noted effects in humans are not known; they should be looked for in both adult life and old age. There is no evidence from the medical records that subjects given potent anti-ChE chemicals (e.g., military nerve agents) experienced the more severe acute effects that were produced in animals with large doses of soman. In particular, there are no reports of respiratory insufficiency or convulsions that could precipitate periods of hypoxia and lead to permanent damage of brain tissue.

JUNCTIONAL NEUROMYOPATHY AND DELAYED NEUROPATHY

Medical records of the vast majority of subjects tested do not refer to induction of the muscle hyperactivity that is associated with acute neuromyopathy in laboratory animals and humans poisoned by OP insecticides. Although there was no systematic attempt to inspect tested subjects for fibrillation or fasciculation of skeletal musculature during or after testing, the doses of anti-ChEs used in relation to the route of application are considered unlikely to have induced muscle damage in most of the subjects. Even if a subacute neuromyopathy had occurred in a few subjects, especially those who reportedly displayed weakness and muscle twitching after exposure, experience with laboratory animals suggests that these changes would have been resolved within weeks.

Although some of the OP agents tested can induce peripheral neuropathy in laboratory animals, the doses needed to induce clinical signs greatly exceed the LD_{50} for that species (105,112, 113). Humans are generally understood to develop neuropathy after receiving doses comparable with those which induce clinical signs in hens (69,121); because the doses used on the test subjects were far below those needed to induce experimental neuropathy, it is most unlikely that any subject experienced delayed onset of distal limb paralysis. However, clinical signs of neuropathy in experimental animals occur some time after the onset of CNS-PNS distal axonopathy, after a particular number of nerve fibers have undergone degeneration. It is therefore not possible to rule out the chance that subclinical pathologic changes occurred in vulnerable nerve-fiber pathways in subjects treated with agents known to be capable of inducing neuropathy. PNS nerve fibers probably undergo regeneration and re-establishment of end-organ connection promptly after the degenerative phase has terminated; damaged CNS fibers are unlikely to regenerate functional connections, but minor damage of this type usually does not induce noticeable functional impairment. Development in the test subjects of anything more than minor and transient sensorimotor manifestations as an expression of such putative damage is considered most unlikely and no such symptoms were recorded. Neurologic examination would reveal signs of long-term CNS changes, such as the Babinski sign (extensor plantar response), hyperactive ankle or knee jerk, and spastic or ataxic gait. Such signs would be permanent attributes of a person who suffered this type of CNS damage, but such changes in the test subjects are considered unlikely.

OTHER ADVERSE HEALTH EFFECTS

Mutagenicity, Carcinogenicity, and Male Reproductive Effects

There is little information on mutagenicity, carcinogenicity, and male reproductive effects, in relation to anti-ChE chemicals. Experimental evidence suggests that malathion does not pose a mutagenic or carcinogenic risk to exposed humans, nevertheless, some scientists disagree with this conclusion (157). The safety of the other compounds could not be confidently determined, because of the absence of laboratory studies. Nevertheless, the Panel is unaware of any reports linking these adverse health effects to single or repeated exposure to anti-ChE agents. Furthermore, there is no suggestion of increased mortality from carcinogenicity, although final judgment on this issue must be reserved until mortality and morbidity data are collected and analyzed.

Information on whether the tested subjects have sired children and on the state of health of their offspring since testing would be helpful in evaluating the possibility of anti-ChE-induced mutagenicity or adverse effects on the male reproductive system.

Cataractogenicity

On the basis of a review of the literature, it appears highly unlikely that single or occasional systemic exposure of young adult subjects to anti-ChE agents would result in the development of initially undetectable, long-term damage to the eye.

Blood Changes

Abnormalities of blood coagulation reported in persons exposed to particular anti-ChEs are considered reversible. Therefore, no long-term adverse effects on hemocoagulation in the test subjects are foreseen.

CONCLUSIONS

The panel concludes that although no evidence has been developed (to date) that any of the anticholinesterase test compounds surveyed carries long-range adverse human health effects in the doses used, the results of an ongoing NAS/NRC morbidity study may shed further light on this issue. The panel therefore is unable to rule out the possibility that some anti-ChE agents produced long-term adverse health effects in some individuals. Exposures to low doses of OP compounds have been reported (but not confirmed) to produce subtle changes in EEG, sleep pattern, and behavior that persist for at least a year. Whether the subjects at Edgewood incurred these changes and to what extent they might now show these effects are not known. If such changes occurred and persisted, they would be difficult to detect now. They could be determined scientifically only by a new study in which EEG, sleep state, and psychologic-test scores were compared with those from nonexposed control subjects. This might be considered, if reasonable suspicion develops, based on responses obtained in the referenced morbidity study, that selected subjects experienced behavioral changes traceable in onset to experimental exposure to the anti-ChE agents.

TABLE 1 RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES

EFFECTOR ORGANS	ADRENERGIC IMPULSES[1]		CHOLINERGIC IMPULSES[1]
	Receptor Type	*Responses*[2]	*Responses*[2]
Eye			
Radial muscle, iris	α	Contraction (mydriasis) ++	——
Sphincter muscle, iris		——	Contraction (miosis) +++
Ciliary muscle	β	Relaxation for far vision +	Contraction for near vision +++
Heart			
S-A node	β_1	Increase in heart rate ++	Decrease in heart rate; vagal arrest +++
Atria	β_1	Increase in contractility and conduction velocity ++	Decrease in contractility, and (usually) increase in conduction velocity ++
A-V node	β_1	Increase in automaticity and conduction velocity ++	Decrease in conduction velocity; A-V block +++
His-Purkinje system	β_1	Increase in automaticity and conduction velocity +++	Little effect
Ventricles	β_1	Increase in contractility, conduction velocity, automaticity, and rate of idioventricular pacemakers +++	Slight decrease in contractility claimed by some
Arterioles			
Coronary	α,β_2	Constriction +; dilatation[3] ++	Dilatation ±
Skin and mucosa	α	Constriction +++	Dilatation[4]
Skeletal muscle	α,β_2	Constriction ++; dilatation[3,5] ++	Dilatation[4] +
Cerebral	α	Constriction (slight)	Dilatation[4]
Pulmonary	α,β_2	Constriction +; dilatation[3]	Dilatation[4]
Abdominal viscera; renal	α,β_2	Constriction +++; dilatation[5] +	——
Salivary glands	α	Constriction +++	Dilatation ++
Veins (Systemic)	α,β_2	Constriction ++; dilatation ++	——
Lung			
Bronchial muscle	β_2	Relaxation +	Contraction ++
Bronchial glands		Inhibition (?)	Stimulation +++
Stomach			
Motility and tone	α_2,β_2	Decrease (usually)[7] +	Increase +++
Sphincters	α	Contraction (usually) +	Relaxation (usually) +
Secretion		Inhibition (?)	Stimulation +++
Intestine			
Motility and tone	α_2,β_2	Decrease[7] +	Increase +++
Sphincters	α	Contraction (usually) +	Relaxation (usually) +
Secretion		Inhibition (?)	Stimulation ++
Gallbladder and Ducts		Relaxation +	Contraction +
Kidney	β_2	Renin secretion ++	——
Urinary Bladder			
Detrusor	β	Relaxation (usually) +	Contraction ++
Trigone and sphincter	α	Contraction +++	Relaxation ++
Ureter			
Motility and tone	α	Increase (usually)	Increase (?)
Uterus	α,β_2	Pregnant: contraction (α); nonpregnant: relaxation (β)	Variable[8]
Sex Organs, Male	α	Ejaculation +++	Erection +++
Skin			
Pilomotor muscles	α	Contraction ++	——
Sweat glands	α	Localized secretion[9] +	Generalized secretion +++
Spleen Capsule	α,β_2	Contraction +++; relaxation +	——
Adrenal Medulla		——	Secretion of epinephrine and norepinephrine

EFFECTOR ORGANS	ADRENERGIC IMPULSES[1]		CHOLINERGIC IMPULSES[1]
	Receptor Type	*Responses*[2]	*Responses*[2]
Liver	α,β_2	Glycogenolysis, gluconeogenesis[10] +++	Glycogen synthesis +
Pancreas			
Acini	α	Decreased secretion +	Secretion ++
Islets (β cells)	α	Decreased secretion +++	——
	β_2	Increased secretion +	——
Fat Cells	α,β_1	Lipolysis[10] +++	——
Salivary Glands	α	Potassium and water secretion +	Potassium and water secretion +++
	β	Amylase secretion +	
Lacrimal Glands		——	Secretion +++
Nasopharyngeal Glands		——	Secretion ++
Pineal Gland	β	Melatonin synthesis	——

Koelle, G.B. In: The Pharmacological Basis of Therapeutics, Goodman, L.S. and Gilman, A, eds.), 5th edition. Macmillan Publishing Co., New York, 1975. Pg. 408.

[1]Responses are designated 1+103+10 provide an approximate indication of the importance of adrenergic and cholinergic nerve activity in the control of the various organs and functions listed.

[2]Dilatation predominates in situ due to metabolic autoregulatory phenomena.

[3]Cholinergic vasodilatation at these sites is of questionable physiological significance.

[4]Over the usual concentration range of physiologically released, circulating epinephrine, B-receptor response (vasodilation) predominates in blood vessels of skeletal muscle and liver; a-receptor (vasoconstriction), in blood vessels of other abdominal viscera. The renal and mesenteric vessels also contain specific dopaminergic receptors, activation of which causes dilatation, but their physiological significance has not been established.

[5]Sympathetic cholinergic system causes vasodilatation in skeletal muscle, but this is not involved in most physiological responses.

[6]It has been proposed that adrenergic fibers terminate at inhibitory B receptors on smooth muscle fibers, and at inhibitory a receptors on parasympathetic cholinergic (excitatory) ganglion cells of Auerbach's plexus.

[7]Depends on stage of menstrual cycle, amount of circulating estrogen and progesterone, and other factors.

[8]Palms of hands and some other sites (“adrenergic sweating”).

[9]There is significant variation among species in the type of receptor that mediates certain metabolic responses.

TABLE 2 Effects of Cholinergics in Normal and Psychotic Persons*

Drug	Effect on Normal Person	Effect on Psychotic Person
Physostigmine antichEs	Depression; Psychomotor retardation	Improvement, particularly of thought disorder; No effect; antagonism of methylphenidate activation
Organophosphorus antichEs	Dysphoria; nightmares; excessive dreaming; hallucinations and delusions; schizoid reactions; auditory hallucinations; paranoid and religious delusions; agression	Some improvement in hebrephrenics; exacerbation in most cases (paranoids)
Choline	Depression	Improvement
Arecoline, oxotremorine		Increased interaction, lucid interval

*Derived from review papers by Singh and Lal (79) and by Karczmar and Richardson (80) and papers of Gershon and Shaw (81), Metcalf and Holmes (82), Duffy and Burchfiel (83), and Karczmar and Ohta (84).

TABLE 3 Summary of Tests Conducted and Records Selected for Anticholinesterase Chemicals

Tox No.	Compound	No. Subjects Tested	No. Records Selected
A-1[b]	Sarin, GB, 1208	246	25
A-2[b]	VX, 1701	740	75
A-3[b]	GA, tabun	26	13
A-4[b]	GF, 1212	21	10
A-5[b]	GD, 1210, soman	83	10
A-6[b]	DFP, diisopropylfluorophosphate	11	5
A-7[b]	EA 3148	32	16
A-9[c]	THA	15	5
A-10[d]	Eserine, physostigmine	138	23
A-11[d]	Prostigmine, neostigmine	22	5
A-12[e]	Hexafluorenium (Mylaxen)	11	11
A-13[d]	Pyridostigmine	27	8
A-14[b]	Malathion	10	5
A-20[f]	Methacholine	9	3
A-21[f]	Urecholine	15	5
	Total	1,406	219

[a]Two sets of records, one based on high dose, another based on random selection; each set contains 219 records.

[b]Organophosphate ester.

[c]Acridine.

[d]Carbamate.

[e]Quaternary ammonium inhibitor.

[f]Cholinergic agonist.

Table 4 Chemical Classification by Cause of Death, Anticholinesterase Chemicals

Causes of Death	Anticho-linesterase Only			Anticho-linesterase Total			Sarin Only			Sarin Total			VX Only			VX Total			Total All Classifications		
	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E
All Trauma	7	10.01	.70	14	19.50	.72	1	2.55	.39	2	3.93	.51	5	6.02	.83	9	12.51	.72	66	94.89	.70
All Disease	12	13.80	.87	17	24.59	.69	5	4.69	1.07	6	7.33	.82	7	7.54	.93	10	14.91	.67	90	107.00	.84
Malignant Neoplasms	6	3.07	1.95	7	5.46	1.28	2	1.05	1.91	2	1.65	1.21	4	1.67	2.40	5	3.29	1.52	26	23.91	1.09
Cardio-vascular System	4	5.65	.71	7	9.84	.71	2	2.04	.98	2	3.21	.62	2	3.00	.67	4	5.84	.69	34	42.03	.81
Cirrhosis of the Liver	1	1.03	.97	1	1.85	.54	1	.34	2.94	1	.53	1.89	0	.57	–	0	1.15	–	7	7.73	.91
Total All Causes*	26	32.58	.80	45	64.01	.70	9	10.87	.83	12	16.89	.71	16	18.07	.89	24	34.63	.69	218	268.38	.81
Total Subjects	602			1317			152			239			362			729			6548		

*This total includes the causes of death listed in this table plus all other causes, including cause not yet determined.

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3

ANTICHOLINERGICS

ATROPINE, SCOPOLAMINE, AND RELATED THERAPEUTIC ANTICHOLINERGIC CHEMICALS*

SITES OF ACTION

Anticholinergic drugs inhibit the actions of acetylcholine (ACh) on autonomic effectors that are innervated by postganglionic cholinergic nerves and on smooth muscles that lack cholinergic innervation; that is, they antagonize the muscarinic actions of ACh. They are therefore known as antimuscarinic agents or muscarinic cholinergic blocking agents. Because the main actions of all members of this class of drugs are qualitatively similar to those of the best-known member, atropine, the terms “atropinic” and “atropine-like” are also appropriately used.

In general, antimuscarinic agents have little effect on the actions of ACh at nicotinic receptor sites. Thus, at autonomic ganglia, where transmission normally involves an action of ACh on nicotinic receptors, atropine produces partial block only at relatively high doses. At neuromuscular junctions, where the receptors are nicotinic, extremely high doses of atropine or related drugs are required to cause any degree of blockade. However, quaternary ammonium analogues of atropine and related drugs generally have some degree of nicotinic blocking activity and consequently are more likely to interfere with ganglionic or neuromuscular transmission in doses only slightly greater than those that produce muscarinic block.

In the central nervous system (CNS), cholinergic transmission appears to be predominantly nicotinic in the spinal cord and both muscarinic and nicotinic at subcortical and cortical levels in the brain (2). Accordingly, many or most of the CNS effects of atropine-like drugs at ordinary doses are probably attributable to their central anticholinergic actions. At high or toxic doses, the central effects of atropine and related drugs consist, in general, of stimulation followed by depression; these are probably due to a combination of antimuscarinic and other actions. There is an increased release and turnover of ACh in the CNS associated with the administration of antimuscarinic drugs; this may result in the activation of nicotinic receptors in the brain and contribute to the central effects of this class of drugs (3). Because quaternary compounds penetrate the blood-brain barrier only poorly, antimuscarinic drugs of this type show little in the way of central effects.

*Most of the information in this section is from Chapter 7 of Goodman and Gilman (1).

PERIPHERAL EFFECTS

Parasympathetic neuroeffector junctions in different organs are not equally sensitive to antimuscarinic agents. However, the relative sensitivity of various parasympathetically innervated organs to blockade by atropinic agents varies little among the drugs. Small doses inhibit salivary and bronchial secretion and sweating. Larger doses cause the pupils to dilate, inhibit accommodation of the eyes, and block vagal effects on the heart, so that the heart rate is increased. Still larger doses inhibit the parasympathetic control of the urinary bladder and gastrointestinal tract, thus inhibiting micturition and decreasing intestinal tone and motility. Even larger doses are required to inhibit gastric secretion and motility. Because only the primary phase of gastric secretion is controlled by the vagus, the remaining hormonally controlled secretion is unaffected. Thus, doses of any antimuscarinic drug that reduce the tone and motility of the stomach and the duodenum and inhibit gastric secretion also invariably affect salivary secretion, ocular accommodation, and micturition.

The drugs produce reversibly the functional equivalent of resection or paralysis of postganglionic cholinergic nerves. The actions and effects of particular antimuscarinic agents usually differ only quantitatively from those of atropine, a belladonna alkaloid, which is considered in detail as the prototype of the group.

CENTRAL EFFECTS

Atropine stimulates the medulla and higher cerebral centers. In doses used clinically (0.5–1.0 mg), this effect is usually confined to mild vagal excitation. The rate and occasionally the depth of breathing are increased, but this effect is probably the result of bronchiolar dilatation and the consequent increase in physiologic “dead space.” With toxic doses of atropine, central excitation becomes more prominent, leading to restlessness, irritability, disorientation, hallucinations, or delirium. With still larger doses, stimulation is followed by depression, coma, and medullary paralysis. The latter may be primarily responsible for a fatal outcome. Even moderate doses of atropine may depress some central motor mechanisms that control muscle tone and movement. This effect has been used to advantage in the management of the tremor and rigidity of Parkinsonism.

Scopolamine in therapeutic doses normally causes drowsiness, euphoria, amnesia, fatigue, and dreamless sleep with a reduction in rapid-eye-movement (REM) sleep. However, the same doses of scopolamine occasionally cause excitement, restlessness, hallucinations, or delirium, especially in the presence of severe pain.

Doses of atropine required to inhibit peripheral responses to choline esters or anticholinesterase (anti-ChE) agents produce almost no detectable central effects. This may reflect difficulty of penetration of the drug into the CNS. In animals, atropine antagonizes the action of ACh applied locally to the cerebral cortex and spinal cord. However, atropine also depresses the effects of noncholinergic stimuli, indicating that the drug has central actions other than blocking cholinergic synapses.

The rise in body temperature due to the belladonna alkaloids is usually significant only after large doses. Nevertheless, in infants and small children, moderate doses induce "atropine fever." In atropine poisoning in infants, the temperature may reach 43°C or higher. Suppression of sweating is doubtless an important factor in the production of the fever, especially when the environmental temperature is high, but other mechanisms also may be important when large doses are taken. It has been suggested that atropine may exert a central effect on temperature regulation; however, animals that do not sweat, such as dogs, do not exhibit fever after atropine.

POISONING BY BELLADONNA ALKALOIDS

The deliberate, or accidental ingestion of belladonna alkaloids or other drugs with atropinic properties is a major cause of poisonings. Infants and young children are especially susceptible to the toxic effects of atropinic drugs (4). Delirium and toxic psychoses, without undue peripheral manifestations, have been reported in adults after instillation of atropine eyedrops.

Fatalities from intoxication with atropine and scopolamine are rare, but they sometimes occur in children, in whom 10 mg or less may be lethal. Idiosyncratic reactions are more common with scopolamine than with atropine, and ordinary therapeutic doses sometimes cause alarming effects. Table 1 lists undesirable responses or symptoms of overdose associated with various doses of atropine.

SYMPTOMS AND SIGNS OF ATROPINE POISONING

Symptoms and signs of atropine poisoning develop promptly after ingestion. The mouth becomes dry and the person experiences a burning sensation; swallowing and talking are difficult or impossible; and there is marked thirst. The vision is blurred, and photophobia is prominent. The skin is hot, dry, and flushed. A rash may appear (more likely in children), especially over the face, neck, and upper part of the trunk; desquamation may follow. Body temperature rises, especially in infants. The pulse is weak and very rapid although tachycardia may be less pronounced in infants and old people. Palpitation is prominent, and blood pressure may be increased. Urinary urgency and difficulty in micturition are sometimes noted. Abdominal distention may develop, especially in infants.

The patient is restless, excited, and confused and exhibits weakness, giddiness, and muscular incoordination. Gait and speech are disturbed. Nausea and vomiting sometimes occur. The behavior and mental signs may suggest an acute organic psychosis. Memory is disturbed, orientation is faulty, hallucinations (especially visual) are common, the sensorium is clouded, and mania and delirium are not unusual (5). The diagnosis of an acute schizophrenic episode or alcoholic delirium has been mistakenly made, and some patients have been committed to psychiatric institutions for observation and diagnosis. The syndrome often lasts 48 h or longer and may be punctuated by convulsions. Depression and circulatory collapse occur only in cases of severe intoxication; blood pressure decreases, respiration becomes inadequate, and death due to respiratory failure follows after a period of paralysis and coma (6).

TREATMENT

When intoxicating doses of atropine have been taken orally, gastric lavage and other measures to limit intestinal absorption should be initiated without delay. Physostigmine, long overlooked as a possible antidote to atropine poisoning, is the rational therapy. For example, the slow intravenous injection of 1–4 mg of physostigmine (0.5–1.0 mg in children) rapidly abolishes the delirium and coma caused by large doses of atropine. Because physostigmine is metabolized rapidly, the patient may again lapse into coma within 1–2 h, and repeated doses may be needed (4,5,7).

MECHANISM OF ACTION

The major action of the antimuscarinic agents is a competitive antagonism of the actions of ACh and other muscarinic agonists. The antagonism can be overcome by increasing sufficiently the concentration of ACh at receptor sites of the effector organ. The receptors affected are those of peripheral structures that are either stimulated or inhibited by muscarine—that is, exocrine glands and smooth and cardiac muscle. Much evidence supports the notion that atropine and related compounds compete with muscarinic agonists for identical binding sites on muscarinic receptors (2,8,9).

ABSORPTION, FATE, AND EXCRETION

The belladonna alkaloids are absorbed rapidly from the gastrointestinal tract. They also enter the circulation when applied locally to mucosal surfaces. Only limited absorption occurs from the intact skin. Atropine disappears rapidly from the blood and is distributed throughout the entire body. Most is excreted in the urine within the first 12 h, in part unchanged. Only about 1% of an oral dose of scopolamine is eliminated as such in the urine, traces of atropine are found in various secretions, including milk. The total absorption of quaternary ammonium derivatives of the alkaloids after an oral dose is only about 10–25 percent (10,11); nevertheless, some of these compounds can cause mydriasis and cycloplegia if applied to the eye.

PHARMACOLOGICAL PROPERTIES OF SYNTHETIC ATROPINE DERIVATIVES

After reports in the late 1950s of the psychotomimetic effects of a number of basic esters of substituted glycolic acids related to scopolamine and atropine, several hundred compounds were synthesized at Edgewood and by its contractors and by academic and industrial researchers. These compounds were intended to be more active centrally than peripherally. Because quaternary salts of these anticholinergic compounds usually penetrate the CNS only with great difficulty, such derivatives were of little interest in the testing program.

The compounds prepared and tested in the Edgewood program are listed in Table 2, and their structures are shown in the master file (Appendix B). The basic structures of these compounds, which are substituted glycolates, are as follows:

$$R_1 - \overset{\displaystyle X}{\underset{\displaystyle R_2}{\overset{|}{\underset{|}{C}}}} - \overset{\displaystyle O}{\overset{\|}{C}} - O - R_3 \text{ (basic structure of glycolates),}$$

where X=-OH, $-CH_2OH$, $-OCH_3$, -Cl, -Br, -I, $-O_2CCH_3$,

R_1 and R_2=hydrogen, phenyl, substituted phenyl, alkyl, cycloalkyl, ethylenic, olefinic, thienyl, and

R_3=acyclic aminoalkyl, cyclic aminoalkyl, bicyclic amino alkyl (e.g., tropine or scopine for atropine and scopolamine, respectively).

Table 3 lists the relative potencies (comparing central with peripheral effects) of many of these derivatives. Note that BZ and EA 3443 exhibit extensive central effects for a long period; the studies at Edgewood emphasized these chemicals, as does this report.

In a comparison of a series of structurally related anticholinergic compounds, there was less discrepancy in potency with respect to peripheral than central actions. Because data on their effects on man are limited, most of the information was derived from animal behavioral studies, which in general predict psychotomimetic actions in man.

BZ and other quinuclidinyl glycolates containing at least one phenyl and a cycloalkyl group in the acid moiety were the most potent and the longest-acting both in animals and in man. The corresponding piperidyl glycolates were one-fifth to one-half as active as the quinuclidinyl esters. Atropine is equipotent (although of shorter duration) to BZ in producing peripheral anticholinergic effects, but its central actions are much less pronounced.

ROLE OF DRUG DISPOSITION IN EXPLAINING DIFFERENCES AMONG ANTICHOLINERGIC COMPOUNDS

ATROPINE

Numerous reports dealing with the disposition of labeled atropine in animals and are reviewed by Wills in Appendix I. Investigators have been only partially successful in their efforts to compare the pharmacologic actions and relative potencies of the various anticholinergic compounds in the CNS with their distribution in brain or their binding to brain components. For instance, BZ has been reported to adsorb to isolated brain mitochondria about 3 times as avidly as atropine (12). The affinity of BZ for peripheral muscarinic receptors was also greater than that of atropine (8), and atropine competed with BZ for specific binding sites in the brain. Because BZ is known to be more potent than atropine and its duration of action is appreciably longer (13), these drug effects could be due to differences in the affinities of the two drugs for specific receptor sites.

BZ

Snyder's group (14,15) used autoradiography to determine precisely the location of binding of $[^3H]$BZ in rat brain. In the cerebral cortex, the greatest binding of the benzilate was in the occipital cortex and the cortex of the cingulate gyrus, with decreasing binding in the cortex in the piriform area and the frontal pole. In the hippocampus, three strata of the archipallium were found to have approximately equal abilities to bind BZ, whereas the white matter of the alveus bound less. The high binding of BZ to the striate body was particularly striking in autoradiographs because of the comparatively low binding activity of the adjoining globus pallidus. Thalamic and hypothalamic nuclei exhibited less binding of BZ, as did the inferior and superior colliculi. Still lower in the binding of this ligand were cerebellar cortex, medulla oblongata-pons, and the cerebral peduncles. Nerve tracts—e.g., the optic chiasma, the cervical spinal cord, and the corona radiata—exhibited the lowest degree of binding.

The areas of the brain that retained the greatest concentrations of the label after intravenous injection of [^{3}H] BZ into cats (16) were motor cortex, sensory cortex, caudate nucleus, lateral geniculate, and medial geniculate. Smaller concentrations were retained in thalamus, hippocampus, hypothalamus, medulla oblongata, colliculi, cerebellar cortex, the pyramids of the medulla, cerebral white matter, and cerebellar white matter.

Both cholinomimetic and anticholinergic compounds reduced the retention of the label after [^{3}H]BZ treatment in some areas of the brain. The cholinomimetic compounds used were 1,2,3,4-tetrahydro-5-aminoacridine (THA) and S-diethylaminoethyl-diethylphosphorothionate, both inhibitors of cholinesterases. For the anticholinergic compounds, displacement of the label probably was related to the substitution of one drug by another.

Zvirblis and Kondritzer (12) found that a mitochondrial fraction prepared from rat brain adsorbed 3–4 times as much BZ as atropine in the physiologic range of pH. The optimal pH for adsorption of BZ was about 8.0, whereas that for atropine was about 9.7; however, at their own optimal pH values, the adsorptions of the two anticholinergic compounds were about the same. THA, Ca^{2+} and PO_4^{2-} decreased the adsorption of the benzilate by mitochondria.

Yamamura et al. (17) studied the adsorption of [^{3}H]BZ on the particulate fraction of homogenates of various regions of the brain of monkey after incubation with [^{3}H]BZ in phosphate buffer at a pH of 7.4. The regions that were the most avid adsorbers of the benzilate, in order of decreasing uptake, were putamen, caudate nucleus, occipital cortex, cingulate gyrus, postcentral gyrus, pyriform cortex, frontal cortex, superior colliculi, thalamus, and inferior colliculi. This distribution of adsorptive sites among various parts of the monkey brain incubated in vitro differs from that for intravenously injected [^{3}H]BZ in the cat (16). The two studies do agree that the caudate nucleus is one of the highest areas of localization of [^{3}H]BZ. Yamamura et al. (17) found that uptake of the label by various areas of brain was correlated with choline uptake and with choline acetyltransferase activity.

Interruption of the septal hippocampal tract and elimination thereby of cholinergic afferents to the hippocampus reduced by about 70% the activity of choline acetyltransferase in homogenates of the hippocampus, but did not alter the binding of [^{3}H]BZ by particles of such homogenates (8). The investigators proposed that presynaptic muscarinic sites that are innervated by the septal hippocampal nerve fibers do not bind the labeled benzilate, whereas postsynaptic muscarinic sites do bind it.

Ketchum (18) provided information that suggests that the brain is the only mouse organ that contains ^{3}H 48 h after intraperitoneal injection of [^{3}H]BZ. Perfusion of [^{3}H]BZ through rat liver had indicated that this organ destroyed the compound quickly; 90% of the compound present originally in the perfusing fluid disappeared from the perfusate within 30 min, and less than 0.2% of the unaltered compound was found in the bile. The identity of the products was not determined.

The effects of BZ and Ditran, compared with atropine and scopolamine, have been examined to elucidate the mechanisms by which these compounds produce their effects. All these compounds have an extremely high affinity for muscarinic receptor sites, so the higher potency and longer duration of action of BZ must be related to other properties.

The long-lasting effects (up to 7 d) of a single injection of BZ in cats (19) raised the issue of possible irreversible interaction of BZ with brain sites. With another anticholinergic drug, [^{3}H] pyrrolidylmethylphenylcyclopentylglycolate (20), a small amount of radioactivity was still measurable 3 d after a single injection. Considering the high affinity of BZ for muscarinic receptors (K_d=10^{-11} M), it would not be surprising to find BZ still attached to brain receptors 5–7 d later, especially because pharmacologic effects of BZ are evident in man after 7–8 d. The prolonged action of BZ, compared with its somewhat shorter duration of binding to CNS structures, is not yet readily explainable.

ANIMAL TOXICOLOGY OF ANTICHOLINERGIC COMPOUNDS

IMMEDIATE EFFECTS

BZ

BZ has been chosen as the paradigm of the various anticholinergic test compounds used at Edgewood. It was used in a large number of volunteers and is probably one of the best documented. Because it is also one of the longest-acting of all the drugs, it is also more likely to cause persistent adverse effects. It was one of the first glycolates used in relatively high doses in man, so more years have elapsed in which long-range consequences might have occurred.

The pharmacology and toxicology of BZ have been studied extensively in animals. Much of the information was derived through a contract with Hazleton Laboratories, Falls Church. A summary of the preclinical pharmacology and toxicology in rats, dogs and monkeys is presented here; a list of other toxicity studies is given in Appendix F. Additional background information is contained in Appendixes G, H, I, J, K and L.

Rats. Repeated intravenous administration of BZ to male albino rats was carried out for 20 daily injections during a 4-wk period. The doses included 0.01, 0.1, and 1.0 mg/kg.

The most significant pharmacologic sign noted was mydriasis. The daily pattern of mydriasis noted after each injection suggested metabolic inactivation of BZ by rats within 24 h. No tolerance to the mydriatic response was, however, apparent during the course of the study. At the high dose there appeared to be a progressive diminution in the intensity of pharmacotoxic signs (other than mydriasis); that suggested development of tolerance or decreased responsiveness to BZ.

Other measured indicators of pharmacologic effect included body weight, food consumption, hematology, gross and microscopic pathologic signs and organ weights. No significant effect attributable to the repeated administration of BZ was found.

There were no indications of liver disease or malfunction directly attributable to BZ in chronic-toxicity studies. In some rats, there were a few small foci of periportal lymphocytic infiltration; but these occurred as often in saline-treated animals.

Dogs. Repeated intravenous administration of BZ to male and female mongrel dogs was carried out for 20 d over a 4-wk period. The dosages were 0.01, 0.1, and 1.0 mg/kg-d. The indicators of effect were gross pharmacotoxic signs, daily body weight, hematology, biochemistry, urinalysis, organ weights, organ-to-body weight ratios, and gross and microscopic pathology.

A minimal number of effects was produced by low dosages between the eleventh and thirteenth injection days. Both the intermediate and high dosages produced mydriasis, ptosis, decreased activity, ataxia, and weakness of limbs. Other signs were less frequently observed. An increase in sedimentation rate occurred at 4 wk in one low- and one high-dosage animal. Liver weight was high in one male from each test group. Testicular atrophy was observed in one male each from the intermediate- and high-dosage groups. A dose of 10 mg/kg induced bradycardia in all of five dogs and resulted in death due to cardiac arrest in two of the animals (21). Dogs given daily intravenous doses of 100 μg/kg for 14 consecutive days had the same LD_{50} as dogs that had not been so exposed, but the interval between injection of the daily dose and the appearance of ataxia increased from 4 min to 14 min during the period of pretreatment. In dogs trained in a conditioned-escape routine and then given graded intravenous doses of BZ, doses up to and including 12.5 μg/kg had no effect on performance. However, a dose of 25 μg/kg resulted in failure to escape by four of four dogs tested (22). None of the experiments summarized in this report demonstrated any persistent effect of exposure to BZ in surviving dogs.

Monkeys. Repeated intravenous administration of BZ at 0.01, 0.1, and 1.0 mg/kg-d to male and female monkeys was carried out for 20 d over a 4-wk period. The indicators of effect were gross pharmacotoxic signs, daily body weight, hematology, biochemistry, organ weights, organ-to-body weight ratios, and gross and microscopic pathology.

The most frequently observed signs were mydriasis at all dosages and decreased activity and ataxia at the two highest dosages. Blood sugar of one monkey given an intermediate dosage and serum transaminase of one monkey given a high dosage were increased at 4 and 2 wk, respectively. The testicular weight and organ-to-body weight ratio were high for one intermediate-dosage animal. All other findings were within control limits.

Cycloplegia persisted in monkeys for more than 7 d after exposure to BZ, whereas most other effects had disappeared after 2 d (18).

EA 3443

Hazleton Laboratories conducted preclinical toxicity studies with EA 3580, EA 3392, and EA 3443. The following information was abstracted for EA 3443.

A total of 20 daily intravenous injections of EA 3443 in male and female monkeys at 0.01, 0.1, and 1.0 mg/kg produced no hematologic or biochemical abnormalities. Organs examined microscopically presented no significant alterations (23).

A total of 20 daily intravenous injections of EA 3443 in mongrel dogs (two dogs per dose) at 0.01, 0.1, and 1.0 mg/kg produced no hematologic, urinary, or biochemical changes. Histopathologic findings were essentially normal (24).

EA 3834

Prompted by a report of hematuria in a subject receiving EA 3834, research was begun on the effects of these drugs on ureteral and bladder motility (25). Microscopic hematuria was shown to occur within several minutes after intravenous administration of EA 3834 and usually cleared within 1 h. The dogs had ureteral or bladder catheters in place and often had a few red blood cells in the urine before administration of the agent. However, the red cells became much more numerous after administration. No additional information is available.

The hematuria was most likely the result of a reduction in renal blood flow. It is not clear that the hematuria was produced by EA 3834.

LONG-TERM AND DELAYED EFFECTS

Atropine

Atropine sulfate was administered intramuscularly to rabbits daily for 100 d. At a dose estimated to be 5% of the LD_{50}, signs of toxicity were seen. Pathologic signs included weight loss, edema of most organs, hepatitis, pulmonary thrombosis, inhibition of spermatogenesis, thymic atrophy, and toxic changes in the gall bladder, spleen, and pancreas. The rabbits' survival of daily administration of atropine beyond the LD_{50} was related to their ability to maintain food intake (26).

Clidinium (Quartzan)

Clidinium was administered intragastrically to dogs at 1,5 and 25 mg/kg, 5 d/wk for 52 wk (27). The animals were observed for survival, behavior, and general physical condition. Blood, liver and kidney function were evaluated. Organs were examined microscopically at the conclusion of tests. Toxic signs of anticholinergic exposure were not seen. Hematologic characteristics were not significantly altered by these prolonged exposures. Clinical-chemistry measures and results of gross and microscopic evaluations of internal organs were within normal ranges.

Administration of clidinium for 1 yr at 5.0, 25, and 50 mg/kg in the diet of rats did not result in drug-related toxicity. Blood counts, clinical-chemistry measures, and results of gross and microscopic studies remained within normal limits (28).

Other Data

No study of the long-term administration of BZ to animals has been uncovered. The only long-term toxicity study with an ester of benzylic acid was with the ester with diethylaminoethanol; no significant pathemas were found in rats during this lifetime feeding study, but the lifetimes of the rats fed the benzilate may have been shortened somewhat. Feeding of the quaternized ester of 3-quinuclidinol to rats for a year (27) and gavaging of dogs with this compound 5 d/wk for a year (29) produced no significant pathemas.

With respect to genotoxicity, BZ has been found to have weak mutagenic activity in yeast cells in culture (30) and to produce gaps and breaks in chromosomes of bone marrow cells; it has not been proved to produce a heritable change in mammalian species. Diethylaminoethylbenzilate was more toxic cytogenetically than BZ, despite its lower anticholinergic activity. Unfortunately, there is too little information on these compounds to assume or exclude genotoxic effects. Although various test systems have been used (point mutations, chromosomal observations, and dominant-lethal effects), most observed effects probably resulted from general drug toxicity, rather than genotoxicity. The drugs have been tested at only a few concentrations, and interpretation of existing data does not permit estimation of the genotoxicity of BZ or even of atropine or scopolamine.

HUMAN TOXICOLOGY OF ANTICHOLINERGIC COMPOUNDS

DOSING CONSIDERATIONS

Atropine

Textbooks generally suggest that death from atropine poisoning may be expected after ingestion of doses above 100 mg. However, a review of the literature for the last 100 yr yielded a probit estimate of about 450 mg/person, based on several hundred cases of accidental poisoning, including some 40 fatalities. Because the oral route is somewhat less effective than the intravenous or intramuscular (and probably the inhalation) route, a conservative figure of 75 mg/person (approximately 900 μg/kg) is a working estimate for the human LD_{50}.

Other Anticholinergic Compounds

Acute toxicity data on the 21 glycolates studied in man in a cool climate show a fairly consistent ratio between the lethal dose in animals and the heart-rate-increasing dose in man. Extrapolation of the animal data therefore allows an estimate of the risk associated with human exposure. Deaths have occurred in seemingly healthy people when atropine has been given at doses only 50 times greater than the heart-rate-increasing dose. Thus, the estimated LD_{50} in man for BZ is about 200 μg/kg, whereas that for EA 3834 is about 60 μg/kg. This represents safety margins of about 35 for BZ and 100 for EA 3834, where safety margin is the ratio between and ID_{50}

In a warm climate, these numbers need correction, because the heat loss from sweating is diminished. It is speculated that fatal hyperthermia or heatstroke could occur at doses very close to the ID_{50} for BZ and not more than 2 or 3 times the ID_{50} for EA 3834.

In the Edgewood tests, no volunteer received BZ at more than 10% of the LD_{50}. Probably no more than 30 or 40 subjects received more than 3% of the LD_{50}.

Few subjects received more than one dose of a glycolate. In the case of BZ, seven subjects were given the ID_{50} on two occasions, 2–3 wk apart. Two subjects received 8.0 µg/kg twice each, in a crossover double-blind study of the efficacy of physostigmine as an antidote. Eight subjects were given 1 µg/kg daily for 7 d (with no appreciable effects), and four received 2 µg/kg on three consecutive days (with cumulative effects approaching incapacitation on the third day.) Thus, fewer than 10% of all subjects received more than a single dose. The figures for other glycolates are lower. The total exposure of any person to glycolate never exceeded 3 times the ID_{50} dose in the entire series of tests.

USE OF ANTIDOTES

Anticholinergic agents are typically used as treatment for anticholinesterase poisoning and vice versa. Physostigmine, tetrahydroaminoacridine, and other cholinesterase inhibitors were used successfully as antidotes in several dozen subjects. The demonstration of physostigmine's effectiveness led to the first controlled study of its ability to reverse delirium due to scopolamine (31). Physostigmine has been used to overcome anticholinergic toxicity.

IMMEDIATE EFFECTS

Acute clinical findings were recorded by involved physicians and nurses when Edgewood volunteers underwent tests with 21 anticholinergic chemicals shown in Table 2–2. About 1,750 subjects were tested with agents in this pharmacologic class, 10% of which were summarized for review. Selection for inclusion in this summary was based on high dose, high frequency of administration, or additional physiologic stress; it was the intent to focus on the extreme cases. The summaries identify the compound administered, dose, route of exposure, and significant acute reactions or clinical findings.

Peripheral and Behavioral Effects

In the brain, the arousal system that maintains alertness during waking hours is depressed by the action of BZ; this leads to drowsiness, which may progress to stupor or even coma at higher doses. Simultaneously, the coordinating motor systems of the cerebellum seem to be affected, and that causes an unsteady gait and pronounced clumsiness. There is a subjective sensation of dizziness which may arise in part from disturbances of the vestibular systems in the brainstem. The modulation of muscle tone is affected, possibly owing to an action on the Renshaw interneurons in the spinal cord, and the results are an abnormal increase in tendon reflexes and a tendency toward stiffness and jerkiness in muscle movement. As the drug penetrates higher in the brainstem, hypothalamic systems show signs of disruption. There is a decrease in drinking and eating. Sleep may be inhibited. A tendency toward increased body temperature appears in a warm environment. Finally, the highest integrative systems of the brain are affected. Memory, attention, problem-solving, and judgment are all seriously impaired. The ability to distinguish reality from imagination is lost, and thoughts and words flow in an uncontrolled manner. For a moment, the subject may seem to be in contact with his surroundings, but within seconds he drifts rapidly into incoherent speech and disconnected action. He is, at this stage, truly incapacitated and cannot function sufficiently even to preserve his life. If not brought under control, the delirious subject can inadvertently become a casualty and may fall prey to a fatal accident.

During the early stage of intoxication, activity is reduced, movements are slow and ineffective, and the subject is generally unable to offer effective resistance and tends to be relatively tractable. Once this period (a few hours) has passed, his behavior becomes increasingly unpredictable, energetic, and dangerous.

In the features thus far described, BZ is a typical glycolate, indistinguishable qualitatively from atropine, scopolamine, EA 3834, EA 3443, EA 3580, EA 302,196, or any other compounds of the 21 in this family studied in volunteers.

EEG Effects

There is a close relationship between the changes in EEG patterns and the behavioral effects of drug administration. With high doses of atropine (10–30 mg), for example, or repeated administration, the EEG shows an increase in slow waves, a decrease in mean frequency, a decrease in the percent time and amplitude of alpha activity, and an increase in the fast frequencies, which can be seen to be ‘riding' on the slow waves. There is a direct association between the EEG fast waves with behavioral restlessness and the EEG slow waves with stupor and cognitive defects. At (‘toxic') doses, patients are in stupor or coma, with high heart rate and lowered blood pressure. The EEG demonstrates persistent high-voltage slow waves, with a minimum of alpha and fast frequencies.

EEG and behavioral effects were modified in a parallel manner by the concurrent administration of antagonistic drugs. For instance, when patients who exhibited a toxic delirium to Ditran or atropine were given tetrahydroaminoacridine, a cholinesterase inhibitor, the stupor was relieved and the EEG showed a decrease in both slow and fast frequencies.

During acute administration of various compounds, the time for recovery varied with dose with low doses, the peak effects of parenteral administration were seen in 0.5 h and lasted up to 6 h; with high doses, the effects persisted for up to 24 h. With toxic doses, a return to baseline occurred in the second day after administration. Followup EEG data are limited, the principal data being reports of atropine toxicity. In these studies, the few statements referring to EEG changes suggest that the effects disappear within a few days of the last exposure.

For the test compounds used at Edgewood, EEG data are available only for BZ, EA 3580, and EA 3834 on five, two, and one patients, respectively. The pretreatment and posttreatment EEG records appeared to be within normal limits and reflected effects observed for other CNS-active anticholinergic drugs. Neuropsychologic effects of the exposures to the agents were transient, and test values reverted to baseline. The EEG changes and neuropsychologic effects were temporally related, and a similar reversion to normal EEG patterns was anticipated. No evidence of persistence of behavioral or EEG effects after these experimental trials was reported by Klapper et al. (32).

BZ Case Reports

Data on the 36 subjects whose records were selected for evaluation from the records of 354 subjects treated with BZ indicate that BZ is active when it is given by the intravenous, intramuscular, aerosol inhalation, or oral route. The fragmentary data available indicate that the higher the dose the greater the effects and the longer their duration. Understanding of the timecourse of effects was confounded by erratic written documentation, which at best was rather sparse, and by the introduction, at various times after exposure to the drug, of treatment with cholinomimetics, such as physostigmine and tetrahydroaminoacridine. Both these treatments ameliorated, at least temporarily and partially, some of the effects of BZ.

Intravenous Injection. BZ doses of 4.8 and 8 μg/kg were used in four subjects. The time course was approximately as follows:

- 1 h: Dry mouth, flushed face, numbness in extremities, and sleepiness.
- 4 h: Above, plus twitching, yawning, clonus and abnormal reflexes, and ataxia.
- 24 h: Above, plus disorientation, hallucinations, and poor performance on psychometric tests.
- 36 h: Hallucinations persisting without treatment.
- 48 h: Diminished nonverbal and motor skills.

Single Intramuscular Doses. BZ doses of 5.0–6.4 μg/kg were used in 15 subjects. These subjects were treated at various times with the antidotes mentioned above. The time course of action of the drug by this route was similar to that by intravenous injection; in fact, more symptoms were reported with this route, but this may have been due to differences in the completeness of reporting. The time course was approximately as follows:

- 10 min: Light-headedness and giggling.
- 30 min: Dry mouth, blurred vision, nausea, chilly sensations, and twitching.
- 1 h: Flushed skin, incoordination, fatigue, unsteadiness, sleepiness, and quivering legs.
- 2 h: Many of the above, plus poor concentration, restlessness, hallucinations, slurred speech, and muscle fasciculations.
- 3 h: Above, plus tremors.
- 4 h: Above, plus difficulty in handling subject and belligerence; pulse, 136.

- 8 h: Above, plus delirium and hallucinations.
- 24 h: Persistent delirium, hallucinations, restlessness, unsteadiness, and increased pulse in some but not all subjects.
- 48 h: Persistent impairment of function in some and return to apparent normal state in others, depending on vigor of antidotal treatment.

Repeated Intramuscular Injection Six Times Over Same Number of Days. This program was used in four subjects. Reporting was extremely fragmentary, but perhaps not much happened, inasmuch as the doses used were quite small—0.5 µg/kg. A maculopapular rash that cleared when treatment was stopped was noted twice, and dysphagia was noted in another subject. No mydriasis was seen. No tolerance could be demonstrated, probably because the dose was below threshold. No cumulative effect was noted.

Aerosol. Doses used ranged from 5.3 to 17.1 µg/kg in eight subjects. Understanding of the clinical manifestations was confounded by the use of antidotes at various times after exposure to the drug. The time course was as follows:

- 30 min: Restlessness, sleepiness, nausea, dry mouth, weakness, and coldness of extremities.
- 1 h: Above, plus dizziness.
- 2 h: Above, plus flushed skin.
- 4 h: Above, plus unsteadiness and disorientation.
- 8 h: Above, plus hallucinations.
- 24 h: Residual confusion, disorientation, hallucinations, and delirium in some, but not all, subjects.
- 48 h: Residual mental impairment, jumpiness, and shakes in some and improvement in others, depending on amount of antidote used.

In one subject, fever and spastic movements of the head were noted; he was treated vigorously with antidotes. At 3 h, he became unresponsive, showed decerebrate rigidity, had a high heart rate, and had urinary retention; he was treated vigorously with antidotes. Five years later (1968), he was hospitalized for microscopic hematuria and was shown by renal biopsy to have focal glomerulitis.

Oral. Doses ranged from 4.5 to 5 µg/kg and were given to five subjects. The major focus seemed to be on following heart rates and the EEG. Clinical manifestations noted with the other routes of administration probably also occurred here. Some heart rates became fairly high (130/min). One subject had an EEG tracing that showed episodes of light sleep with spindling.

Summary. In spite of the sketchy records, BZ appears to be an anticholinergic hallucinogen quite comparable with JB-329 (Ditran) or others of the JB series or with scopolamine. The onset of effects is usually rapid, regardless of the route of administration, and the duration is often a function of the dose. Although pharmacokinetic data on these compounds in man are unavailable, there is some evidence of a dose-response relationship. Without treatment to counter the effects of these strong central anticholinergic agents, some effects may persist for several days. For instance, one subject who had received BZ displayed hyperthermia, tachycardia, and spastic movements for a few hours, and required vigorous treatment. He was discharged 6 d after exposure, well oriented and with normal appearance. No further information is available on him; the Veteran's Administration did not find his military records. Another subject developed signs of decerebrate rigidity with limb twitching. Two separate medical opinions were recorded: toxic encephalopathy and BZ delirium. The subject was discharged 6 d after exposure. EEG tracing 20 d after exposure was normal. His VA hospital record provided no useful information.

EA 3834

EA 3834 is somewhat distinctive from the other test compounds. The intravenous ID_{50} is approximately 5.7 μg/kg base equivalent, about the same as that for BZ. Although the time of onset of severe effects of EA 3834 is inversely related to dose (about 35 min for the ID_{50} and about 10 min for 3 times the ID_{50}), the duration of severe effects is roughly constant within this dose range, namely 6–9 h. The constancy of recovery time from EA 3834, which is rather unusual for these drugs, reduces the period of medical care required for higher-dose casualties. In contrast, doubling the ID_{50} prolongs BZ incapacitation about 40 h, as it does for EA 3443 and EA 3580.

Physostigmine has proved effective as an antidote to EA 3834, as it has with many other glycolates. Within 20 min of a single intramuscular injection of 1 mg of physostigmine salicylate per 18 g of body weight, the stuporous subject becomes alert and able to perform at close to his normal level.

Possible renal toxicity has been reported in two men after the use of this compound. One volunteer who had received the agent intravenously had red blood cells in his urine shortly after the completion of his test. Extensive workup failed to uncover any definite kidney disease or lesion to account for the bleeding, which persisted intermittently for a year after exposure. Such a phenomenon had not been noted in any previous glycolate study. It may be related to a pre-existing abnormality that did not show up in routine screening. No additional information is available on the second patient. Animal studies had suggested that such bleeding could indeed result from EA 3834, from BZ, and even from atropine. On careful review of the experiment, it was concluded that the bleeding could be ascribed to techniques used and animal selection. In man, the effect of atropine on renal function was investigated, and no abnormalities were observed. Testing of EA 3834 was cautiously resumed in human subjects, and renal function was monitored with frequent pretest and posttest urine examinations. In a total of 69 subjects, including those who participated in field tests, no additional cases of bleeding were encountered, even though the subjects received up to 3 times the dose administered to subjects with hematuria. A decrease in glomerular filtration was observed in some subjects, but this also occurred in a control population. Nevertheless, continued vigilance concerning possible renal effects seems warranted.

Overall Evaluation of Immediate Adverse Effects

In general, in all the testing programs there were few violent responses or injuries, largely because of surveillance and safety precautions taken at Edgewood. The only other laboratory abnormalities encountered in the Edgewood records involved an increase in liver enzyme values of two volunteers within about 2 wk of exposure to CAR 302,368. These abnormalities reversed after a few weeks, and it was unclear whether the changes resulted from the drug administration. Several cases of pyuria after exposure to EA 3580, or related drugs, and running of an obstacle course, were reported, but the drug etiology is unclear.

LONG-TERM AND DELAYED EFFECTS

Delayed effects of the acute cholinergic drugs were extremely rare. One person reported by Klapper et al. (32) experienced "flashbacks" while still at Edgewood Arsenal. Edgewood records showed that he received EA 3834 intravenously. Medical records on this subject obtained from the VA were voluminous, but did not disclose any significant findings. Six-month followup studies performed by Hart and Balter (33) on related glycolates failed to demonstrate significant cognitive changes. Volunteers in a prospective psychometric study who had received EA 3167 showed no evidence of residual drug effects.

Two nonvolunteers—one military (a chemist) and one civilian (a pharmacologist)—were accidentally exposed to unknown doses of EA 3167, the most persistent of the glycolates studied. In each case, subjective and objective observations of performance over a period of 6–12 mo indicated that mild, but nontrivial, impairment of cognitive function could be discerned for approximately 6 mo, after which seemingly full recovery ensued. These patients were of superior intellect and had occupations that required optimal cognitive function for them to be successful. It is possible that in less demanding assignments they might not have been aware of residual deficits. Similar persistent decrements were not reported by BZ volunteers.

An additional ancedotal report (L.G.Abood, personal communication, 1982) concerns an evaluation of the psychologic state and well-being of three subjects who had been exposed to BZ during the preceeding 10 yr. Subject A had an oral dose of approximately 5 mg during the summer of 1969; subject B, an oral dose of approximately 10 mg in June 1972; and subject C, an oral dose of 5–10 mg in October 1976. All three were graduate students who had surreptitiously undertaken self-experimentation and who had been engaged in laboratory experiments with the drug. All experienced hallucinations, delirium, confusion, amnesia, and the full spectrum of psychologic, neurologic, and autonomic responses associated with the drug. Within 2–4 h after ingestion, all three were hospitalized in the psychiatric ward at the University of Rochester, Strong Memorial Hospital and retained for various periods (3–6 d).

Regarding the possible long-range effects of the drug experience, all three subjects were having difficulties both adjusting to graduate education and in their personal lives immediately before taking BZ. Subjects A and C had entertained the possibility of either dropping out or transferring to another university, largely because of inadequate academic performance. Within a few months after the drug experience, all three demonstrated definite improvement in both academic performance and psychologic well-being. They all appeared to be more out-going, communicative, and enthusiastic about graduate school. This assessment was shared by a number of faculty members and fellow students. They all eventually received Ph.D. degrees with distinction. They are now pursuing an active, productive research and postgraduate education. They all appear well-adjusted and well-motivated and seem to be leading productive, self-fulfilling careers. None of the subjects has experienced any adverse sequelae that might be attributable to the drug experience.

In the absence of additional reports of long-range consequences in subjects who had received the anticholinergic test compounds, data have been sought on delayed adverse reactions to other anticholinergic drugs used therapeutically, such as Ditran and atropine.

Since the introduction in 1958 of Ditran for the treatment of depressed patients (34), the number of persons receiving the drug for depression and other psychiatric disorders has exceeded 2,000 (34–37). During 1957–1963, Ditran was used in at least 200 people, many of whom were private patients. The treatment involved the intramuscular administration of 10–30 mg of Ditran to a depressed patient—a dose that generally produces the full spectrum of behavioral and neurologic effects attributable to the centrally active anticholinergics. The patient's psychiatric condition was closely observed and evaluated for the next few months. The progress of some of the private patients was followed for years after treatment, in some cases for 5 yr or more. In no instance was there any complaint of sequelae or delayed psychopathologic symptoms. The drug ameliorated depression for a period of a few months in a number of patients.

Hundreds of patients have received huge doses of atropine and scopolamine (up to 250 mg), sometimes given three times a week for up to 4 mo, and this form of therapy continues in Eastern Europe today. A chronic behavioral syndrome of toxicity appears unlikely, and single or even multiple exposures to the anticholinergic drugs used in the volunteers, frequently at low doses, are deemed insufficient to stimulate a persistent toxic syndrome. Of course, individual susceptibility to acute effects, which may trigger a long-term effect, cannot be excluded.

The question of occasional long-lasting or late-appearing residual effects is not easily answered. There is very little evidence that these might occur in man, in that so many persons have been exposed to these agents. The new case of glomerulitis several years after exposure in all likelihood was coincidental. Animal studies have been equivocal, but they have suggested the possibility of acute effects on the kidney and liver in some instances.

"Flashbacks" are difficult to explain, but they may result from a heightened sensory awareness after exposure to a drug. They have been of little clinical consequence, even though reported to be associated with a number of mind-altering drugs, including marijuana.

It should be emphasized that careful screening of volunteers for testing with the anticholinergic agents was probably essential, to avoid psychiatric complications after the hallucinatory episode.

If a subject has latent psychopathology, the drug experience may precipitate the latent tendencies and create problems later. In one such instance, a subject receiving Ditran at a university medical school required psychotherapy for 2 mo after the drug episode. Psychiatric interviews and psychologic tests before the drug session indicated that this subject had borderline paranoid and homosexual tendencies; otherwise, he appeared normal. The subject was amnesic during the manifestation of these symptoms; but the complications ensued after other divinity students in his dormitory reminded him of his aberrant behavior under the influence of Ditran. The severe embarrassment resulting from this confrontation may have precipitated the anxiety and loss of self-confidence that required psychotherapy.

In summary, one cannot be certain that long-term adverse effects never follow exposure to anticholinergic agents, but it seems most unlikely.

MORTALITY

The deaths of 222 among some 6,620 soldiers have been analyzed. Table 2–4 shows the standardized mortality ratios (SMRs) comparing the observed numbers of deaths and the numbers expected for the U.S. population categorized by age and calendar year. Most of the SMRs for the various test groups are somewhat less than unity, although many of the groups are relatively small. The values for BZ, scopolamine, atropine, and "anticholinergic only" are 0.87, 2.50, 1.76, and 1.06, respectively. It is interesting that atropine and scopolamine, drugs with a long history of therapeutic use, had SMRs exceeding unity, whereas the four candidate chemical warfare agents (BZ, EA 3834, EA 3443, and EA 3580) had SMRs less than unity. This suggests that exposure to the test anticholinergic chemicals may not be related to a subsequent increase in mortality. For anticholinergic with or without other drugs, an SMR of 0.73 is statistically significant and probably represents a selection artifact, inasmuch as volunteers for these studies were especially screened for good health and thus would be expected to have lower than average mortality.

The mortality data do not reveal a cause for concern that significant numbers of the men exposed to the anticholinergics suffered an untimely death. Because the participants were all especially selected for these studies on the basis of their health records, there were relatively few deaths in all groups. It is impossible to determine how much less than 1.0 the SMR values should have been. Comparison of the values of the various groups would not be expected to reveal treatment effects unless these were sizable, which was not the case. However, a small drug effect might be masked by this type of data analysis. For the same reasons, further subdivision of the deaths by cause does not provide useful data. Mortality is discussed in greater detail in Chapter 4.

SUMMARY

The Panel has examined the assembled data on the possibility of long-term adverse effects associated with the testing of various anticholinergic compounds in human volunteers at Edgewood Arsenal.

Although there are extensive descriptions of the acute CNS effects of the compounds, there is a paucity of information on delayed adverse effects. Animal experiments have not demonstrated significant long-term effects; and chronic administration of other anticholinergic drugs, such as atropine and scopolamine, which have been used therapeutically in man in high doses for years, has not been associated with chronic adverse effects. Indeed, there are anecdotal reports of improvement in behavioral patterns after such treatment. Because the CNS effects of the anticholinergic test compounds appear to be qualitively similar to those of atropine or scopolamine (although they may differ quantitatively in potency and duration of CNS effects), no additional long-term toxicity is anticipated. The apparent lack of an effect on mortality of the test subjects also suggests that such an effect is unlikely. Morbidity data are required to determine whether the subjects have had more health problems than those anticipated in a corresponding control group. This information is not yet available.

In a few persons, some relatively short-term adverse effects were reported after conclusion of the chemical testing (hematuria, hepatitis, and hallucinogenic flashbacks). It is unclear whether these effects resulted from the drug administration or occurred randomly and coincidentally. The extremely low incidence of such reports makes it doubtful that the anticholinergic test substances played a role in inducing these effects. However, the data are not sufficient to answer this question with certainty.

CONCLUSIONS

No firm evidence has been seen that any of the anticholinergic test compounds surveyed produced long-range adverse human health effects in the doses used at Edgewood Arsenal. More intensive study is required to confirm this conclusion. The high frequency of uncontrolled variables makes evaluation of behavioral effects difficult.

On the basis of available data, in the judgment of the panel, it is unlikely that administration of these anticholinergic compounds will have long-term toxicity effects or delayed sequellae. An ongoing morbidity study should provide more definitive information once it is completed.

TABLE 1 EFFECTS OF ATROPINE IN RELATION TO DOSE

Dose, mg	Effects
0.5	Slight cardiac slowing; some dryness of mouth; inhibition of sweating
1.0	Definite dryness of mouth; thirst; increase in heart rate, sometimes preceded by slowing; mild dilatation of pupil
2.0	Rapid heart rate; palpitation; marked dryness of mouth; dilated pupils; some blurring of near vision
5.0	All the above symptoms marked; speech disturbed; difficulty in swallowing; restlessness and fatigue; headache; dry, hot skin; difficulty in micturition; reduced intestinal peristalsis
10.0	Above symptoms more marked; rapid and weak pulse; iris practically obliterated; vision very blurred; skin flushed, hot, dry, and scarlet; ataxia, restlessness, and excitement; hallucinations and delirium; coma

Table 2 Summary of Exposures to Anticholinergics and Records Selected for Review of Acute Effects of Anticholinergic Chemicalsa

Tox No.	Agent code	No. volunteers	Total exposures[b]	No. records selected
B1	BZ (EA 2277)[c]	292	358	36
B2	EA 3443[c]	101	101	25
B3	EA 3580[c]	130	133	27
B4	Scopolamine	534	636	42
B5	Atropine	444	738	34
B6	EA 3167[c]	2	4	4
B7	Ditran	9	13	12
B8	EA 4929 (benzetimide)	18	18	10
B9	27349	50	50	10
B10	226,086	21	21	8
B11	302,196	52	56	15
B12	301,060	29	29	9
B13	302,282	8	8	8
B14	302,368	5	5	5
B15	302,537	18	18	8
B16	302,668	39	39	11
B18	Benactizine	16	26	9
B21	M-Scopolamine	72	114	21
B22	M-Atro (atropine methyl nitrate)	18	50	10
B23	EA 3834	144	173	33
B25	TAB, BAT	24	24	6
1 anticholinergics		1,752[b]	2,614	343

[a]Chemicals are identified in the master file (Appendix B).

[b]Some volunteers received more than one chemical.

[c]EA 3167, long-lasting; EA 3580, potent; EA 3443, skin penetrant; BZ, chemical-agent candidate.

Table 3 Human Pharmacologic Characteristics of 14 Glycolic Acid Esters (Based on Edgewood Arsenal Studies, 1960–1971)

Compound	Sources[a] of information	No. subjects	MED[b] (mg/kg)	ID_{50}[c]	Duration,[d] h	Relative Potency[e] Peripheral	Central
226,086	EA report	21	(1.5)	(2.0)	(18–36)	(2–4)	(10.0)
EA 3443	EATR 4066	101	1.2	3.4	48–72	2.3	6.2
EA 3580	231, 265, 269	136	1.4	3.9	16–24	2.6	6.0
EA 3167	248	24	2.8	4.1	(96–240)	(2–4)	(5.6)
BZ	228, App K	354	2.3	5.2	48–72	5.4	4.0
EA 3834	25, 254, 255	171	2.0	5.7	10–15	1.0	3.7
27349	EATM 114–2	50	3.0	6.0	10–15	(2.0)	3.4
301,060	250	29	3.0	(6.0)	(12–20)	(4.0)	(3.4)
302,668	259	39	(4.0)	8.9	10–15	0.9	2.4
302,282	273	20	4.4	12.5	6–10	(2.0)	1.7
Scop	166	637	9.4	20.2	5–10	0.8	1.0
302,196	261	56	(12.0)	28.9	4–6	0.7	0.7
Ditran	166	22	54.0	100.0	4–8	0.2	0.2
MeScopolam.	166	66	5.0	(400)	5–10	3.6	0.5
Atropine	166	602	63.6	152.4	8–12	1.0	0.1

[a]Refers to reference numbers in Appendix I except EA numbers.

[b]Dose required to produce 25% decrement in Number Facility (NF) in 50% of volunteer population (when administered intravenously or intramuscularly).

[c]Dose required to produce 90% decrement in NF scores in 50% of volunteers.

[d]Average time in hours from administration intravenous or intramuscular to near recovery (consistently above 75% of baseline on NF) in subjects receiving ID_{50}.

[e]Peripheral potency is in comparison to atropine (based on ED_{50} to produce maximal heart rate of at least 100 beats/min.). Central potency is in comparison with scopolamine (based on ID_{50}).

Numbers in parentheses are approximations based on limited information.

Observed and Expected Deaths Among Test Subjects by Classification of Chemical Administered and Underlying Cause of Death

	Anticholinergic[a]						BZ only[a] (N=136)			Scopolamine only[a] (N=85)			Atropine only[a] (N=62)		
	Only (N=570)			Not only (N=1,179)											
	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.
Underlying cause of death	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.
All trauma	10	10.0	1.00	11	20.6	0.53	2	3.0	0.67	2	1.3	1.54	1	1.0	1.00
All diseases	5	8.1	0.62	5	14.9	0.34	3	3.9	0.77	0	0.7	-	1	0.7	1.43
Malignant neoplasms	1	1.8	0.56	3	3.3	0.91	0	0.9	-	0	0.2	-	1	0.2	5.00
Cardiovascular disease	3	2.8	1.07	2	4.8	0.42	2	1.6	1.25	0	0.2	-	0	0.2	-
Cirrhosis of the liver	1	0.6	1.67	0	1.2	-	1	0.3	3.33	0	0.1	-	0	0.1	-
Total deaths, all causes[b]	19	18.0	1.06	20	35.5	0.56	6	6.9	0.87	5	2.0	2.50	3	1.7	1.76

	EA 3443 only[a] (N=28)			EA 3580 only[a] (N=45)			EA 3834 only[a] (N=64)			Total (N=6,620)		
	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.
Underlying cause of death	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs	Exp.	Exp.	Obs.	Exp.	Exp.
All trauma	0	0.6	-	0	0.8	-	1	0.9	1.11	86	128.2	0.67
All diseases	1	0.5	2.00	0	0.6	-	0	0.4	-	105	146.9	0.71
Malignant neoplasms	0	0.1	-	0	0.1	-	0	0.1	-	30	32.9	0.91
Cardiovascular disease	1	0.2	5.00	0	0.2	-	0	0.1	-	41	58.0	0.71
Cirrhosis of the liver	0	0.0	-	0	0.0	-	0	0.0	-	7	10.5	0.67
Total deaths, all causes[b]	1	1.1	0.91	0	1.4	-	1	1.2	0.83	222	275.1	0.81

[a]Only: This chemical and none other, except for innocuous and control substances. Not only: This and one or more other test chemicals.

[b]Includes causes of death listed here and all other causes, including those not yet determined.

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4

MORTALITY

METHOD OF ANALYSIS

The testing of chemical agents in human subjects at Edgewood which began in 1955 continued for more than 20 yr. During that period, the volunteers participated in an average of 3.1 tests each; 29% participated in only one test, and 2% participated in 10 or more tests. Because most of the volunteers participated in more than one test, the date of the most recent test was arbitrarily set as the date on which followup by the Medical Followup Agency of the National Research Council and the ascertainment of mortality began. Followup continued through December 31, 1980. The period of followup ranged from 4 to a maximum of 26 completed years.

The experience of 6,620 men is examined here. One-hundred were excluded from the study because of erroneous identification or because some essential datum, such as date of birth, was unknown. Among those studied, there were 222 deaths.

Deaths during followup were identified by matching the volunteers against a computerized file of veterans maintained by the Veterans' Administration (VA), which includes the date of death of every veteran for whom the VA has received a claim for a burial allowance and other veteran death benefits. Both name and service number were used for matching. If a death certificate showing the cause of death could not be found in the VA claim folder for a deceased veteran, a copy of the certificate was requested from the vital statistics office of the jurisdiction where the death occurred. The underlying cause of death was coded for analysis. The 31 deaths for which death certificates had not been received by the cutoff date for the receipt of data for analysis—March 31, 1982—have been classified as “cause of death unknown.” These 31 deaths are distributed in similar proportions between the men who received potentially hazardous substances (15% of 134 deaths) and the men who did not (13% of 88 deaths).

The groups of volunteers cannot be compared for mortality solely on the basis of numbers of observed deaths. One must also take into account the number of years that each group has been followed and the ages of the men being observed. The volunteers in the early test years were about the same age as men used in tests conducted during the later years (Table 4–1). However, the men tested in the early years not only are under observation for a much longer period than men used in the most recent tests, but also have grown older and have had a longer followup. Consider two volunteers of the same age at testing, one whose followup began in 1955 and the other in 1970. The first man will have aged 15 yr by the time the second man is tested and will have been exposed to the risk of dying for an additional 15 yr at the end of followup. Thus, the proportion of volunteers who die during followup will decrease for each succeeding test group, in the absence of any drug effect.

The testing of drugs spanned a period of 21 yr. However, some chemical agents were tested earlier than others (Table 4–2). The testing of LSD and derivatives was concentrated in the early years; more than half the total number of doses had been administered by the end of 1959. At the other extreme were the approved drugs, innocuous substances, and control substances, which were administered late in the testing period. One can therefore expect a much higher proportion of men in the LSD tests to have died than of the men who received approved drugs, innocuous chemicals, or control substances, even if LSD and its derivatives have no adverse effects on survival.

If one calculated the numbers of deaths expected among a test group of men, on the basis of their ages and the numbers of years they were followed, one would have a yardstick for assessing the significance of the number of deaths observed during followup. The ratio of observed deaths to expected deaths, the standardized mortality-ratio (SMR), can be used to compare the mortality experiences of two or more groups. Expected deaths by underlying cause were calculated on the assumption that the volunteers experienced U.S. male, age-specific death rates for each calendar year of followup.

RESULTS

Because most of the volunteers participated in two or more tests, an analytic problem arises: If one of the test substances, say Substance A, produced detectable adverse effects, such effects would also tend to appear among men who received Substance B if there were many men tested with both substances A and B. Accordingly, data on men who were tested with any particular substance are shown in two nonoverlapping groups: those who received “only” that substance (whether once or more than once) and those who received “not only” that substance but other active substances as well.

Observed and expected numbers of deaths and the ratios of observed to expected deaths, the SMRs, by underlying cause of death in volunteers who were exposed to chemicals of various types, are shown in Table 4–3. As noted above, a distinction has been made between men who received only one type of chemical and those who were exposed to two or more types (the “not only” groups).

The SMR for the total experience, 0.81, indicates that observed mortality for all causes was only 81% of expectation. That reflects the requirement that a man meet minimal health standards to be accepted into the Army; the screening process results in a death rate that is lower than that of U.S. males in general, and this effect persists for many years. It is evident that men who were tested with active substances had been subjected to additional screening, so these men constituted an unusually fit group and might be expected to have unusually low mortality for a number of years. This phenomenon is identical with the “healthy-worker” effect that is seen in most studies of occupational group. Men who were exposed to two or more types of chemicals experienced lower SMRs than those exposed to only one type of compound, again because of selection, a man had to be in exceptionally good health to participate in multiple tests.

No “all causes” SMR exceeded 1 by an amount greater than can reasonably be attributed to chance. The high SMR, 1.11, representing an 11% excess of observed deaths, occurred in the “no test” group—men who were not exposed to any chemical agent, possibly because of some health defect detected in the examination that preceded testing. The highest SMR, 1.20, occurred in the small group of men exposed only to the cholinesterase reactivators and represents an excess over expectation of less than a single death. The only other SMR to exceed unity occurred in men who were exposed to the anticholinergic chemicals only. Men who were exposed to anticholinergic chemicals plus other chemicals (which sometimes included substances to counteract the effects of the anticholinergic chemicals) had an SMR, 0.56, which is significantly below unity. However, when 212 men exposed to low single doses and 319 to high single doses were examined separately, no dose effect was apparent. Had the anticholinergic chemicals increased the risk of dying, one would have expected that increase to be larger in men with larger single exposures.

No evidence of a deferred, drug-induced mortality increase was seen when the experiences of the first 10 and remaining 16 follow-up years were examined separately. Of the 14 categories of experimental chemicals studied, only two had an SMR exceeding unity during the last 16 follow-up years. One of these (cholinesterase reactivator, only) was clearly due to small numbers, one observed death with 0.8 deaths expected, while the other, 47 observed versus 39.7 expected deaths, was seen in the 1,719 volunteers who were not exposed to any of the chemicals, the "no test" group. We can state with 95-percent certainty that the true SMR for these men during the first 10 years falls between 0.69 and 1.36, while the limits for the last 16 years are 0.83 and 1.54, respectively.

Most of the experimental chemicals were administered to too few volunteers to be examined separately for an influence on mortality. However, a few were considered of sufficient interest to justify being examined in that way (Table 4–4). The specific chemicals are grouped by type. The SMRs for these individual compounds range from a low of 0.80 (nine observed, 11.3 expected deaths) for the men tested with sarin only, to a high of 2.50 (five observed, two expected) for scopolamine only. Atropine, a therapeutic anticholinergic similar to scopolamine, had the next highest SMR, 1.76 (three observed, 1.7 expected). Of the eight deaths of men who received one or the other of these two therapeutically similar compounds, three were attributed to trauma (one accident, one suicide, and one other trauma), and one was due to cancer; the causes of the other four will not be known until the death certificates are obtained.

The SMRs become much more erratic when attention is directed to the underlying causes of death, because of the small expected number. If 0.5 deaths are expected, the SMR will be 0.0, 2.0, or 4.0, if only zero, one, or two deaths are observed.

Trauma was the underlying cause of 86 deaths, compared with 128.2 expected, for an SMR of 0.67. These volunteers had a significantly lower death rate from injury than that experienced by U.S. males in general. This tendency is seen in every category of test chemical, with two exceptions—the 570 men who received anticholinergic agents only (10 observed, 10.0 expected, SMR=1.00) and the 607 men who received cholinesterase reactivators (11 observed, 11.0 expected, SMR=100). In each instance, deaths due to homicide contributed to the excess. It would be difficult to attribute an increase in deaths due to trauma to effects of the two types of chemicals, because the excess is not seen among all volunteers exposed to them. No excess is seen in the anticholinergic “not only” group or in the cholinesterase reactivator “only” group.

Three diseases were responsible for more deaths than expected among all volunteers; cancer of the respiratory tract, leukemia and aleukemia, and renal disease. All three renal-disease deaths were in the no-test group; the excess is clearly not a drug effect. The excess of respiratory-cancer deaths derives in part from the control and no-test groups; that suggests that the excess is general, not drug-related. Deaths due to respiratory cancer were responsible for an SMR of 1.11 in a group of World War II and Korean Conflict veterans (1). It is possible that the proportion of cigarette-smokers is higher among veterans than in the U.S. male population in general. The third disease causing more deaths than expected is leukemia and aleukemia (four observed, 2.8 expected, SMR =1.43). One death occurred in the innocuous-chemical and-control group. Of the other three who died of leukemia or aleukemia, two had received anticholinesterase agents, two cholinesterase reactivators, and one each irritants, cannabis, and an unclassified chemical (included under total experience in Table 4–3). When the total volunteer experience is divided into the 1,984 men who did not receive potentially hazardous chemicals and the 4,636 men who did, the average annual death rates from leukemia are similar—3.4 and 4.0 per 100,000 person-yr of observation, respectively.

What is the chance that an increased risk of death will actually be detected? After choosing a level of significance at which the null hypothesis (no increased risk) will be rejected (P .05, say), the power of a comparison is the probability that, in fact, the number of deaths observed will be large enough to constitute a significant excess over the number expected if there were no increased risk.

Power can be large if the relative risk is large or if the sample is sufficiently large. If the sample is small, then only fairly large relative risks will be detected with high probability. In particular, if 10 or more deaths would be expected on the null hypothesis, a relative risk of 2.0 would be detected in more than 80 percent of trials (power greater than 0.80). The same power is achieved if three deaths are expected only if the relative risk is at least 3.0, and if only two deaths are expected at U.S. population rates, the relative risk would have to be at least 4.0 to achieve a power of 0.80. When the number of men exposed to a given chemical are small enough or the condition at increased risk rare enough to produce an expected value of only 0.1 deaths, failure to demonstrate a significant increase would mean only that the relative risk is less than 30. From a statistical point of view, the experience being studied is incapable of demonstrating risks of dying increased less than three- or four-fold.

It can be concluded that, over the time span examined here, there is no evidence that volunteer participation in the testing programs had any long-term adverse effect on mortality.

Table 1 Numbers of Men Tested by Year of Birth and Year of Beginning of Followup

Year of Birth	1955– 1959	1960– 1964	1965– 1969	1970–	Total
	No. Men:				
Before 1920	35	17			52
1920–1924	55	22	2		79
1925–1929	118	78	1		197
1930–1934	417	139	17		573
1935–1939	491	837	77	17	1,422
1940–1944	47	978	805	69	1,899
1945–1949		31	1,191	571	1,793
1950–1954			18	516	534
After 1954			1	70	71
Total	1,163	2,102	2,112	1,243	6,620
	Mean Year of Birth:				
	1934	1939	1945	1950	1942
	Mean Age at Beginning of Followup, yr:				
	23.9	23.5	22.4	22.8	23.1

Table 2 Median Years in Which Categories of Chemicals were Administered

Category	Year
Anticholinesterases	1962
Anticholinergics	1968
Cholinesterase reactivators	1968
Irritants	1967
Cannabis	1965
LSD derivatives	1959
Approved drugs	1971
Innocuous chemicals and controls	1971

Table 3 Observed and Expected Deaths among Test Subjects by Chemical Administered and Underlying Cause of Death

	Anticholineaterase[a]						Anticholinergic[a]						Cholinesterase reactivator[a]					
	Only (N=507)			Not only (N=958)			Only (N=570)			Not only (N=1,179)			Only (N=83)			Not only (N=607)		
Underlying cause of death	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	7	11.7	0.60	13	19.8	0.66	10	10.0	1.00	11	20.6	0.53	1	1.4	0.71	11	11.0	1.00
Accidents	4	7.5	0.53	7	12.5	0.56	6	6.2	0.97	6	12.8	0.47	1	0.9	1.11	5	6.9	0.72
Homicide	1	1.9	0.53	3	3.1	0.97	3	1.6	1.88	2	3.2	0.63	0	0.2	0.0	4	1.8	2.22
Suicide	0	2.1	0.0	0	3.7	0.0	0	1.9	0.0	1	4.0	0.25	0	0.3	0.0	0	2.1	0.0
All diseases	13	17.4	0.75	10	21.9	0.46	5	8.1	0.62	5	14.9	0.34	1	1.1	0.91	4	10.0	0.40
Malignant neoplasms	5	3.9	1.28	5	4.8	1.04	1	1.8	0.56	3	3.3	0.91	0	0.3	0.0	3	2.2	1.36
Respiratory tract	1	1.1	0.91	2	1.2	1.67	0	0.4	0.0	2	0.6	3.33	0	0.1	0.0	1	0.5	2.00
Leukemia, aleukemia	0	0.3	0.0	2	0.4	5.00	0	0.2	0.0	0	0.4	0.0	0	0.0	–	2	0.2	10.00
Diseases of cardiovascular system	6	7.3	0.82	4	8.4	0.48	3	2.8	1.07	2	4.8	0.42	0	0.4	0.0	1	3.6	0.28
Chronic nephritis, other renal	0	0.2	0.0	0	0.3	0.0	0	0.1	0.0	0	0.2	0.0	0	0.0	–	0	0.1	0.0
Cirrhosis of the liver	1	1.3	0.77	0	1.7	0.0	1	0.6	1.67	0	1.2	0.0	0	0.1	0.0	0	0.8	0.0
Total deaths, all causes[c]	21	29.2	0.72	28	41.6	0.67	19	18.0	1.06	20	35.5	0.56	3	2.5	1.20	17	21.0	0.81

	Irritant[a]						Cannabis			LSD derivative[a]			drugs[a]		
	Only (N=855)			Not only (N=1,046)			Total (N=252)			Only (N=103)			Only (N=159)		
Underlying cause of death	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	8	16.0	0.50	10	19.6	0.51	1	5.1	0.20	0	2.5	0.0	0	2.4	0.0
Accidents	5	10.0	0.50	7	12.2	0.57	1	3.2	0.31	0	1.6	0.0	0	1.5	0.0
Homicide	1	2.5	0.40	1	3.1	0.32	0	0.8	0.0	0	0.4	0.0	0	0.4	0.0
Suicide	1	3.0	0.33	2	3.8	0.53	0	1.0	0.0	0	0.4	0.0	0	0.5	0.0
All diseases	7	15.0	0.47	9	17.6	0.51	2	4.8	0.42	1	4.6	0.22	0	1.8	0.0
Malignant neoplasms	1	3.3	0.30	4	3.9	1.03	1	1.1	0.91	0	1.0	0.0	0	0.4	0.0
Respiratory tract	0	0.8	0.0	1	0.9	1.11	0	0.2	0.0	0	0.3	0.0	0	1.0	0.0
Leukemia, aleukemia	0	0.3	0.0	1	0.4	2.50	1	0.1	10.00	0	0.1	0.0	0	0.0	–
Diseases of cardiovascular system	3	5.5	0.55	3	6.3	0.48	1	1.7	0.59	0	2.0	0.0	0	0.6	0.0
Chronic nephritis, other renal	0	0.2	0.0	0	0.2	0.0	0	0.1	0.0	0	0.1	0.0	0	0.0	–
Cirrhosis of the liver	1	1.1	0.91	0	1.3	0.0	0	0.4	0.0	1	0.3	3.33	0	0.1	0.0
Total deaths, all causes[c]	18	31.0	0.58	23	37.1	0.62	3	9.8	0.31	1	7.1	0.14	1	4.2	0.24

	Innocuous chemical and control, exclusively (N=106)			No test (N=1,719)			Total experience (N=6,620)		
Underlying cause of death	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	1	1.7	0.59	31	33.5	0.93	86	128.2	0.67
Accidents	1	1.0	1.00	20	21.1	0.95	54	80.6	0.67
Homicide	0	0.3	0.0	4	5.4	0.74	15	20.5	0.73
Suicide	0	0.3	0.0	2	6.2	0.32	5	24.0	0.21
All diseases	2	1.2	1.67	43	42.4	1.01	105	146.9	0.71
Malignant neoplasms	2	0.3	6.67	6	9.6	0.63	30	32.9	0.91
Respiratory tract	1	0.0	--d	3	2.6	1.15	10	8.5	1.18
Leukemia, aleukemia	1	0.0	--d	0	0.8	0.0	4	2.8	1.43
Diseases of cardiovascular system	0	0.4	0.0	20	17.3	1.16	41	58.0	0.71
Chronic nephritis, other renal	0	0.0	–	3	0.5	6.00	3	1.7	1.76
Cirrhosis of the liver	0	0.1	0.0	3	2.9	1.03	7	10.5	0.67
Total deaths, all causes[c]	3	2.9	1.03	84	75.8	1.11	222	275.1	0.81

[a]Only: Man received this classification of chemical and none other, except for innocuous and control substances.

Not only: Man received this classification of chemical and one or more other experimental chemicals.

[b]Includes injury undetermined, whether accidentally or purposely inflicted, and injury resulting from operations of war.

[c]Includes the causes of death listed in this table plus all other causes, including cause not yet determined.

[d]Indeterminate, because of zero denominator.

Table 4 Observed and Expected Deaths among Test Subjects by Chemical Administered

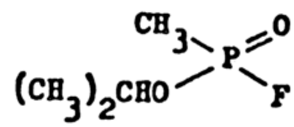
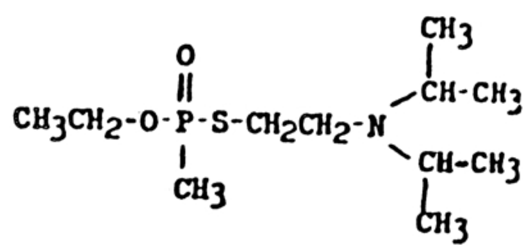
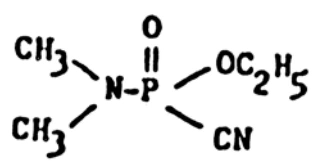
		No. Deaths		
Chemical	No. Subjects	Observed	Expected	O/E
Anticholinesterase only	507	21	29.2	0.72
Sarin only	149	9	11.3	0.80
VX only	281	12	14.5	0.83
Remainder of group	77	0	3.3	0.0
Anticholinergic only	570	19	18.0	1.06
BZ only	136	6	6.9	0.87
EA 3443 only	28	1	1.1	0.91
EA 3580 only	45	0	1.4	0.0
Scopolamine only	85	5	2.0	2.50
Atropine only	62	3	1.7	1.76
EA 3834 only	64	1	1.2	0.83
Remainder of group	150	3	3.8	0.79
Irritant only	885	18	31.0	0.58
Mustard only	68	3	3.0	1.00
Remainder of group	817	15	28.0	0.54

REFERENCE

1. Keehn, R.J., 1980: Follow-up Studies of World War II and Korean Conflict Prisoners. III. Mortality to 1 January 1976, Am. J. Epidemiol. 111:194–211.

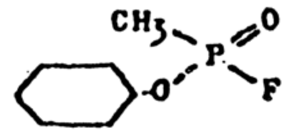
APPENDIX A

MASTER FILE—ANTICHOLINESTERASE CHEMICALS[a]

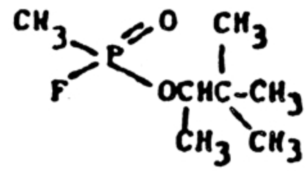
	Structural Formula
Tox Num A001 EA Num EA 1208 Military Desig GB Chemical Name Isopropylmethylphosphonofluoridate CAS Num 107–44–8 Common Name Sarin Chemical Type Organophosphorus ester	
Tox Num A002 EA Num EA 1701 Military Desig VX Chemical Name Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate CAS Num 5–782–69–9 Chemical Type Organophosphorus ester	
Tox Num A003 EA Num EA 1205 Military Desig GA Chemical Name Ethyl N,N-dimethylphosphoramidocyanate CAS Num 77–81–6 Common Name Tabun Common Name Taboon A Common Name Trilon 83 Common Name Gelan I Other Num MCE Chemical Type Organophosphorus ester	

[a]Tox Num=arbitrary NRC designation; EA Num=Edgewood Arsenal designation.

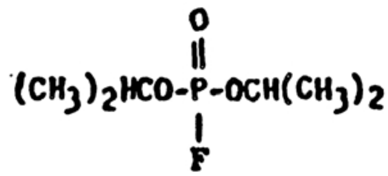
Tox Num A004
EA Num EA 1212
Military Desig GF
Chemical Name Cyclohexyl methylphosphonofluoridate
CAS Num 329–99–7
Chemical Type Organophosphorus ester



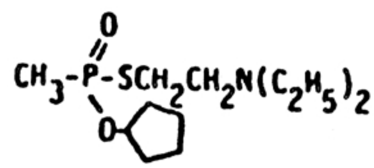
Tox Num A005
EA Num EA 1210
Military Desig GD
Chemical Name Pinacolyl methylphosphonofluoridate
CAS Num 96–64–0
Common Name Soman
Other Num T.2107
Chemical Type Organophosphorus ester



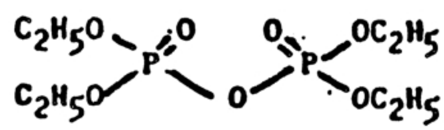
Tox Num A006
Abbreviated Name—PF3
EA Num EA 1152
Military Desig DFP
Chemical Name Diisopropyl fluorophosphate
CAS Num 55–91–4
Other Num T.1703
Chemical Type Organophosphorus ester



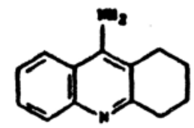
Tox Num A007
EA Num EA 3148
Chemical Name Cyclopentyl S-2-diethylaminoethyl methylphosphonothiolate
Chemical Type Organophosphorus ester



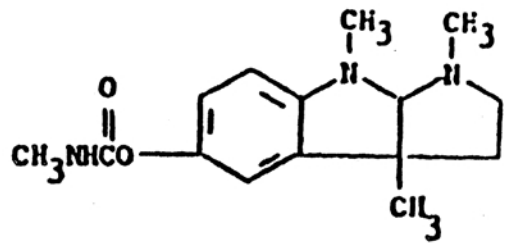
Tox Num A008
EA Num EA 1285
Chemical Name Tetraethyl pyrophosphate
CAS Num 107–49–3
Common Name TEPP
Chemical Type Organophosphorus ester



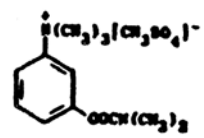
Tox Num A009
Abbreviated Name—THA
Chemical Name 9-Amino-l,2,3,4-tetrahydroacridine
CS Num CS 12602
CAS Num 321–64–2
Common Name Tacrine
Common Name Romotal
Chemical Type Acridine



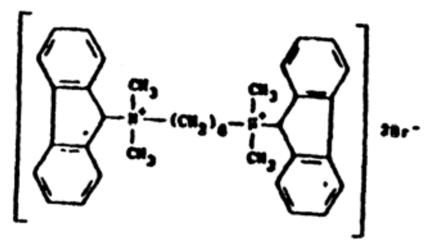
Tox Num A010
Chemical Name Physostigmine
CS Num CS 58525
CAS Num 57–47–6 (free base)
CAS Num 64–47–1 (sulfate)
CAS Num 57–64–7 (salicylate)
Common Name Eserine
Chemical Type Carbamate



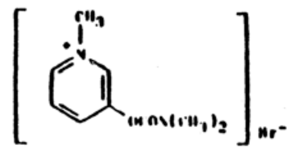
Tox Num A011
Chemical Name m-(Hydroxyphenyl)trimethylammonium methylsulfate dimethylcarbamate
CAS Num 51–60–5 (methyl sulfate)
CAS Num 114–80–7 (bromide)
CAS Num 59–99–4
Common Name Prostigmin
Common Name Neostigmine methyl sulfate
Chemical Type Carbamate



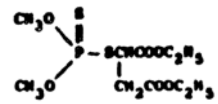
Tox Num A012
Chemical Name Hexamethylenebis[9-fluorenyldimethylammonium bromide]
CAS Num 317–52–2
Common Name Mylaxen
Common Name Hexafluorenium bromide
Chemical Type Curare



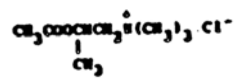
Tox Num A013
Chemical Name 3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate
CAS Num 155–97–5
Common Name Pyridostigmine bromide
Common Name Mestinon
Chemical Type Carbamate



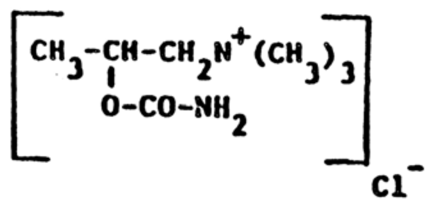
Tox Num A014
Chemical Name S-[1,2-bis(ethoxycarbonyl)ethyl]-O,O-dimethyl phosphorodithioa
CAS Num 121–75–5
Common Name Malathion
Chemical Type Organophosphorus ester



Tox Num A020
Pharm Class Direct cholinergic
Chemical Name (2-Hydroxypropyl)trimethylammonium chloride acetate
CAS Num 62–51–1
Common Name Mecholyl chloride
Common Name Methacholine
Chemical Type Choline ester

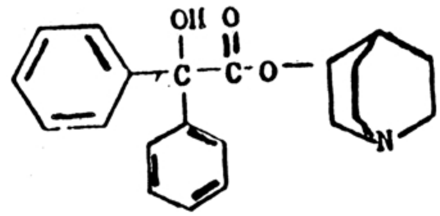
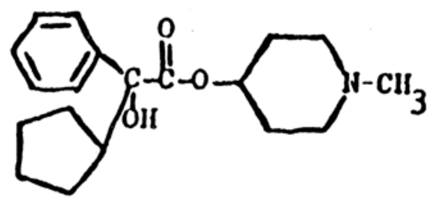
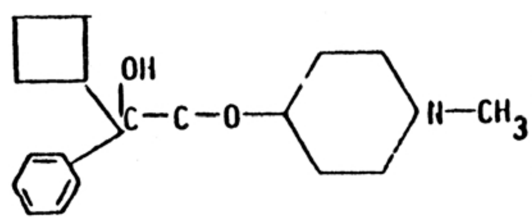
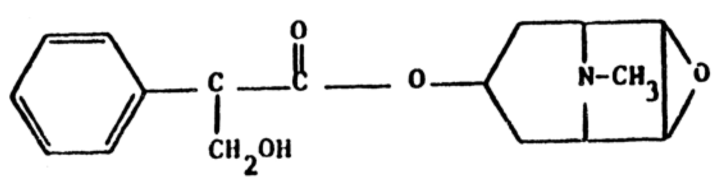


Tox Num A021
Pharm Class Direct cholinergic
Chemical Name (2-Hydroxypropyl)trimethylammonium chloride carbamate
CAS Num 590–63–6
Common Name Urecholine chloride
Common Name Bethanecol chloride
Chemical Type Quaternary ammonium carbamate



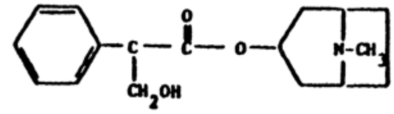
APPENDIX B

MASTER FILE—ANTICHOLINERGIC CHEMICALS[a]

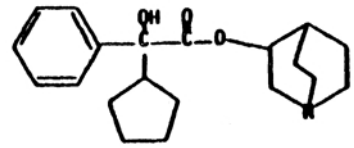
	Structural Formula
Tox Num: B001 Abbreviated Name: QNB EA Num: EA 2277 Military Desig: BZ Chemical Name: 3-Quinuclidinyl benzilate CS Num: CS 4030 CAS Num: 13004–56–3 (hydrochloride) CAS Num: 6581–06–2 (free base) CAS Num: 24572–08–5 (hydrobromide) Other Num: R02–3308 Chemical Type: Glycolic acid ester	
Tox Num: B002 EA Num: EA 3443 Chemical Name: N-Methyl-4-piperidyl cyclopentylphenylglycolate CAS Num: 37830–21–0 Chemical Type: Glycolic acid ester	
Tox Num: B003 EA Num: EA 3580 Chemical Name: N-Methyl-4-piperidyl cyclobutylphenylglycolate CAS Num: 54390–94–2 Chemical Type: Glycolic acid ester	
Tox Num: B004 Cont Num: 300113 CAS Num: 51–34–3 (free base) CAS Num: 55–16–3 (hydrochloride) Common Name: scopolamine Common Name: Hyoscine Common Name: Sominex Chemical Type: Glycolic acid ester	

[a]Tox Num arbitrary NRC designation; EA Num Edgewood Arsenal designation; Cont Num contractor's designation.

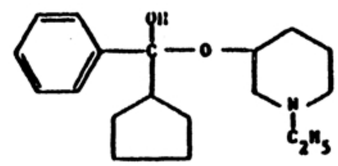
Tox Num: B005
CAS Num: 51–55–8 (free base)
CAS Num: 55–48–1 (sulfate)
CAS Num: 33952–38–4 (hydrochloride)
Common Name: Atropine
Chemical Type: Glycolic acid ester



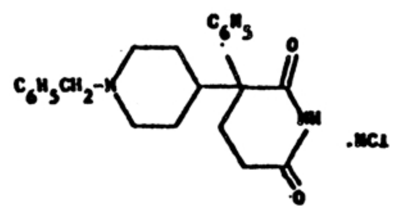
Tox Num: B006
EA Num: EA 3167
Chemical Name: 3-Quinuclidinyl phenylcyclopentylglycolate
CAS Num: 26758–53–2
CAS Num: 29125–55–1 (hydrochloride)
Chemical Type: Glycolic acid ester



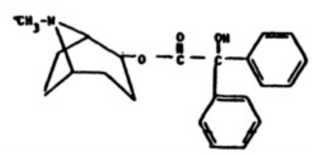
Tox Num: B007
Chemical Name: 1-Ethyl-3-piperidyl α-cyclopentyl-α-phenylglycolate hydrochloride
CS Num: CS 4297 (free base)
CS Num: CS 62025 (hydrochloride)
CS Num: CS 4298 (hydrochloride)
CAS Num: 8015–54–1
Common Name: Ditran
Other Num: JB 329 (free base)
Other Num: JB 840 (hydrochloride)
Chemical Type: Glycolic acid ester



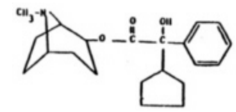
Tox Num: B008
Chemical Name: dl-2-(1-Benzyl-4-piperidyl)-2-Phenylglutarimide hydrochloride
CAS Num: 14051–33–3
Common Name: Benzetimide hydrochloride
Common Name: Dioxatrine
Other Num: R4929
Other Num: MCN-JR4929
Other Num: 4929
Chemical Type: Substituted glutarimide



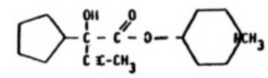
Tox Num: B009
Chemical Name: L-2-α-Tropinyl benzilate hydrochloride
CS Num: CS 27349
Cont Num: 219758
CAS Num: 64520–33–8
CAS Num: 64471–12–1 (free base)
Chemical Type: Glycolic acid ester



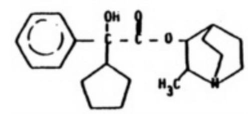
Tox Num: B010
Chemical Name: L-2-α-Tropinyl L-cyclopentylphenylglycolate
Cont Num: 226086
CAS Num: 64471–85–8
Chemical Type: Glycolic acid ester



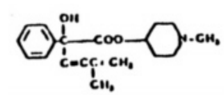
Tox Num: B011
Chemical Name: N-Methyl-4-piperidyl cyclopentyl-(1-propynyl)-glycolate
Cont Num: 302196
CAS Num: 53034–67–6
Chemical Type: Glycolic acid ester



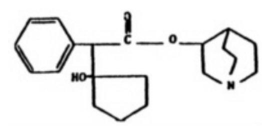
Tox Num: B012
Chemical Name: cis-2-Methyl-3-quinucidinyl cyclopentylphenylglycolate
Cont Num: 301060
Chemical Type: Glycolic acid ester



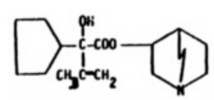
Tox Num: B013
Chemical Name: 1-Methyl-4-piperidyl phenyl-(3-methylbut-1-yn-3-enyl)-glycolate
Chemical Type: Glycolic acid ester
Cont Num: 302282



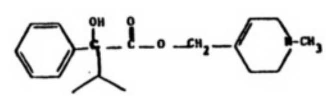
Tox Num: B014
Chemical Name: 3-Quinuclidinyl (1-hydroxycyclopentyl) phenylacetate
Cont Num: 302368
Chemical Type: Glycolic acid ester



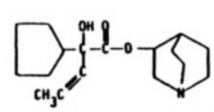
Tox Num: B015
Chemical Name: 3-Quinuclidinyl cyclopentyl-(2-propenyl)-glycolate
Cont Num: 302537
Chemical Type: Glycolic acid ester



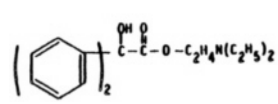
Tox Num: B016
Chemical Name: 4-(1-methyl-1,2,3,6-tetrahydropyridyl)-Methyl-Isopropylphenylglycolate
Cont Num: 302668
Chemical Type: Glycolic acid ester



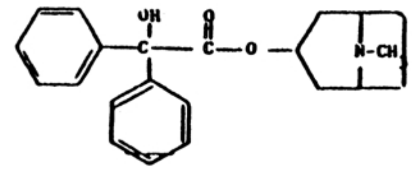
Tox Num: B017
Chemical Name: 3-Quinuclidyl cyclopentyl-(1-propynyl)-glycolate
Cont Num: 302212
Chemical Type: Glycolic acid ester



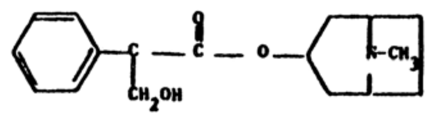
Tox Num: B018
EA Num: EA 2092
Chemical Name: 2-Diethylaminoethyl diphenylglycolate
CAS Num: 57–37–4
Common Name: Benactyzine (hydrochloride)
Chemical Type: Glycolic acid ester



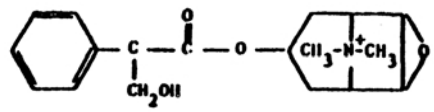
Tox Num: B019
Abbreviated Names: BAT; BETE; BTE
Chemical Name: Tropine benzilate
CAS Num: 3736–36–5
Chemical Type: Gycolic acid ester



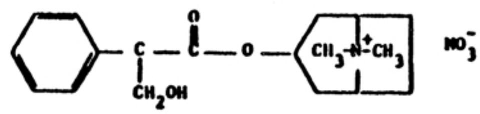
Tox Num: B020
Pharm Class: Anticholinergic
Chemical Name: D-Tropine tropate
CAS Num: 55–47–0 (hydrochloride)
CAS Num: 50700–39–5 (hydrobromide)
Common Name: D-Hyoscyamine
Chemical Type: Glycolic acid ester



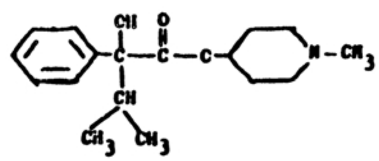
Tox Num: B021
CAS Num: 155–41–9
CAS Num: 6106–46–3 (nitrate)
Common Name: Methyl scopolamine
Common Name: Methscopolamine bromide
Common Name: Pamine (bromide)
Chemical Type: Glycolic acid ester



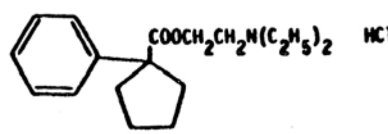
Tox Num: B022
CS Num: 60450
CAS Num: 52–88–0
Common Name: Atropine methyl nitrate
Common Name: Eumydrin
Chemical Type: Glycolic acid ester



Tox Num: B023
EA Num: EA 3834
Chemical Name: N-Methyl-4-piperidyl isopropylphenyl-Glycolate
Chemical Type: Glycolic acid ester

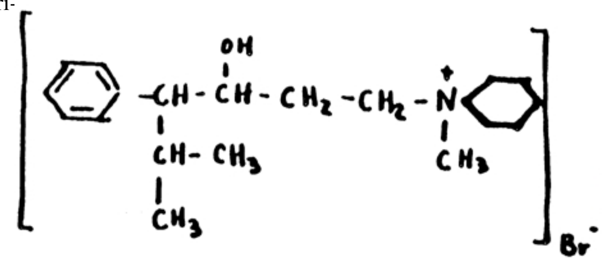


Tox Num: B024
Chemical Name: 2-Diethylaminoethyl 1-phenylcyclopentanecarboxylate hydrochloride
CAS Num: 125–85–9
Common Name: Caramiphen hydrochloride
Common Name: Panparnit
Chemical Type: Basic ester

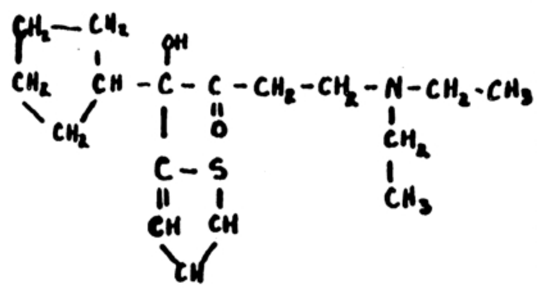


Tox Num: B025
Abbreviated Name: TAB
Common Name: Mixture of TMB4 (D4), atropine (B5), and benactyzine (B18)
Chemical Type: Glycolic acid ester

Tox Num: B026
Chemical Name: 1-(3-Hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide
CAS Num: 520–20–7
Common Name: Darstine
Common Name: Mepiperphenidol
Chemical Type: Piperidinium halide



Tox Num: B027
Chemical Name: -Cyclopentyl- -hydroxy-2-thiopheneacetic acid, 2-diethylaminoethyl ester hydrochloride
CAS Num: 3737–35–7
Other Num: WIN 2299
Chemical Type: Glycolic acid ester



APPENDIX C

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINESTERASE PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinesterase chemicals

Research and experimental case file on volunteers (case report summaries, high dose and random samples)

Anticholinesterase chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, random-sample case report summaries

Elements of final report, submitted by Panel members, dated January 21, 1982

Complete list of volunteers (anticholinesterase chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

APPENDIX D

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINERGIC PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinergic chemicals

Research and experimental case files on volunteers (case report summaries, high dose samples)

Anticholinergic chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, summary of digest-report investigation of two cases of reported flashback

Elements of final report, submitted by Panel members, dated January 15, 1982

Complete list of volunteers (anticholinergic chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

APPENDIX E

CASE-FILE DATA ON EDGEWOOD SUBJECTS*

SUMMARY OF VOLUNTEER TESTING WITH GB (A1)

A total of 246 subjects was tested with GB under various conditions. Twenty-five subjects were selected for review, representing approximately 10% of the test group. Selections were based upon the following criteria:

	No Treatment (9 subjects)
Route:	Intravenous (3.0–4.0 μg/kg) 9 subjects
	No symptoms
	Treatment (PAMCL) (9 subjects)
	Dizziness, frontal headache, blurred vision lethargy, nausea, stomach pain, vomiting.
	Treatment (7 subjects)
Route:	Intravenous (3.0–4.0 μg/kg) (P_2S) 2 subjects
	Intravenous (3.0–4.0 μg/kg) (TMB_4) 3 subjects
	Whole-body exposure to vapor (Atropine) 1 subject
	Whole-body exposure to vapor (Pyrbenzamine) 1 subject
	Rhinorrhea, chest tightness, wheezing.

SUMMARY OF VOLUNTEER TESTING WITH VX (A2)

A total of 740 subjects was tested under various conditions with VX. Seventy-five subjects were selected for review, representing 10% of the test group. Selections were based upon the following criteria:

	No Treatment (52 subjects)
Route:	Oral (single dose; 3.5 μg/kg) 1 subject
	Percutaneous (single dose; 5–45 μg/kg) 22 subjects
	Oral (multiple dose) 29 subjects
	Treatment (23 subjects 2-PAM, Atropine, Regitine
Route:	Intravenous (1.2–2.0 μg/kg) 7 subjects
	Intramuscular 3 subjects
	Oral (4.25–4.5 μg/kg) 3 subjects
	Percutaneous (5.0–45 μg/kg) 10 subjects

The vast majority of these subjects reportedly experienced little or no effect from the drug. In 2 cases, the cholinesterase (RBC) level was less that 20% of normal in subjects that received multiple doses, but in each case the dosing was stopped and the cholinesterase levels returned to normal limits within 6–7 days post exposure. No visible effects of the drug were observed in any of these subjects. One subject (A2(BN)) received 30 ug/kg, percutaneously, showed some effects (headache, nausea, ChE 15% of normal, nervous) and was treated with multiple doses of atropine and 2-PAM. Subject recovered and RBCChE was 97% of normal when discharged.

Another subject (A2(BA)) received 1.5 μg/kg intravenously, developed twitching (fasciculation?) of leg muscles, perspiration of palms and soles of feet 15 min after injection when RBC ChE was 87%

*This represents selection of high-dose subjects. A second set of records of equal number was also procured, on the basis of random selection.

of normal (dropping to 17% at 1h). By 30 min, hands and feet were cold and the eyes felt “heavy.” A third subject (A2(BS)), who received 3.2 µg/kg intramuscularly, was dizzy, nauseated, and very drowsy after 2h. During the next 10 hours, the subject lacked concentration and stared vacantly into space. Slight dizziness and blurred vision persisted at 26h when he was treated with 2-PAM and recovered.

Some others reportedly experienced dizziness, light-headedness, nausea, vomiting, “stiff jaw,” headache, and ptosis lasting for at least 24h after exposure.

SUMMARY OF VOLUNTEER TESTING WITH GA (A3)

A total of 26 subjects was tested under various conditions with GA. Thirteen (13) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

	Treatment (10 subjects)
Route:	Intravenous (2.0–3.0 µg/kg)+(PAMCl, Pretreatment) 3 subjects
	Intravenous (1.0–2.0 µg/kg)+(PAMCl, Post Treatment) 2 subjects
	Intravenous (3.0 µg/kg)+(P_2S, Pretreatment) 1 subject
	Intravenous (3.0 µg/kg)+(P_2S, Post Treatment) 2 subjects
	Intravenous (3.0 µg/kg)+(PAMCl+TMB4, Pretreatment) 1 subject
	Intravenous (3.0 µg/kg)+(PAMCl+P_2S, Pretreatment) 1 subject

All subjects responded to pre- or post-exposure treatment with PAMCl or P_2S when exposed to GF. ChE levels recovered after initial depression within 24 hours or less; they were approaching normal levels by 1 to 2 days post-exposure. Treatment with PAMCl and P_2S or PAMCl and TMB4 did not alter or significantly improve the recovery. None of these subjects reportedly had any serious or prolonged effects from the drug.

SUMMARY OF VOLUNTEER TESTING WITH GD (A5)

A total of 83 subjects was tested under various conditions with GD. Ten (10) subjects were selected for review, representing approximately 12% of the test subjects. Selections were based upon the following criteria:

	No Treatment (4 subjects)
Route:	Percutaneous (8.5–90 µg/kg) 4 subjects
	Treatment (6 subjects) Atropine, PAMCl, P_2S, TMB-4
Route:	Intravenous (1.0–2.0 µg/kg) 6 subjects

Blood cholinesterase measurements were made on the 4 subjects that received GD by the percutaneous route. None of these subjects showed any significant decrease in their ChE levels over a 2-week period. No complaints or severe effects reportedly were observed in any of the subjects tested by this route at the doses used. Two of the subjects who received GD by the intravenous route, although treated with oxime and/or atropine showed significant decrease in the ChE levels, and exhibited the “usual physiological responses” from this agent. Weakness and muscle contractions were also experienced. They also developed persistent vomiting and nausea to a point of dehydration. One subject was referred to Walter Reed Hospital for psychiatric observation and diagnosed for anxiety reaction, acute agitation and hysterical reaction; he was later released for duty.

SUMMARY OF VOLUNTEER TESTING WITH DFP (A6)

A total of 11 subjects was tested with DFP under various test conditions. Five (5) subjects were selected for review, representing approximately 45% of the test group. Selections were based upon the following criteria:

	No Treatment (1 subject)
Route:	Intramuscular (16.0 μg/kg) 1 subject
	Treatment (4 subjects)
Route:	Intramuscular (40–55 μg/kg) (PAMCl) 3 subjects
	Intramuscular (65 μg/kg) (PAMCl+Atropine) 1 subject (Weak, shaky knees, dizziness, nausea, blurred vision, perspiration).

SUMMARY OF VOLUNTEER TESTING WITH EA 3148 (A7)

A total of 32 subjects was tested under various conditions with EA 3148. Sixteen (16) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

	No Treatment (13 subjects)
Route:	Intravenous (0.7–1.15 μg/kg) 13 subjects
	Treatment (3 subjects)
Route:	Intravenous (0.7 μg/kg) (PAMCl) 1 subject
	Intravenous (1.15 μg/kg) (Scopolamine) 2 subjects

Subjects with large doses experienced a rapid, severe depression of RBC ChE following agent administration. For example, volunteer #3799 (case #1385), who received 1.15 μg/kg EA 3148, showed 22% of normal RBC ChE values at 15 min after dosing and 0% after 48h; this recovered to 88% of normal at 72d post-exposure.

Of the subjects that received no additional treatment, two subjects who had received the highest dose (1.15 μg/kg) showed toxic signs with 5 to 8 minutes post-exposure. These subjects felt dizzy, weak, tired, sweating, with hands and feet very moist. Within 2 hours post-exposure, these subjects reportedly were resting, eating well and feeling fine. Anorexia, poor sleep, fatigue, unusual dreams, dizziness, euphoria, blurred vision, increased salivation, restlessness are recorded in an Army report summarizing the experience with EA 3148 (CRDL. Tech. Memo 2–31). The report also notes one individual whose schizoid personality seemed to be exaggerated by the drug. Four individuals had decrements on the test of numerical facility.

Subjects treated with PAMCl or Scopolamine did not have any severe drop in ChE, and there were no long lasting effects of the drug observed.

SUMMARY OF VOLUNTEER TESTING WITH THA (A9), TACRINE

A total of 15 subjects was tested under various conditions with THA. Five (5) subjects were selected for review, representing approximately 33% of the test group. Selections were based upon the following criteria:

	No Treatment (2 subjects)
Route:	Intravenous (24 mg, total) 1 subject
	Oral (250 mg, total) 1 subject
	Treatment (3 subjects)
Route:	Oral (192–200 mg, total) (Atropine) 2 subjects
	Oral (250 mg, total) (PAMCl+Atropine) 1 subject

The subjects' blood cholinesterase levels were monitored continuously, along with EEG, EKG, respiratory rate and volume with pneumotact, and blood pressure. There were no significant changes in the parameters measured. Untreated subjects were asymptomatic at the dose levels given, and even though there was some drop in ChE (28% of normal) in plasma, the RBC ChE was unaffected. Subjects treated with atropine alone, or with PAMCl, responded well to relief of severe parasympathomimetic reactions. No residual effects of the drug reportedly were observed in any of the subjects tested.

SUMMARY OF VOLUNTEER TESTING WITH PHYSOSTIGMINE (A10)

A total of 138 subjects was tested with physostigmine and 22 were selected for review, representing approximately 16% of the test group. The criteria for selection was based upon the following: Dose, Route, Frequency and Treatment.

	No Treatment (6 subjects)
Route:	Intramuscular, single dose (28.0 μg/kg) 2 subjects
	Intramuscular, multiple dose (15–30 μg/kg) 4 subjects
	Treatment (16 subjects)
Route:	Physostigmine in single (45 μg/kg) and multiple doses over 1 to 2 days was administered intramuscularly in subjects that had been previously dosed with a variety of drugs such as:
	BZ (7.4–14.5 μg/kg) aerosol inhalation, 2 subjects
	3834 (2.0 mg) percutaneous, 1 subject
	Atropine (125 μg/kg) intramuscular, 3 subjects
	Prolixin (15.0–23.0 μg/kg) intramuscular, 6 subjects
	302668 (10.0 μg/kg) intravenous, 1 subject
	302196 (75.6 μg/kg) oral, 1 subject
	TAB (90 mg total) intramuscular, 1 subject
	Pretreatment with methyl scopolamine (1.0 mg) 1 subject

Only 2 subjects (A10J) and (A10K) who received doses of BZ and were subsequently treated with physostigmine, showed any prolonged central effects (hallucinations, disorientation, confusion) lasting 4 to 6 days post-exposure. Both subjects were asymptomatic and appeared normal when discharged from test. One subject (A10–0) was exposed to Prolixin (23.0 μg/kg) and then treated with multiple doses (1.0 mg×7 doses) over a 2-day period, intramuscularly: At 27 hours post-exposure, the subject complained of blurred vision, and facial expression was mask-like, tongue “thick” and jaws open. By 28 hours post-exposure, subject was having an oculo-gyric crisis, mouth to one side, and left foot tremulous and turned inward.

Subject was further treated with Cogentin, intravenously (1.0 mg and 1.3 mg), in two doses. Within 30 minutes post-treatment, subject was relaxed and dozing and, by 31 hours post-exposure, the subject appeared normal. At 50 hours post-exposure, the subject was asymptomatic and was discharged at 73 hours post-exposure with no complaints.

SUMMARY OF VOLUNTEER TESTING WITH PROSTIGMINE (A11)

A total of 27 subjects was tested with prostigmine as a part of the esophageal motility studies. Seven (7) subjects were selected for review, representing approximately 26% of the test group. Selections were based upon the following criteria:

	No Treatment (1 subject)
Route:	Intramuscular (0.5 mg) 1 subject
	Treatment (6 subjects)
Route:	Intramuscular (1.5 mg) (Atropine/PAMCl) 6 subjects

The esophageal motility studies (EPP) were performed by the subjects before and after administration of the drug. ChE levels were also determined in many of the subjects. One subject developed severe cramps in the abdomen, necessitating termination of the study (A11B). All other subjects reportedly tolerated all procedures very well.

SUMMARY OF VOLUNTEER TESTING WITH HEXAFLUORENIUM (MYLAXINR) (A12)

A total of 11 subjects was tested with Mylaxin as a part of an esophageal test. 11 Subjects were selected for review, representing 100% of the test group. Selections were based upon the following criteria.

	No Treatment (11 subjects)
Route:	Intravenous (0.4 mg/kg) 11 subjects

The majority of subjects tested experienced a sensation of “warmness” in the stomach and nausea very shortly (2–10 minutes) after receiving the drug, followed by vomiting within 5–15 minutes. The plasma ChE levels in all subjects were depressed while RBC ChE levels were not significantly affected. Esophageal motility studies disclosed marked spasm of the lower 2/3 during deglutition. This coincided with depression of the plasma ChE. There were no prolonged or long-lasting effects reported in any of these subjects as a result of this drug. All subjects reportedly had recovered within 24 hours post-exposure.

SUMMARY OF VOLUNTEER TESTING WITH MALATHION (A14)

A total of 10 subjects was tested with 1.1% malathion powder dusted over the entire body for up to 10 days. Five (5) subjects were selected for review, representing 50% of the test group. All of these subjects reportedly were asymptomatic and developed no signs of intoxication. One subject (A14A), showed low RBCChE which could not be ascribed to laboratory error; however, there were no other significant drops in this subject's ChE levels. All other subjects showed no significant decreases in cholinesterase.

SUMMARY OF VOLUNTEER TESTING WITH METHACHOLINE (A20)

A total of 9 subjects was tested with mecholyl alone or with EA 1729. Several of these subjects also received other drugs such as neostigmine, urecholine, PAMCl, epinephrine by various routes. Three (3) subjects were selected for review, representing 33% of the test group.

Although these subjects were tested or treated with several of these drugs, as well as mecholyl, their vital signs reportedly returned to normal and they were all in good condition by the end of the testing period. There were no prolonged or delayed effects from this drug reported in these subjects.

SUMMARY OF VOLUNTEER TESTING WITH URECHOLINE (A21)

A total of 15 subjects was tested with urecholine under various conditions, under the (EPP). Five (5) subjects were selected for review, representing 33% of the test group. Selections were based upon the following criteria:

	No Treatment (2 subjects)
Route:	Subcutaneous (5.0 mg) (single dose) 1 subject
	Subcutaneous (5.0 mg) (multiple dose) 1 subject
	Treatment of Multiple Compounds (3 subjects)
Route:	Subcutaneous (5.0 mg) (PAMCl+Prostigmine) 1 subject
	Subcutaneous (5.0 mg) (Prostigmine+Curare+PAMCl) 1 subject
	Subcutaneous (5.0 mg) (Tubocurare+Prostigmine+Atropine) 1 subject

EPP were performed before and after administration of the drug. ChE was also monitored in some cases. All subjects tolerated these procedures very well, and there were reportedly no untoward effects of the drugs in any of the subjects tested.

APPENDIX F

EXCERPTS FROM BZ DATA

Contract DA-18–108–405-CML-826

Hazelton Laboratories Falls Church, Virginia

CONTENTS

APPENDIX G

SUMMARY OF ACUTE- AND CHRONIC-TOXICITY DATA IN VARIOUS MAMMALS WITH THE ANTICHOLINERGIC TEST AGENTS (DITRAN, BENACTYZINE, EA 2545, EA 3167, EA 3443, EA 3392, EA 3580, EA 3834, and 226,086)

by

Leo G.Abood, Ph.D.*

Studies were carried out by Lakeside Laboratories (now incorporated into Merrill Dow Pharmaceuticals, Inc.) in rodents and dogs. The results can be summarized as follows.

TOXICITY

Acute. The intravenous LD_{50} for male rats was 28 mg/kg, and that for male mice was 45 mg/kg.

Chronic. At 8 mg/kg (twice a day) for 8 wk, there was no change in weight of rats; at 300 mg/kg, there was a 12% decrease in weight, compared with controls. In dogs there was no significant change at 3.75 mg/kg.

BLOOD (RATS)

At 300 mg/kg, hemoglobin was reduced by 8%, compared with controls. Sedimentation rates increased by a factor of 3. There was a slight shift in the lymphocyte-to-neutrophil ratio. Leukocyte count increased by about 50% over 8 wk. Terminal red cell count was reduced by 21%.

HISTOPATHOLOGY

At 300 mg/kg, very few abnormalities were observed in rats. An increase in heart weight occurred in some rats; other organ weights remained normal. There were no histopathologic signs at 300 mg/kg; occasional slight changes were due to infection.

In dogs, there were no histopathologic signs attributable to Ditran at 37.5 mg/kg (highest dose). The blood picture was essentially unchanged.

OTHER DRUGS

Acute- and chronic-toxicity tests were performed by various qualified laboratories at Edgewood laboratories on the various test agents used in the Edgewood study in human volunteers. Acute toxicity was usually studied in mice, rats, cats, dogs, and monkeys to determine the LD_{50} and to observe pharmacologic effects, such as autonomic changes, changes in motor activity, ataxia, prostration, and convulsions. All the compounds were similar in the spectrum of their pharmacologic effects and differed mainly in relative potency and

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LD_{50}. For the most part, their LD_{50s} corresponded to central nervous system potency.

Chronic toxicity was usually studied over a period of a month, with single daily injections of at least 3 doses of the test agent, the largest dose being near the LD_{50}. Essentially no gross pathologic effects were noted at any of the doses. Occasionally, at nearly lethal doses, slight changes were observed in the weights of some organs—liver, heart, spleen, and thymus. There were also slight changes in blood cells (e.g., increased leukocyte counts) and the Paneth cells of the intestine (decreased counts).

CONCLUSION

On the basis of the acute- and chronic-toxicity data, the agents were deemed to be safe for human trials, particularly for acute studies of the type performed at Edgewood. It should be noted, however, that no attempt was made to observe any long-term behavioral effects, after either acute or chronic drug administration. Behavioral studies are difficult to perform, requiring a battery of complex operant and psychophysical measuring devices. Even with behavioral testing, it is questionable whether subtle long-range behavioral changes could be detected in animals, particularly in acute studies.

APPENDIX H

EVALUATION OF DATA FROM SHORT-TERM GENETIC TESTING OF ANTICHOLINERGIC CHEMICALS

by

Virginia C.Dunkel, Ph.D.*

A number of short-term tests can be used to determine the genotoxic potential of chemicals. These tests use both prokaryotic and eukaryotic cells and measure such end points as gene mutations, chromosomal aberrations, and interactions with critical macromolecules. It is widely recognized that no test can detect all genotoxic compounds, and multiple end points are required to provide a reliable assessment of genotoxicity. Information from several tests can be combined to reveal two important toxic effects: carcinogenesis and mutagenesis.

There are reports of studies in which short-term tests have been used to determine the genotoxic potential of the anticholinergic compounds 3-quinuclidinyl benzilate (BZ), atropine, and scopolamine. The objective of this discussion is to evaluate the data available on these compounds, to determine whether they are genotoxic. Such information would aid in the overall assessment of the potential of these drugs to produce long-term adverse health effects.

3-QUINUCLIDINYL BENZILATE

In the reported studies of (BZ), the test systems used have had three different end points: point mutations, chromosomal aberrations, and dominant-lethal effects.

In the point-mutation studies (1), Saccharomyces cerevisiae was used as the target cell, and the effects of the chemical were tested in a direct in vitro assay without metabolic activation and in a host-mediated modification. In the direct reverse-mutation assay, there were increases by a factor of 2–6 in the number of His^+ revertants and by a factor of 2–7 in the number of trp^+ revertants over the spontaneous frequencies. In contrast, there were no reported increases in the number of His^+ or trp^+ revertants in the host-mediated assay.

Cytogenetic analyses for chromosomal aberrations were carried out on cells from mice (1) and Chinese hamsters (12) treated with BZ and on human peripheral lymphocytes (1) treated in vitro. It was reported that BZ did not induce translocations and did not increase the number of abnormal metaphases in spermatocytes from male mice.

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With bone marrow cells from treated mice, an increase in the frequency of abnormal metaphases was reported, but there is no information on the type of abnormality observed. Similarly, in the studies with human peripheral lymphocytes, "aberrant cells" were observed at a single concentration of BZ (10^{-4} M), but again the type of aberration was not identified. Lower concentrations had showed no effects, and higher concentrations were reported to be toxic. In the studies with bone marrow cells from BZ-treated Chinese hamsters, Sram (2) reported that there was a dose-related increase in the number of gaps and that the frequency of breaks was increased, but not dose-related. That the occurrence of gaps is not a useful measure of the clastogenic potential of a chemical has been substantiated in other studies (3). In addition, although it was concluded that the frequency of breaks observed was not dose-related, it appears from the data that there were increasing numbers with increasing dose, which plateaued and then began to decrease at the highest doses tested.

The final system used in the evaluatin of BZ was the dominant-lethal assay (1). The only effect observed in these studies was a decrease in the fertility of the males at the highest dose tested. Such an effect is indicative only of the toxic effects of the test compound.

Overall, at most a marginal response might be indicated by the results of the point-mutation assays with Saccharomyces cerevisiae and the possible presence of cytogenic effects—breaks in Chinese hamster bone marrow cells. The significance of these effects, however, is open to question, and further studies are required to confirm or negate the original observations. Since these studies were reported, a substantial amount of information has been obtained on the predictive capacity of short-term tests, and other assays, such as of gene mutation in bacteria and mammalian cells and of unscheduled DNA synthesis, might provide a better base of information for reaching a conclusion about the potential genotoxicity of BZ.

SCOPOLAMINE

The studies reported on scopolamine are also limited and inconclusive, even though the capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells has been tested.

No effects were observed in either the DNA-damage assay (4) or the point-mutation assay in Salmonella (5), but the data available on neither of these systems can be considered sufficient for evaluation. In the DNA-damage assay, the compound was treated at only two doses and only in the absence of an exogenous metabolic activating system. The testing in Salmonella typhimurium (the Salmonellamicrosome (test), although conducted both with and without metabolic activation, was at a single concentration, and only two test strains were used. In testing of a compound for its ability to induce mutations in this assay, it is necessary to use a range of concentrations and at least four of the five test strains (6).

In the studies carried out to determine whether scopolamine had the capacity to induce chromosomal aberrations, three different types of mammalian cells were used: HeLa and BSC-K cell lines and human peripheral leukocytes (7). No effects were observed in either the human leukocytes or the BSC-K cells, but chromatid aberrations were observed in HeLa cells at a final 1% concentration of the compound. The aberrations were gaps and breaks, but there was no evidence of exchange figures, such as translocations. As indicated previously, the presence of gaps is not a significant measure of clastogenic potential, and the presence of breaks in a single cell line does not establish the mutagenic potential of a chemical.

The available data on scopolamine are insufficient, and a conclusion cannot be drawn as to whether this chemical is potentially genotoxic.

ATROPINE

As is the case with BZ and scopolamine, reports are available on the testing of atropine for its capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells. With one exception, these studies are deficient in one respect or another, and no definitive conclusion can be drawn from them. The problems include lack of incorporation of a metabolic activation system in assays in which the target cell does not have full metabolic capabilities (4,8); lack of a range of doses up to and including one at which toxic effects are observed, that reaches the level of solubility, or that is recognized as sufficient for testing (4,9); lack of specification of the dose used (10); and inclusion of a test method, induction of chromosomal aberrations in grasshopper spermatocytes (11), with which experience in genetic toxicology is limited.

In the single study in which there is adequate information, McCann et al. (12) reported that atropine does not induce point mutations in the Salmonella/microsome assay. The compound was tested at up to 5,000 μg/plate, both with and without metabolic activation, in test strains that can reveal both base-pair and frameshift mutations. This negative result, however, does not in itself provide sufficient information to justify a conclusion about the genotoxic potential of the compound.

CONCLUSIONS

The lack of adequate data, including test results, and limitations in the test systems used preclude a definitive assessment of the genotoxic potential of BZ, scopolamine, and atropine. Only through further studies can a conclusion be reached about the genotoxic potential of these chemicals.

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APPENDIX I

DIGEST REPORT—ANTICHOLINERGIC CHEMICALS

"TAB" Mixture

Discussion

Note: The chemical nomenclature may differ from that given in the master file. The EA or other number serves for identification of the compound in the master file.

APPENDIX I

DIGEST REPORT ANTICHOLINERGIC CHEMICALS

by

J.Henry Wills, Ph.D.

INTRODUCTION

The Board on Toxicology and Environmental Health Hazards of the National Research Council provided 58 reports that are related to the administration of anticholinergic compounds to human volunteers by personnel and contractors of the Chemical Corps Medical Laboratories at Army Chemical Center, Md., and its successor organizations at Aberdeen Proving Ground, Edgewood, Maryland. Those reports recorded studies of the responses of humans, mostly men, to 19 individual substances and to a mixture of atropine, benactyzine, and the oxime, trimedoxime (TMB-4). Two of the 19, benzetimide (Dioxotrine) and mepiperphenidol (Darstine), are N-alkylated derivatives of piperidine, but the other 17 are esters. Anticholinergic substances not mentioned in these reports, but said to have been administered also to volunteers, are N-methyltropinylphenylcyclopentylglycolate, 3-quinuclidinyl-1-hydroxyphenylcyclopentylacetate, and N-diethylaminoethylphenylcyclopentylcarboxylate.

The 17 esters represented in the 58 reports mentioned above belong to seven series of compounds: esters of tropic acid, of benzylic acid, of phenylisopropylglycolic acid, of phenylcyclopentylglycolic acid, of 3-quinuclidinol, of N-methyl-4-piperidinol, and of N-diethylaminoethanol.

Esters of tropic acid occur in nature and have a comparatively long history of use as drugs. Atropine (DL-hyoscyamine) and its optical isomers, the D- and L-hyoscyamines, and scopolamine (L-hyoscine) and its optical isomer, D-hyoscine, have been obtained from such plants as deadly nightshade (Atropa belladonna), jimsonweed (Datura stramonium), henbane (Hyoscyamus niger), horsenettle (Solanum carolinense), and various species of Scopolia. The L isomers of both esters are more potent than the D isomers.

Both esters have peripheral and central anticholinergic activities; i.e., they interfere with the actions of acetylcholine at peripheral neuroeffector junctions on smooth and cardiac muscles and on cells of glands that produce external secretions and at synapses between some neural elements. Scopolamine is especially potent in the last regard, whereas atropine and L-hyoscyamine are especially effective in blocking the action of acetylcholine at peripheral neuroeffector junctions. Even at neuromuscular junctions on skeletal muscles, which atropine usually is considered not to affect significantly, a sufficiently high concentration (10^{-4} M) of this drug in vitro caused a shortening of the duration of end-plate currents (1).

Quaternization of these alkaloids removes much of their ability to penetrate into the central nervous system and to affect its functions. However, the same variation in chemical structure increases the weak ability of these materials to interfere with the action of acetylcholine at the motor end plates of skeletal muscles.

Toxic psychoses and delirium from ingestion of atropine and scopolamine have been known for many centuries; descriptions of the effects of these drugs antedate by long times the recognition of their mechanism of action. For example, henbane was recommended in the Ebers papyrus of about 1550 B.C. for the relief of abdominal distress.

After the first isolation of atropine from plant material in 1832, the actions of this group of alkaloids were identified rapidly. By 1875, Ringer (2) was able to give the following description (excerpted) of the actions of atropine (belladonna):

> Belladonna employed either internally or externally checks or even suppresses the secretion of the glands. This at least is true of the mammary, sudoriparous and salivary glands, and possibly of other glands.
>
> Its influence on the secretion of the submaxillary glands has been fully worked out. This gland receives branches from the chorda tympani nerve which is endowed with two sets of fibres, one acting immediately on the cells, the other causing the blood-vessels to dilate, being vaso-inhibitory. Belladonna acts through the nerves distributed to the cells, for after the injection of atropia, if the chorda tympani nerve is irritated, the vessels of the submaxillary gland become distended as usual, but the gland does not secrete. The paralyzing effect of atropia is antidoted by physostigma, for after the injection of physostigma, irritation of the chorda tympani causes the gland to secrete.
>
> Dropped into the eye, applied to the skin in its neighborhood, or taken by the stomach, preparations of belladonna very speedily produce extreme dilatation of the pupil. This is one of the most characteristic effects of belladonna.
>
> A full dose of belladonna produces great dryness of the tongue and roof of the mouth, extending down the pharynx and larynx, inducing consequently some difficulty in swallowing, with hoarseness, and even dry cough; and a large dose will sometimes induce dryness of the Schneidarian membrane, and dryness of the conjunctiva, with much injection.
>
> Belladonna often relieves colic of the intestines; and is especially serviceable in the colic of children.
>
> After a considerable dose of belladonna, the face becomes much flushed, the eye bright, dry, and injected, the pupil dilated, the sight dim and hazy, while the power of accommodation in the eye for

> distance is lost. The mind and senses are peculiarly affected. The ideas, at first rapid and connected, become incoherent and extravagant; there is often decided delirium, with pleasing illusions. Sometimes the patient is possessed with constant restlessness, keeps continually moving, and cannot be quieted. A kind of somnambulism is occasionally observed; thus cases are recorded where, under the influence of belladonna, the patient for a long time performs the movements customary to his occupation; thus, it is narrated of a tailor that he sat for hours moving his hands and arms as if sewing and his lips as if talking, but without uttering a word.
>
> The delirium may be furious and dangerous, requiring the patient to be restrained; nay, it is recorded of one poisoned by this drug that so violent did he become that he was ordered to be confined in a mad-house.
>
> The first effect of belladonna on the pulse is to increase its quickness, fullness, and force to the extent even of 50 to 60 beats in the minute . Meuriot is of opinion that belladonna paralyses the peripheral branches of the vagal nerve, and by this means accelerates the heart's action.

Ringer had this to say about the effects of hyoscyamus (scopolamine):

> Thus it produces dryness of the mouth and throat, dilatation of the pupil, presbyopia, lightness and swimming in the head, delirium and hallucinations, a drunken gait, and often a strong desire to fight. Sometimes there is aphonia, and often sleepiness, with oppressive disagreeable dreams. A red rash has been observed after large doses. The pulse at first is much lessened in frequency but soon recovers itself, sometimes becoming even quicker than before the medicine was taken.
>
> Hyoscyamus is generally used to produce sleep when opium disagrees. It has also been employed in neuralgia.

It was only after the role of acetylcholine in the functioning of the autonomic nervous system, in neuroeffector transmission to the various entities innervated by that system and to skeletal muscles (3–11), and in synaptic transmission within some areas of the central and peripheral nervous systems (12–17) had been demonstrated that the proximate mechanism of action of atropine, scopolamine, and other compounds of similar activity could be understood. These compounds seem to attach to the same receptors to which acetylcholine usually attaches itself and thus to interfere with the ability of acetylcholine to produce changes in the properties of cellular membranes.

The actions of atropine and scopolamine to block synaptic transmission within some areas of the central nervous sytem and to induce coma were applied to the treatment of mental disease by Forrer (18) and Goldner (19). In 1950, Forrer published an account of a study of 16 schizophrenic patients treated intramuscularly with large doses of atropine sulfate on 3 days of each week for a long period. The doses of atropine sulfate were increased gradually during a course of treatment from 20–32 mg to 212 mg, to maintain the achievement of unconsciousness, with hyperreflexia and development of the Babinski sign, despite a gradually developing tolerance to the drug.

In 1957, Miller et al. (20) reviewed the cases of 206 mental patients treated in this general way, including 148 who had been ill for 2 yr or more before treatment. At the time of that report, the technique was to inject atropine sulfate at 3:00 a.m., so that the patient would recover sufficiently from the effect of the drug to be able to eat lunch and take part in organized activities in the hospital during the afternoon and evening of the same day. Four treatments were given each week, instead of the three that had been given initially. The desired state of coma was produced within 30–60 min of intramuscular injection, and spontaneous recovery, with complete clearing of the sensorium, required 6–9 h after the injection. Of 206 patients treated, 115 (55.8%) were considered to have improved moderately or markedly, 60 (29.1%) to be unimproved, and 31 (15.0%) to have improved only slightly. Six months after completion of a course of injections, 115 patients (55.8%) were still improved moderately or markedly.

In a series of about 500 patients treated with large doses of atropine, with about 10,000 individual inductions of coma, there was one death (21). This fatality was attributed to the replacement, without notification of the physician, of the specially trained nursing and technical personnel on the treatment ward with persons who were not thoroughly familiar with the procedures for caring for comatose patients, and particularly the procedures for controlling febrile reactions. The patient died of uncontrolled hyperthermia.

Goldner (19) reported that he had used intramuscular doses of 5–50 mg of scopolamine in treating beneficially an unspecified number of mental patients, but then had switched to atropine because of its greater availability. He reported on a group of 20 patients treated with atropine-toxicity therapy, 13 (65%) of whom were considered to have benefited moderately or markedly. Goldner also gave a limited comparison of the results of electroconvulsive therapy (ECT) and of atropine-toxicity therapy, stating that several patients who had not benefited from ECT improved on substitution of atropine-induced coma.

Wada et al. (22) reported on 51 psychoneurotic and psychotic patients treated with intramuscular injections of 10–220 mg of atropine sulfate every other day during a period of 3–7 wk. The average dose was 30–50 mg. The longest period of observation mentioned in the paper was 6 mo. Of the 51 patients, 28 (54.9%) were considered to have improved detectably. The proportion that improved among the psychotic patients (53.3%) was smaller than that among psychopathic and psychoneurotic ones (66.7%). The greatest alterations in symptoms and signs of the patients' diseases were in psychomotor excitement, hallucination, euphoria, anxiety, compulsion, and delusion, Wada et al. stated that improvement, when it occurred, began with the third to the seventh injection and increased with later injections. If no improvement occurred by the tenth injection, it was not likely to appear. The authors concluded that atropine-toxicity therapy is a relatively safe type of shock therapy, but stated that it was successful in only about 53.3% of their psychotic patients and that its efficacy probably had been overestimated by Schwarz (23). Schwarz had concluded that 25% of 155 cases of psychoneurosis and psychosis had improved markedly with atropine-toxicity therapy and that another 32% had improved moderately.

Miller et al. (20) described the phenomena experienced by patients given large doses of atropine to induce a toxic coma:

> The various stages are passed through quietly and comfortably. Neurologically, there are in order: progressive muscular incoordination, decreased pain sensitivity, and hyperreflexia with the development of the Babinski sign. On the psychological side, we observed clouding of the sensorium, disorientation, loss of time-space relationship, distortion of perception by illusions and hallucinations, confusion, and coma which proceeds to but does not go further than the early fourth stage in one and one-half hours after the drug is administered.
>
> Although the sensorium is markedly clouded, there is no cessation of psychological phenomena. Patients undergoing treatment indicate by their actions, and occasionally by word, that psychic processes continue even during the coma. Illusions seem to be experienced, inasmuch as patients attempt to pick up the bedclothing with their fingers and later report that they saw, and attempted to pick up, flowers, bugs, snakes, et cetera. Conversations may be held with absent persons—indicative of an hallucinatory state. With both atropine-induced illusions and hallucinations, highly significant past events in the patient's life seem to provide the major psychic stimuli. Behavior is correlated with psychological phenomena. Circumoral movements, including sucking and illusory “eating” and “smoking,” are commonly observed. When a sufficient degree of motor coordination is retained to accomplish the necessary movement, a progressive, coordinated, and purposeful series of events may often be observed. For example, a pantomime of striking a match, lighting a cigarette, and subsequently of smoking that cigarette is not infrequently observed. Affective lability and corresponding rapid alternation of facial expressions suggestive of pain, sadness, querulousness, and euphoria are commonly observed.

This clinical experience indicating that even large and temporarily incapacitating doses of atropine and scopolamine had no persistent deleterious effects on the health of human beings made it seem that the administration of other, possibly more incapacitating, anticholinergics to human volunteers was not unethical or immoral if two conditions had been fulfilled. These were that a compound to be administered to volunteers had been tested adequately in experimental animals to determine that the probability of its having persistent ill effects on health was small and that the volunteers had been informed as fully as possible of the possible effects of the compound. In the cases of compounds that had been administered to man previously, the effects could be described quite accurately; if the only previous recipients had been experimental animals, the description of possible effects had to be somewhat hypothetical but usually could be reasonably accurate.

Before a discussion of the specific groups of anticholinergic compounds, it may be worth while to have a general view of the procedure of the experiments carried out with human volunteers at Edgewood Arsenal and followed also by contractors. The following description is a composite from two sources: a report from Edgewood Arsenal (24) and a report by a contractor (25).

Test subjects were selected in two stages. Groups of possible volunteers were acquainted with the general nature of the experimental program through a briefing, during which they could ask questions about the general conditions and procedures of the studies. Those who volunteered to take part in the experimental program after this general briefing were given thorough medical examinations, including psychologic or psychiatric assessment. The volunteers considered acceptable for a particular experiment were then given a specific briefing and an opportunity to volunteer to take part in that study.

The studies were carried out in air-conditioned areas from which hazards of accidental injury had been eliminated as thoroughly as possible (e.g., glass panels in doors were replaced with wood, electric outlets and cables shielded, and sharp corners padded). Beds and toilet, messing, and recreational facilities were provided within the research areas. The research was conducted under surveillance of qualified physicians, who administered all injections or oral doses of the test substances. When volunteers were exposed to inhalation of vapors or aerosols, the concentrations of these substances in the air to be breathed by the subjects were established by appropriate personnel. Breathing of the contaminated air by the volunteers was observed and supervised by physicians. After an exposure to an agent, the volunteers were observed continuously for 8–24 h, and then intermittently for up to 14 d. In a few cases, followup studies were made 6 mo or more after the exposure.

ESTERS OF TROPIC ACID

This group contains five of the substances said to have been administered to volunteers, but only three of these are mentioned in the 58 reports related to studies of the effects of anticholinergic compounds on human beings: atropine, scopolamine, and methylscopolammonium. The other two substances in this group that were administered to volunteers are D-hyoscyamine and methylatropinium. Atropine and scopolamine appear, respectively, in 18 and 11 reports, whereas methylscopolammonium salts appear in only three.

Atropine and scopolamine differ only in the basic moiety esterified with tropic acid. Scopolamine and the hyoscines have an oxygen bridge inserted between the two carbon atoms in the dihydropyrrole ring of tropine that are not adjacent to the nitrogen atom, forming scopine, whereas atropine and the hyoscyamines have no such bridge. The facts that atropine is a racemic mixture of D- and L-hyoscyamines, that scopolamine is L-hyoscine, and that the L forms of both hyoscyamine and hyoscine have much greater biologic effectiveness than the D forms mean that atropine sulfate, with only 50% of its active isomer, has only about 60% as much active material per unit of weight as scopolamine hydrobromide, despite the addition of an oxygen atom in the bridge in the latter molecule.

STUDIES IN ANIMALS

Several relatively recent reviews of the actions and potencies of the solanaceous alkaloids are available (26–32). Generally, they give a great deal of information about the medically useful or undesirable actions of the compounds without dealing with their toxicity in any quantitative manner. Table I–1 contains a summary of information on the LD_{50} doses for experimental animals of the five compounds in the group of esters of tropic acid, collected from a variety of published sources (33–41) and from internal reports of Bristol Laboratories, Lederle Laboratories, Maltby Laboratories, the Squibb Institute for Medical Research, Strasenburgh Laboratories, the Upjohn Company, and Sterling-Winthrop Research Institute.

Albanus (42) studied the effects of subcutaneous injections of 13 anticholinergic compounds on central and peripheral cholinergic activities in the dog, recording such factors as the appearance of ataxia and of what he called “obstinate progression” (i.e., the failure of the animal to pull back after encountering an obstacle in its path), the light reflex of the iris, salivation, and heart rate. The compounds administered to dogs included atropine, scopolamine, and a methylatropinium salt. A dose of atropine at 0.1 mg/kg produced no significant alterations in the dogs, but a dose of 0.2 mg/kg produced ataxia in three of four dogs and increased their heart rate by 76%. A dose of 0.3 mg/kg made five of five dogs ataxic and caused three of five to engage in obstinate progression. A dose of 0.5 mg/kg rendered seven of seven dogs both ataxic and out of contact with the environment, as indicated by obstinate progression. This dose also abolished the light reflex of the iris, blocked salivation between 45 and 180 min after the dose, and increased heart rate by 89%.

Scopolamine was considerably more active than atropine in this series of experiments, a dose of 0.03 mg/kg producing ataxia in six of six dogs and increasing heart rate by 24%. A dose of scopolamine at 0.05 mg/kg rendered four of four dogs ataxic and caused them to engage in obstinate progression. This dose also resulted in abolition of the light reflex of the iris, paralysis of salivation between 45 and 135 min after the dose, and a 59% increase in heart rate.

The methylatropinium salt was much less effective than either of the native alkaloids. A dose of 2.5 mg/kg produced ataxia in none of four dogs. A dose of 10 mg/kg induced ataxia in five of eight dogs and obstinate progression in three of eight. It abolished both the light reflex of the iris and salivation between 45 min after the dose and the end of the period of observation, at 315 min after the dose. Doubling that dose induced both ataxia and obstinate progression in four of four dogs.

The only study of the subchronic toxicity of members of the group of esters of tropic acid found is one by Boyd and Boyd (43) with atropine. Groups of rabbits were given daily intramuscular injections of atropine sulfate at 44–118 mg/kg for 100 d. The daily dose required to cause death within 100 d was 78±5 mg/kg, whereas that required to cause death within 10 d was 127±9 mg/kg. The Boyds obtained a relationship between the number (y) of daily injections to produce death and the size of the daily dose (x): $\log y=2.35-0.343 \log x$. The principal sublethal effects noticed in the rabbits were (in the approximate order of their appearance): increased intracolonic temperature, decreased drinking, decreased production of urine, and decreased growth.

Three other papers were related to a special type of subchronic toxicity induced by atropine: alteration of cells, embryos, and fetuses exposed to the chemical. Ishidate et al. (44) exposed cultures of fibroblasts from Chinese hamsters to atropine sulfate at 250 ug/ml in saline for 48 h. At the end of the period of incubation, 1% of the cells were polyploid. Of cells examined for chromosomal aberrations after incubation with atropine for 24 h, 1% had gaps and breaks in their chromosomes. These percentages of polyploidy and of chromosomal aberration were only slightly greater than those in cultures to which plain saline had been added. Examination of atropine sulfate for carcinogenicity in animals by the method specified in Survey of Compounds Which Have Been Tested for Carcinogenic Activity (U.S. Public Health Service Publication 149) and for mutagenicity in bacteria yielded no evidence that atropine had either activity.

Butros (45) explanted 21- to 22-h chick blastoderms onto agar-containing substrates with atropine at 40–100 µg/ml for 24 h. Blastoderms exposed at 40–80 µg/ml had slight but dose-related injury to the developing neural tube. Those exposed at 90 ug/ml had arrested development with pycnosis and slight cytolysis of the cells of the brain wall and with somites that were smaller than normal and that had slightly opaque cells. Blastoderms exposed at 100 µg/ml had their growth arrested and their brain walls wrinkled and folded, the cells evincing early cytolysis. In some of these blastoderms, the neural tube was completely cytolyzed; in others, the neural tube was open at both its ends. Some 85% of the embryos had normal beating heart tubes; some heart primordia were incomplete and had lateral gaps.

Grunfeld and Balazs (46) reported that pregnant rats given daily subcutaneous injections of atropine sulfate at 0.1 or 0.5 mg/kg-d from day 11 to day 18 of pregnancy had low heart rates, hypothermia, and greater than usual slowing of the heart by a dose of pilocarpine for about a week after the end of administration of atropine. The progeny of these rats had tachycardia for 2 wk after birth and mydriasis for a week after their eyes opened. No long-lasting effects on either the dams or the pups were reported.

Scopolamine, added to the culture medium in a Petri dish at 1 mg/plate after incubation with microsomes prepared from livers of rats, produced no change in the cultural characteristics of Salmonella typhimurium strains TA 98 and TA 100 (47). In agreement with this finding of nonmutagenicity of scopolamine for bacteria, a study by Vrba (48) with HeLa cells, human leukocytes, and monkey renal cells revealed that scopolamine hydrobromide at a final concentration of 1% in contact with these cells induced aberrations of the chromatids in the HeLa cells, but not in the monkey renal cells or the human leukocytes. Vrba concluded that changes in chromatids in HeLa cells do not prove that a chemical is mutagenic in man.

Campbell and Ramirez (49) gave three pregnant rats daily intraperitoneal injections of scopolamine at 1.2 mg/kg. Three other groups of two pregnant rats each were given nothing other than the usual food and water or daily intraperitoneal injections of physiologic saline or scopolamine at 2.4 mg/kg. All experimental procedures began on the tenth day of pregnancy and were continued until parturition. Some of the offspring of these dams, when 120 d old, were deprived of water for 18 h and then placed in a conflict situation in which they were exposed to continuous electric shock of variable voltage whenever they tried to drink. The voltage required to inhibit drinking was recorded. Offspring of the three dams that had received daily doses of scopolamine at 1.2 mg/kg were said to have tolerated electric shock better than those of dams under either of the other regimens. No data were presented.

Lavallee (50) collected screening data on scopolamine with dogs and monkeys for comparison with the data from experiments with human volunteers performed at or for Edgewood Arsenal. In dogs, the incapacitating dose of scopolamine had been estimated to be less than 100 mg/kg. In monkeys, with only two animals, only a single dose of scopolamine had been used. The incapacitating dose was clearly above that dose, which had been 32 µg/kg. In man, the incapacitating dose in 50% of the subjects was said to be about 10 µg/kg. The agreement between the values for man and for the experimental animals was not good. Indeed, when a series of seven compounds was examined for comparative effectiveness in man and in dogs and monkeys, the comparative grading in man did not agree with those in the other animals.

In addition to the more or less standard actions of anticholinergic compounds due to their ability to interfere with the actions of acetylcholine on muscarinic receptors (resulting in acceleration of the heart; relaxation of smooth muscles in bronchi and bronchioles, intestinal tract, urinary tract, iris and ciliary body of the eye, bile ducts and gall bladder, and some cutaneous blood vessels; and decrease or abolition of secretion by glands of the gastrointestinal tract and skin), these substances may have pronounced effects on the central nervous system, as has been mentioned earlier. A large amount of research with experimental animals has been directed toward elucidation of the basic mechanisms involved in these actions.

Much of the research has been oriented around one of the basic tenets of pharmacology and toxicology: that a chemical entity affects particular structures in the body because they contain particular aggregates and arrangements of molecules and atoms that establish a special affinity between the structures and the chemical (51). As a corollary, the particular aggregate and arrangement of atoms in a molecule of a chemical entity may condition its affinity for a given site, or receptor. Thus, isomeric forms of a molecule may have different affinities for a given receptor, as has been found to be true for the D and L isomers of hyoscyamine and hyoscine (38). Also, fairly small differences between two molecules may make extensive differences in their abilities to react with particular sites in the body. For example, Graham and Lazarus (34) found that methylatropinium nitrate had approximately the same activity as atropine sulfate in preventing cholinergic stimulation of isolated rabbit intestine, but that it was some 3 times as lethal to mice after intraperitoneal injection. They also found that methylatropinium nitrate had a more pronounced inhibitory effect on the rabbit cardiovascular system than atropine sulfate; this difference between the two compounds certainly contributed to, and may explain, the greater lethality of methylatropinium nitrate. There are indications that quaternization of atropine did not alter extensively its affinity for the receptor on intestinal smooth muscle, but did increase the affinity for receptors on components of the cardiovascular system and perhaps other structures.

Schallek and Smith (52) reported that intravenous injection of atropine at 0.01 mg/kg into dogs anesthetized with thiopental had no detectable effect on their EEGs or their cardiovascular systems. A dose of 0.1 mg/kg increased the predominant frequency in the EEGs of three of five dogs and induced tachycardia in two of five. A dose of 1.0 mg/kg decreased the predominant frequency of the EEGs in four of five dogs and caused hypotension in one of five. Still higher doses increased these actions and also induced bradycardia. Similar effects by atropine and scopolamine in curarized cats and monkeys had been reported previously (53,54), and stimulation followed by depression by atropine and scopolamine of maze-running by rats had been seen by Macht (55).

In 1963, Zvirblis and Kondritzer (56) found that mitochondria isolated from 0.2 g of brain of young adult white rats adsorbed between 8.4 and 12.6% of the [^{14}C]atropine in 4 ml of atropine solutions with concentrations of 0.063–0.500 µg/ml. When the mitochondria isolated from 0.4 g of brain were immersed in the same concentrations of atropine, the adsorption was between 8.0 and 13.5%. If the lower of the last two values is erroneous (it was obtained with the second lowest concentration of atropine) and the correct value should be about 13.3% between the values for the lowest and the next higher concentrations, the range of adsorption by the larger amount of mitochondria would be 11.4–13.5%. Even with substitution of the hypothetical value in the second series, increasing by 100% the mass of brain substance represented by the isolated mitochondria seems to have increased the adsorption of atropine by no more than a mean of about 13%. Aggregation of mitochondria in the higher concentration of these organelles may have reduced the effective surface for adsorption of atropine. In these experiments, Zvirblis and Kondritzer found also that 3-quinuclidinyl benzilate was adsorbed by mitochondria 2.6–3.3 times as avidly as was atropine.

In 1973, Farrow and O'Brien (57) performed a study somewhat similar to that of Zvirblis and Kondritzer, using atropine and muscarone as the adsorbates and various fractions isolated from a homogenate of rat brain as the adsorbents. They found that both adsorbates—but not decamethonium, dimethyltubocurarine, or nicotine—were bound reversibly on mitochondria in a crude fraction. Muscarone was bound to the extent of only about 0.37 times the binding of atropine. Atropine bound especially to fractions that consisted of presynaptic and postsynaptic membranes, membranes of uncertain origin, microsomes, and synaptosomes. It bound also to homogenates of liver and to mitochondria prepared from liver, but not to homogenates of lung or kidney. Muscarone had a similar pattern of binding to various fractions, except that it did not bind to mitochondria from liver.

Hiley and Burgen (58) used [^{3}H]propylbenzilyl-choline mustard as a model muscarinic agonist to examine the concentrations of muscarinic receptor in various regions of dog brain. Synaptosomes and membranes prepared from the head of the caudate nucleus, the proreal gyrus, the uncinate gyrus, the suprasylvian gyrus, the lingual and suprasplenial gyri, and the anterior sylvian gyrus had the largest uptakes of the model agonist, whereas those prepared from the dorsal and ventral halves of the lumbar spinal cord, the subcortical white matter, the pes pedunculi, the corpus callosum, and the cerebellar cortex had the smallest uptakes.

Hiley and Burgen (58) measured also the choline acetylase and acetylcholinesterase activities of selected areas of the brain, in an attempt to determine whether the concentration of muscarinic receptors in a location was related to either of these enzyme activities. The amount of the labeled propylbenzilylcholine mustard bound was not well correlated with either the choline acetylase or the acetylcholinesterase activity; there was a modest correlation between binding of the model agonist by synaptosomes and membranes prepared from nine areas of brain and the choline acetylase activities of the same areas, but it was far from precise.

Burgen et al. (59) determined that hyoscine was 10 times as active as atropine in vitro in reducing the uptake of [^{3}H]propylbenzilylcholine mustard by synaptosomes from rat cerebral cortex. A methylatropinium salt was only slightly less active than atropine, but had a very flat dose-action curve: an increase in its concentration by a factor of 10 increased the percent change in uptake of the propylbenzilylcholine mustard only from 64% to 67%. These investigators also reported that the muscarinic receptor in cerebral cortices of the mouse, the guinea pig, the dog, the pig, and the macaque behaved very similarly to that in the rat.

In 1974, the finding of Zvirblis and Kondritzer (56) of a particularly large affinity of the muscarinic receptors in brain for 3-quinuclidinyl benzilate was applied to the assay of the quantity of muscarinic receptor in a sample of tissue (60,61). Intramuscular injection of atropine 30 min before intravenous injection of [^{3}H]3-quinuclidinyl benzilate decreased the binding of the quinuclidinyl benzilate in various regions of the brain. Although the corpus striatum had the highest uptake of the labeled substance, the cerebral cortex was not far behind. The percent decreases by atropine in the uptake of the ligand were approximately equal for the corpus striatum and the cerebral cortex. The hippocampus, which came next in magnitude of benzilate binding, was also next in magnitude of the change in binding of the benzilate caused by atropine. Indeed, in the six-membered series of corpus striatum, cerebral cortex, hippocampus, hypothalamus, pons-medulla oblongata, and cerebellum, there was complete agreement in rank order for benzilate binding and reduction by atropine of benzilate binding. Administration to rats of mecamylamine, phenobarbital, or L-dihydroxyphenylalanine before intravenous injection of [^{3}H]3-quinuclidinyl benzilate had no effect on benzilate binding in the six areas of the brain.

In these experiments, the highest concentration of labeled benzilate bound to protein was in the microsomal fraction of a homogenate of whole brain, and the next highest was in the mitochondrial fraction, the protein of the microsomal fraction binding nearly 2.5 times as much of the label as that of the mitochondrial fraction. The protein of the nuclear fraction bound only about one-twelfth as much of the labeled benzilate as that of the microsomal fraction and about one-fifth as much as that of the mitochondrial fraction. The muscarinic receptor substance(s) in the rat brain appears to be fairly generally distributed in membranes of subcellular components and to be localized especially in the corpus striatum, the cerebral cortex, and the hippocampus, the last of these three binding nearly 3 times as high a concentration of labeled benzilate as the next most active structure in such binding, the hypothalamus.

Because both muscarinic anticholinergic agonistic drugs (scopolamine, isopropamide, and atropine) and muscarinic agonistic drugs (oxotremorine, acetylcholine, methacholine), but not nicotinic anticholinergic drugs (mecamylamine, hexamethonium, and D-tubocurarine) or nicotinic agonistic drugs (dimethylphenylpiperazinium and nicotine), strongly inhibited the binding of labeled benzilate by homogenates of rat brain, binding of this compound was proposed as an indicator of the presence of muscarinic receptors in particular brain structures. In the nine-membered series of corpus striatum, cerebral cortex, hippocampus, superior-inferior colliculi, pons-midbrain, thalamus, hypothalamus, cervical cord-medulla oblongata, and cerebellar cortex, Yamamura and Snyder (61) found, as had Hiley and Burgen (58) with binding of labeled propylbenzilylcholine mustard, that binding of labeled benzilate was not well correlated with either the choline acetylase or the cholinesterase activity of the particular region, but was slightly better correlated with choline acetylase activity than with that of cholinesterase. Farrow and O'Brien (57) had concluded that binding of labeled atropine to various subcellular components of the rat brain could not be accounted for by binding to acetylcholinesterase.

Farrow and O'Brien (57) had found also that scopolamine was a much more potent inhibitor of the binding of labeled atropine to synaptic membranes than a variety of nicotinic anticholinergic and agonistic drugs; the next most active inhibitor used by these investigators had been carbamylcholine chloride, found by Yamamura and Snyder (56) to be less effective than acetylcholine and methacholine in reducing binding of labeled 3-quinuclidinyl benzilate.

In later work, Snyder's group (62,63) used autoradiography to determine more precisely the locations of binding of [^{3}H]3-quinuclidinyl benzilate within the various regions studied earlier. In the cerebral cortex, the most active areas of benzilate binding were the occipital cortex and the cortex of the cingulate gyrus. The cortex in the piriform area was less active in this regard, and that of the frontal pole still less so. In the hippocampus, three strata of the archipallium were found to have approximately equal abilities to bind 3-quinuclidinyl benzilate, but the white matter of the alveus had much less activity of this sort. The striate body also had high binding activity for this ligand, which was particularly striking in the autoradiographs because of the comparatively low binding activity of the adjoining globus pallidus. Thalamic and hypothalamic nuclei had low binding activities for 3-quinuclidinyl benzilate similar to those of the inferior and superior colliculi. Still lower in activity in binding this ligand were cerebellar cortex, medulla oblongata-pons, and the cerebral peduncles. Nerve tracts—e.g., the optic chiasma, the cervical spinal cord, and the corona radiata—had the lowest binding activities evaluated by Snyder et al.

On the basis of the research outlined in the preceding paragraph, one would expect anticholinergic compounds to produce changes in affect and motor and autonomic regulatory activities. Krieger (64) has found that a dose of atropine that was sufficient to block the circadian increase in the plasma concentration of 17-hydroxycorticosteroids during the night did not prevent stimulation of secretion of 17-hydroxycorticosteroids by the adrenal cortex after administration of ACTH or of stressors, such as insulin and piromen. Thus, the effect of atropine seems to have been exerted not on the adrenal cortex, but rather on the pituitary, the controller of activity by the adrenal cortex through modifiable release of ACTH.

Paton and Rang (65), building on indications in previous papers (66–69) that atropine antagonized the action of acetylcholine in tissues by combining reversibly with specific receptors, found evidence of the existence, in the longitudinal muscle of the small intestine of the guinea pig, of two binding sites for atropine with different capacities for it. The equilibrium constant for binding atropine amounted to slightly more than 5.01×10^{-7} M, or about 1,180 pmol/g of muscle. For methylatropinium salts, binding seemed to be to one site with reasonably definite binding properties and to an ill-defined series of other sites with much higher equilibrium constants. Methylatropinium was found to be about 2.34 times as active as atropine in blocking the receptors in this smooth muscle from access by acetylcholine.

Yamamura and Snyder (70) found that these muscarinic receptors resembled those in brain in having an affinity for [^{3}H]quinuclidinyl benzilate that was greater than that for atropine, was the same as that for scopolamine, and was slightly less than that for methylatropinium salts. Scopolamine was about 3 times as active as atropine and about 1.7 times as active as a methylatropinium salt in preventing contraction of the longitudinal muscle of the ileum. The activity of 3-quinuclidinyl benzilate in this regard was the same as that of the methylatropinium salt.

Sayers and Bürki (71) determined the activities of a group of 13 psychoactive compounds, including atropine, in four different test systems, among which was assay of interference with binding of labeled 3-quinuclidinyl benzilate to a homogenate of rat brain. The agreement between interference with binding and interference with stimulation by acetylcholine of isolated guinea pig ileum was good. The agreements between interference with benzilate binding and interference with production of tremor in mice by intraperitoneal injection of a standard dose of oxotremorine or dilatation of the pupil of the eye of the mouse were less good.

Witter et al. (72) compared the distributions in the rat brain of tritium-labeled atropine sulfate and methylatropinium nitrate injected intraperitoneally at 10 mg/kg. The rats were killed 30 min after the injections. Livers, kidneys, and brains were collected. Neither atropine nor the quaternized alkaloid was bound to a very great extent in basal ganglia, but both were bound rather extensively in pons-pyramidal tract, preoptic area, and cerebellum. The principal disagreements between the localizations of the two compounds were in the septum, where atropine was bound relatively poorly and the quaternized alkaloid was bound comparatively well (atropine was still bound at nearly twice the concentration at which the methylatropinium radical was bound), and in the hypothalamus and the cortex. In the last two areas, the alkaloid was bound comparatively extensively, whereas the atropinium radical was bound comparatively poorly. In all regions of the brain, atropine was bound to an extent that was 1.9–3.5 times that to which the atropinium compound was bound. In both the liver and the kidney, as well as in plasma, there was a higher concentration of the atropinium radical than of atropine itself. There seems to be no general decrease in the ability of the atropinium ion to move in the body, therefore, but rather a relatively poor ability of that ion to penetrate to or into the cells of the central nervous system. This difference between the alkaloid and its quaternized form may be related simply to the lipoid-rich nature of the central nervous system.

In 1952, Michaelis et al. (73) reported that atropine given before or after DFP did not affect acetylcholine concentration in the cortex of rabbit brain and that DFP increased that concentration by more than 100%. Despite this negative report on the influence of atropine on acetylcholine concentration in the brain, Berry and Stotz (74) stated in 1956 that intraperitoneal injection of atropine at 6 mg/kg decreased the mean concentration of acetylcholine in the rat brain from 365 ug/g to 204 ug/g at 30 min after injection. The depressant effect of atropine on acetylcholine concentration in the brain was confirmed by Giarman and Pepeu in two papers (75,76). In the first, they reported that intraperitoneal injection of equal doses (50 mg/kg) of atropine and scopolamine into rats produced the same mean percent decreases in acetylcholine concentration in the brain within 10 min after injection. A dose of atropine (400 mg/kg) that produced alternation of central excitation and depression, tremors, and convulsions within 10 min produced a slightly smaller mean depression of acetylcholine concentration than the smaller dose. The difference was not significant, however.

In the second paper (76), the investigators compared the effects on brain acetylcholine concentration in the rat caused by a series of doses of atropine sulfate with those caused by two doses of hyoscine hydrobromide and one dose of methylatropinium nitrate at 30 min after intraperitoneal injections of the drugs. The three lowest doses of atropine sulfate (1, 5, and 25 mg/kg) produced dose-related mean decreases in acetylcholine concentration, but the highest dose (50 mg/kg) produced a smaller mean decrease than the two next lower doses. A dose of methylatropinium nitrate equimolar with a dose of atropine sulfate of 2.5 mg/kg resulted in a slightly smaller decrease in the mean acetylcholine concentration than the atropine dose of 1.0 mg/kg. A dose of hyoscine hydrobromide equimolar with atropine sulfate at 2.5 mg/kg resulted in a lowering of the mean acetylcholine concentration nearly midway between those resulting from doses of 5 and 25 mg/kg of atropine sulfate.

In a study of the time course of the decrease in the acetylcholine concentration in the brain in the rat, Giarman and Pepeu (76) found that the maximal change occurred at about 60 min after intraperitoneal injection of hyoscine hydrobromide and that injections of this compound at 1 mg/kg three times per day for 6 d resulted in progressive decreases in acetylcholine concentration during the first 3 d. Later, the concentration increased slightly, so that at 145 h after the first injection of hyoscine hydrobromide (1 h after the nineteenth injection) the mean value was 60% greater than the lowest one, observed at 73 h following the first injection (1 h after the tenth injection). The greatest change in acetylcholine concentration was in the cerebrum (a decrease of 35%), accompanying changes in the rostral mesencephalon and in the caudal mesencephalon, with the metencephalon being below the level of significance.

Aquilonius et al. (77) studied the effects of atropine and of methylatropinium nitrate on the release of acetylcholine by the cortex of the rat brain (by vividiffusion) and on motor activity (jiggle cage). Atropine sulfate and methylatropinium nitrate at 0.25–10 mg/kg were injected into one jugular vein. Although doses of methylatropinium nitrate of 0.25–0.75 mg/kg produced slightly greater release of acetylcholine into the collecting cups than the same doses of atropine sulfate, the former drug in doses up to 10 mg/kg produced a maximal increase in motor activity of 2.33 times basal activity, whereas atropine sulfate produced an increase in motor activity of 6.75 times basal activity.

By using atropine and methylatropinium labeled with tritium, Aquilonius et al. (77) found that injection of equimolar doses of the two compounds into a jugular vein resulted after 30–60 min in nearly equal concentrations of the labels from the two compounds in not only the brain and cerebrospinal fluid but also the plasma. Despite the fairly marked differences between the fractions of the injected doses of the two compounds that entered the cerebrospinal fluid and brain, the fractions of the doses that entered the fluid in the collecting cups were not very different after 30 min; after 60 min, they were somewhat more different, but perhaps not significantly so. To explain the appearance of the methylatropinium compound in the vividiffusion cup and in the cerebrospinal fluid, the investigators adopted the suggestion of Szerb (78) that such charged compounds as the quaternized alkaloid may pass from the blood into the subarachnoid fluid and both seep into a vividiffusion cup and cause superficial layers of cortical cells to release acetylcholine.

Following the finding that septal lesions result in increased concentrations of acetylcholine in the hippocampus, Rommelspacher and Kuhar (79) studied the effects of muscarinic cholinergic agonists and antagonists on the acetylcholine concentration in the rat hippocampus after lesions of the septum had been produced electrolytically. The drugs were injected intraperitoneally just before administration of ether for creation of the septal lesions. The rats were killed 20 min after the lesions had been made. Doses of 5 and 10 mg/kg of atropine and scopolamine resulted in decreases (atropine) and increases (scopolamine) in the mean concentration of acetylcholine in the hippocampus. Smaller doses of atropine (0.1 and 1.0 mg/kg) may have induced increases in the acetylcholine concentration; only two rats received each of these doses.

Berger et al. (80) induced hippocampal after-discharges by electric stimulation of the fornix and found that doses of atropine at 1–2 mg/kg, presumably injected intravenously, doubled the duration of the after-discharges induced by a standard stimulus. This result may have been due to increased release of acetylcholine.

Frances and Jacob (81) found that eserine and arecoline, on the one hand, and atropine and scopolamine, on the other, had oppositely directed effects on the acetylcholine concentration in the mouse brain. Eserine and arecoline induced increased concentrations, whereas atropine and scopolamine induced decreases. These data indicate that a subcutaneously injected dose of scopolamine about one-fifteenth that of atropine administered similarly produced the same decrease in acetylcholine concentration in the brain as the larger dose of atropine. In the case of scopolamine, the percent decrease in acetylcholine concentration was a linear function of the log dose of the alkaloid in the range of doses used—0.3–3 mg/kg. Atropine at 1 to about 8 mg/kg yielded a linear relationship between percent decrease in acetylcholine concentration and log dose. At 10–30 mg/kg, there was no further effect on acetylcholine concentration by atropine, although the maximal change induced by this compound was only about two-thirds that induced by the largest dose of scopolamine used. There was a linear relationship between the percent decrease in acetylcholine concentration and activity (jiggle cage) in rats given scopolamine at 3 mg/kg or atropine at 10–30 mg/kg.

Herink et al. (82) used septal lesions to modify the motor activity of rats, as had Rommelspacher and Kuhar (79). An intraperitoneal injection of atropine at 1 mg/kg in normal rats increased aggressiveness within 5 min, whereas in rats with septal lesions it decreased aggressiveness. Atropine decreased defecation, an index of emotion, in both types of rats.

Usui and Iwahara (83) used freely moving male rats with recording electrodes in the dorsal hippocampus and in contact with the dura over the frontal and occipital cortices and found that atropine at 5–30 mg/kg injected intraperitoneally delayed the first appearance and shortened the first episode of paradoxic sleep. Doses greater than 10 mg/kg usually prevented paradoxic sleep entirely. The regularity of the theta rhythm from the hippocampus was markedly disturbed in paradoxic sleep without rapid eye movements; when paradoxic sleep was accompanied by rapid eye movements, theta activity was increased in both frequency and amplitude. Theta activity associated with body movement was not altered by atropine. The hippocampus seems to have two systems for generating theta activity, one that contains muscarinic cholinergic receptors and is susceptible to modification by atropine, and one that is not susceptible to alteration by atropine.

Takeyasu et al. (84) gave male rats two intraperitoneal injections of atropine at 3 mg/kg each day for 4 wk, after which the rats were not given atropine for 5–8 d. At the end of the withdrawal period, the effect of subcutaneous atropine (1–20 mg/kg) on motor activity was evaluated. Previously unatropinized rats were subjected to the same doses of atropine while their activity was monitored. Both the naive and the atropine-adapted rats yielded dose-related curves of increased activity as the dose of atropine was increased; the curve for naive rats was considerably higher than that for the atropine-adapted ones. Takeyasu et al. found evidence that the masses of muscarinic cholinergic receptors in the cerebral cortex, the hippocampus, and the corpus striatum were all increased by the repeated daily doses of atropine. They suggested that the smaller response to atropine in the atropine-adapted rats than in the naive ones might be due to this factor. (Both curves in Figure 1 of the paper by Takeyasu et al. tend to become parallel to the dosage axis at different levels of activity, rather than converging as required by their hypothesis.)

In dogs, intravenous injections of atropine at 0.5–1.5 mg/kg produced a syndrome of decreased alertness and responsiveness to commands, stumbling and slipping, swaying, incoordination and ataxia, splaying of the hind legs, blundering into obstacles and attempting to progress forward despite the presence of an obstacle, whining or barking, agitated pawing, apparent pursuit of unreal moving objects, collapse, and sleep from which the dogs could be aroused temporarily by handling or noise (85). Recovery from a 1-mg/kg dose of atropine required about 5 h. Intravenous injections of tetrahydro-5-aminoacridine [THA] at 1 mg/kg and yohimbine at 0.5 mg/kg were found to produce some recovery from the effects of atropine, THA being more effective than yohimbine [86]. These authors did not find that small intravenous doses of physostigmine or neostigmine (0.02 mg/kg) were significantly antagonistic to the central effects of anticholinergic drugs.

Because the usual dosage of atropine is small, its metabolism could not be studied effectively until fairly recently, when methods for labeling the drug with radioactive isotopes and for measuring the concentrations of the isotopes in biologic samples became available. Evertsbusch and Geiling (87) seem to have been the first investigators to use ^{14}C-labeled atropine. Their atropine was prepared by extraction from Atropa belladonna grown in an atmosphere of $^{14}CO_2$, so that the position of the label was unknown. The most sensitive method for determining atropine had been the mouse eye assay, with which Pulewka (88) and Tonnesen (89) were able to determine that only a fraction of a dose of atropine administered to man (by mouth or by subcutaneous injection) appeared unchanged in the urine. Evertsbusch and Geiling (90) found that a portion of the label from randomly labeled [^{14}C]atropine appeared in the expired air as $^{14}CO_2$.

In 1953, Gosselin et al. began an intensive study of the metabolic fate of atropine made by esterifying tropinol with tropic acid that contained an atom of ^{14}C in the position between the benzene ring and the carboxyl group. Mice were the first animals studied (over 100 were used), followed by rats, a few cats, and finally eight human beings. The original group of investigators separated into three parts before the entire series of studies was completed, and one of those parts studied the fates of two differently labeled atropines in man.

The first part of the research to be reported was the study of the metabolic fates of labeled tropic acid and atropine in mice and rats (91,92). Mice and rats given intraperitoneal injections of alpha-[^{14}C]tropic acid at 1 mg/kg excreted in their urine only a single labeled compound that had essentially the same chromatographic characteristics as authentic tropic acid. The excretion of ^{14}C administered in this form was complete within about 2.5 h in the mice and within slightly more than 3 h in the rats.

When the labeled atropine was administered to both mice and rats by both intravenous and intraperitoneal injections, the cumulative excretion of ^{14}C in the urine by the mouse was always considerably greater than that by the rat. The label from alpha-[^{14}C]atropine injected intravenously into mice appeared in their urine slightly more promptly and to a somewhat greater extent than that from labeled atropine that had been injected intraperitoneally or subcutaneously or that had been administered by gavage. The curve for the clearance of ^{14}C from the body of the mouse with time elapsed after subcutaneous injection of the labeled alkaloid required three simultaneous exponential equations to represent the observations after a period of latency of about 45 min, during which excretion of ^{14}C followed none of the three exponential relationships. In addition to atropine itself, at least three other substances containing appeared in the urine of the mouse.

At 30 min after intravenous injection of labeled atropine into mice, the structures found to have the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the duodenum and jejunum. By 4 h after injection, the structures with the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the ileum. At 24 h after injection, the structures with the highest concentrations of ^{14}C were the colon and cecum, the gall bladder and the bile, and the stomach. (In all cases, the hollow viscus and its contents are designated by the name of the viscus alone.) The comparatively high concentration of the label in the stomach 24 h after injection may indicate that the mice had ingested some of their feces, the contents of the colon and cecum having high concentrations of ^{14}C at that time. No $^{14}CO_2$ was found in the expired air.

It is clear that the principal routes of removal from the body of the atropine label used by Gosselin et al. (91,92) were the urine and the feces, the intestinal route of excretion being comparatively minor. Whatever the mode of administration of the labeled atropine to mice, 80–90% of the ^{14}C was excreted in the urine. The consistent presence of the gall bladder and its contents among the structures with the highest concentrations of the label indicates that the label enters the gastrointestinal tract after secretion by the liver into the bile, which then flows into the small intestine through the bile duct and the common duct. The progressive shift of the segment of the intestinal tract with the highest concentration of ^{14}C from the duodenum and jejunum to the ileum and then to the large bowel reflects the movement of a bolus of an especially high concentration of the isotope down the tract during a period of about 24 h.

The failure to find $^{14}CO_2$ in the expired air of the mice indicates that the tropic acid moiety in the atropine molecule is not metabolized—in accord with the finding that labeled tropic acid itself was excreted without loss in the urine. The finding of atropine but no tropic acid in the urine of the mice indicates that hydrolysis of the ester did not occur rapidly. The latter finding was somewhat unexpected because an esterase capable of hydrolyzing atropine is present in the livers of many vertebrate species, although it or a similar enzyme appears in the sera of only a few species (93–95). This enzyme is also able to hydrolyze L-hyoscyamine, tropinyl benzoate, and caramiphen (95).

The rat was found (96) to excrete in its urine substances containing ^{14}C derived from the labeled atropine with approximately the same paper chromatographic retardation factors (Rfs) as those found previously in the urine of the mouse. The rat differed from the mouse in excreting about 40% of the label in its feces, compared with about 10% for the mouse. The guinea pig, unlike the other mammals studied, excreted in its urine a large part of the ^{14}C in a form that was probably tropic acid or some similar molecule (Rf slightly below that for tropic acid added to urine from the guinea pig). The majority of the ^{14}C in the urine of the cat was excreted in the form of atropine, only two other small peaks of radioactivity being evident on paper chromatograms. The rat and the cat, unlike the mouse and the guinea pig, excreted almost as much of the label in feces as in urine. In the rat and the cat, the livers were more active both in initial uptake and in maintaining a high uptake until 4 h after intravenous injection of labeled atropine than those of the mouse and the guinea pig. Increased transfer of the isotope from the blood into the bile by the liver in the former two species probably explains its greater fecal excretion by these animals than by the other two species.

Despite the quantitative differences in excretion of labeled atropine by different routes in the mouse and the rat, Gabourel and Gosselin (97) proposed that atropine follows the same metabolic pathways in the rat as in the mouse. They studied mice given intravenous injections of alpha-[^{14}C]atropine and found that approximately 25% of the dose was excreted in the urine as atropine, more than 50% as glucuronides, and the remainder as intermediate oxidation products and, probably, tropic acid esters with altered tropyl moieties. Although they were unable to prove that the aromatic ring of the tropic acid moiety was hydroxylated, they proposed that hydroxylation of this ring, initially at the 4-position and then at the 3-position, resulted eventually in the production of monoglucuronides and diglucuronides. However, Phillipson et al. (98) found that incubation of atropine or hyoscine with a preparation of microsomes from guinea pig liver led first to the dealkylation of the nitrogen atom of the tropyl or the scopyl moiety and sequentially to production of the N-oxides and N-hydroxynoratropine or N-hydroxynorhyoscine. It seems possible that conjugation with glucuronic acid from uridine-5-diphospho-alpha-D-glucuronic acid under the catalytic influence of uridine diphosphate glucuronyl transferase occurred in the course of the oxidative series of reactions mentioned earlier and resulted in the formation of an N-glucuronide of noratropine. Such a reaction is known to occur with secondary aromatic amines, such as noratropine.

Werner and Schmidt (99) studied the types of chemical reactions undergone by [^{14}C]atropine introduced into mice, rats, guinea pigs, rabbits, cats, and monkeys. Their general conclusion was that, although various species differed quantitatively in the relative amounts of particular end products of atropine metabolism, the chemical changes undergone by the molecule in the various species were the same: hydrolysis of the ester linkage, hydroxylation of the benzene nucleus, glucuronidation, and oxidation to CO_2.

Kalser et al. (100) found that bile of rats into which [^{14}C]atropine had been injected intravenously might contain up to five peaks of radioactivity of comparatively low Rf: 0.02, 0.08–0.09, 0.16, 0.22–0.23, and 0.68. The substances represented by Rfs of 0.08–0.09 and 0.16 were present in the bile in the highest concentrations. No tropic acid or unaltered atropine was found in bile. Metabolites of atropine appeared in the bile within 10 min after intravenous injection of that alkaloid. The plasma of these rats contained only unchanged atropine in concentrations great enough to be detectable.

Isolated, perfused livers of rats exposed to [^{14}C]atropine injected into the perfusion fluid excreted about 60% of the ^{14}C into the bile during 4 h (101). All the metabolites were more polar than atropine itself, but they were not identified.

Winbladh (102) presented data on the distribution of atropine labeled randomly with ^{3}H in newborn pups,

pups at 3 and 6 wk of age, pups at 3 mo of age, and adult dogs (10 mo old or older). After subcutaneous injection of labeled atropine at 0.5 mg/kg, the isotope was removed from the blood plasma much more slowly in the newborn pups than in the adult animals. The pups of other ages had intermediate half-times for removal from the plasma, the greatest step of difference being between newborn pups and those 3 wk old. The half-times for removal of the label from the plasma after intravenous injection of labeled atropine were not strikingly different from those measured after subcutaneous injections.

In newborn pups, the organs that initially took up the highest concentrations of the label were the kidneys and the liver, the kidneys having a higher concentration. The liver maintained a higher proportion of its early concentration of ^3H until 8 h after the injection than the kidneys, however. Pups 6 wk old behaved qualitatively like the newborn ones, but in the adult dogs the initial uptake of the label by the liver was considerably greater (by up to 80%) than that by the kidneys. Of the ^3H in the urine, 50–90% was in the form of unchanged atropine. In brain, Winbladh (102) found a progressively decreasing concentration of atropine (the only form in which the label was found in brain) as serial samples were taken from the cortex to the vicinity of the lateral ventricles.

Harrison et al. (103) used atropine labeled with ^3H in the para position of the benzene ring in the tropic acid moiety of atropine to follow the disposition of that alkaloid in the rat. They found that the mean half-times for removal of the label from various tissues after intraperitoneal injection were as follows: plasma, 44 min; heart and kidney, 54 min; liver, 74 min; brain, 76 min; and fat, 101 min. The half-times were determined from the slopes of the decay curves.

Two fairly recent papers on the metabolic fate of atropine in man have been published (104,105); an excellent summary of the work was presented by Kalser (106). The two subjects in the first of these papers excreted within 24 h 85% of an intramuscularly injected dose of 2 mg of alpha-[^{14}C]atropine. A urine sample collected from one of the subjects between 1.5 and 4 h after injection contained substances that produced four small, interconnected peaks of radioactivity on a paper chromatogram that were apparently removed by incubation of the urine with bacterial beta-glucuronidase. Only three clear peaks of radioactivity appeared on the paper chromatograms of this urine after it had been exposed to glucuronidase. Two of these had Rfs that agreed with those for atropine (the major peak) and for tropic acid (a small peak). The identity of the third peak, with an Rf below that for atropine, was not determined. After alkaline hydrolysis of this sample of urine, the only radioactive substance detected in the chromatogram had an Rf similar to that of tropic acid; thus, the tropic acid portion of the ester seems not to have been altered metabolically to any important extent.

The second paper (105) used two samples of [^{14}C]atropine; one was labeled on the methyl group attached to the nitrogen atom of the tropine moiety, and the other was labeled at the two carbon atoms in the ring structure of the tropine moiety adjacent to the carbon atom involved in the ester linkage. Two male and two female subjects received intramuscular injections of 2 mg of one of these labeled atropines. One subject received individual injections of each labeled atropine on two different days. When the methyl-labeled atropine was administered, $^{14}CO_2$ appeared in the expired air. Administration of the other labeled atropine did not result in the excretion of $^{14}CO_2$, nor had this substance been found in the air expired by the only one of the subjects in the first of these papers (104) whose expired air was examined. One concludes, therefore, that the only part of the atropine molecule that is subject to complete oxidation is the methyl group on the nitrogen atom of the tropine moiety. As for excretion of the label in the urine, the two samples of atropine used by Kalser and McLain yielded similar results. There was rapid excretion of ^{14}C during the first 4 h after injection that was nearly linear with time, but by 16 h after injection the rate of excretion of ^{14}C in the urine had decreased to a much lower rate that was also nearly linear with time. The low rate persisted for up to 48 h after injection in the one subject who received each of the labeled atropines on separate days.

Kalser and McLain (105) found that glucuronidation was an important metabolic pathway in the early excretion of atropine from the body, but that this type of reaction seemed not to be particularly important after 4 h from the time of injection of atropine. Referring to the work of Phillipson et al. (98), one may suppose that glucuronidation occurs when noratropine is produced by oxidative dealkylation of the nitrogen atom of the tropine moiety of atropine. The paper by Kalser and McLain indicated that excretion of $^{14}CO_2$ in the expired air reached a peak about 75 min after the intramuscular injection of atropine and suggested that by 4 h after such a dose it may have been fairly low again. Possibly, therefore, glucuronidation and oxidative dealkylation proceed contemporaneously and in a coupled fashion.

The two labeled atropines used by Kalser and McLain (105) yielded the same chromatographically distinguishable ^{14}C-containing substances in the urine excreted by their subjects. There were four principal peaks of radioactivity, all of which appeared to consist, at least in part, of glucuronides at the early times of collection of urine. Peaks of radioactivity with Rfs similar to those of labeled atropine and tropine were the most prominent ones in the paper chromatograms of the urine of these subjects. In samples of urine collected during the first 6 h after a dose of labeled atropine, a component of Rf 0.70 seemed to be converted to one of Rf 0.55 by incubation with beta-glucuronidase from beef liver.

In summarizing this work on the metabolism of atropine by man, Kalser (106) used Tonnesen's data (89) on the urinary excretion of atropine by five subjects to show that there is a fairly broad range of rates of excretion of atropine; during the 12 h after ingestion of atropine, these five subjects excreted 15.5–42% of the ingested dose in their urine. It is not surprising, therefore, that one subject given labeled atropine intramuscularly excreted only 35.3% of the injected dose during the first 8 h, whereas the five experiments reported by Kalser and McLain (105) yielded cumulative excretion of injected atropine in urine during the same period between about 63% and 112% of the injected amount. After omission of the highest value, which came from urine samples that the investigators thought might have been handled improperly, there is still a variation between 63% and 83%. (Three experiments with three different subjects yielded closely similar excretion rates, with a mean of about 80% of the injected dose. A fourth experiment with one of the same subjects yielded the low value of 63%.)

When the data on ^{14}C still in the body at various times after intramuscular injection of tropine-labeled atropines were plotted and analyzed to yield two linear regressions in time, the derived half-times ranged between 1.3 and 2.1 h for the early parts of the curves and between 12.5 and 38.0 h for the later parts. When the data for the subject studied most extensively by Gosselin et al. (104) after intramuscular injection of tropine-labeled atropine were treated similarly, the half-times were 1.8 and 16.5 h. Apparently, the two types of labeling led to very similar estimates of the rate of removal of atropine from the body.

Two other studies provide somewhat similar findings. In one (107), coma-producing doses of atropine sulfate were injected intravenously, [^3H]atropine being mixed with unlabeled atropine to permit a dose of 100 uCi of ^3H to be included in the total dose of atropine sulfate. One psychopath, one epileptic, and 13 schizophrenics were studied. Graphs of data from three patients were presented in the paper. One man received a total dose of 70 mg of atropine sulfate (1.06 mg/kg), another man received 150 mg (2.8 mg/kg), and the third patient, a woman, received 270 mg (4.5 mg/kg). All injections were said to have been intravenous, but the curve for the concentration of the isotope in the blood of the woman is like that from a subcutaneous injection. During the 24 h after injection, the recoveries of the label in the urine, from the lowest dose to the highest, were 27.1%, 48.0%, and 81.1%. The concentrations of the isotope in the blood of the two men decreased rapidly during the first 15 min and more slowly thereafter; in the woman, the concentration increased to a maximum at about 15 min after the injection and then decreased moderately slowly. In all three patients, the concentration of the isotope in the blood became zero by or soon after 24 h after injection.

Hayden et al. (108) used a radioimmunoassay method to measure the removal of atropine from human plasma in four subjects given intravenous injections of 0.32 mg of atropine. They found a very rapid decrease in the plasma concentration during the first 5 min after injection, followed by a slower decrease. By 4 h after injection, the atropine concentration in the plasma was only about 2.25% of that present 5 min after injection. The initial period of rapidly changing concentration of atropine in the plasma may be a period of mixing in the plasma and equilibration between the plasma and various tissues.

A paper by Virtanen et al. (109) presented graphs of concentration of atropine in the serum of two women given 1.3 mg of atropine intravenously. There was an initial very rapid decrease in concentration, estimated by a radioimmunoassay method, followed by a slower decrease. The half-times for removal of atropine from the blood in these two women were 2.09 and 1.86 h. These times are similar to those calculated by Kalser and McLain (105) for the early parts of their curves. The half-times in the paper by Gaszner et al. (107) correspond more nearly with those calculated by Kalser and McLain for the later parts of their curves.

Because tropic acid was the only substance found after alkaline hydrolysis of urine from subjects given tropate-labeled atropine and a substance that was either tropine or a close relative was the only one found in large quantities after alkaline hydrolysis of urine from subjects given tropine-labeled atropine, it is apparent that atropine did not undergo drastic metabolic modification in human subjects. Oxidative dealkylation, with excretion of $^{14}CO_2$ in the expired air, probably led to the temporary formation of noratropine. This compound may then have been glucuronidated directly or been oxidized further to the hydroxylamine form and then glucuronidated. The finding that glucuronidation became a relatively unimportant reaction within about 4–6 h after the injection of atropine into muscle suggests that there is some sort of coupling between oxidative dealkylation of the tropine moiety of atropine and glucuronidation; in other words, glucuronidation may depend on the presence of some particular concentration of substrate, whether that substrate be noratropine or hydroxynoratropine.

HUMAN DATA FROM THE GENERAL LITERATURE

Gordon and Frye (27) collected from the literature reports on 11 deaths of humans considered to be due to atropine. Five of these involved application of solutions of atropine to the eyes—one person was thought to have received 1.6 mg of atropine and another 18.1 mg.

Four deaths were related to ingestion of atropine. One person had taken 198 mg of atropine with 100 mg of morphine. A 3.5-yr-old child had taken 540 mg of atropine and 1,350 mg of phenobarbital. Another person was reported to have taken a daily dose of tincture of belladonna equal to 1.2–2.5 mg of atropine for 6 yr before death (probably the highest dose for most of the time); there was nothing in the report to indicate clearly that death was attributable to long-term ingestion of atropine (110). Two deaths were connected with injection of atropine, in one case of 32 mg. On the other side of the picture, Alexander et al. (111) reported that an adult had survived after ingesting 1,000 mg of atropine; Mackenzie and Piggott (112) told of three children weighing 14–24 kg who lived after taking 600 mg of atropine.

Reports of cases of serious, nonfatal intoxication by tropic acid esters indicate that recovery from such episodes is reasonably rapid. For example, Sims (113) reported the case of a 67-yr-old woman who had applied belladonna plasters to her back for a month to relieve backache. During this time, the skin to which the plasters had been applied became excoriated. On the day of admission to a hospital, the patient had disturbed her husband in the early morning by wild and incoherent mutterings. On admission, she had two plasters on her back and was delirious and flushed. Her pupils were dilated equally and unreactive to light. Her arms were held in flexion and the legs in extension. After removal of the plasters from her back, she regained consciousness within about 12 h. Her pupils remained dilated for more than 36 h, however. She was released from the hospital as fully recovered, including healing of her skin, on the tenth day after admission.

Intoxication by scopolamine with similar signs was reported by Beach et al. (114). A 35-yr-old woman had taken 12 Sominex tablets (equivalent to 3 mg of scopolamine). She was in stupor with occasional clonic convulsions. Her pupils were dilated, her mouth was dry, her skin was hot to touch, her pulse was rapid, and she reacted vigorously to minor stimuli. When she was put to bed, her hands and fingers made repetitive plucking movements at the bedsheet. Apparent visual hallucinations, evidenced by avoidance movements, accompanied by screaming and severe disorientation began to abate after 12 h. The patient was discharged on the fourth day after admission to a hospital.

Stoll (26) compared the actions of atropine and methylatropinium nitrate on several functions of the human body. Methylatropinium nitrate at 10 μg/kg produced mean increases (four subjects) in pulse rate twice those produced by the same dose of atropine. In a series of experiments in which subjects were given identical doses (20 μg/kg) of the two drugs, they were found to have almost identical effects on pulse rate. Methylatropinium nitrate induced somewhat greater increases in the mean systolic and mean diastolic blood pressures and a considerably greater increase in the minute volume of the heart than atropine. Atropine increased the consumption of oxygen, whereas methylatropinium nitrate decreased it. Methylatropinium nitrate was about twice as effective as atropine in increasing skin temperature.

Cullumbine et al. (115) studied the effects of atropine sulfate on healthy men. Twenty men each received 1 mg of atropine sulfate by subcutaneous and intramuscular injection and 0.5 mg by intravenous injection. Forty men received double doses by the same three routes of administration. The first sign of action by atropine in many subjects was a slight, temporary decrease in heart rate, followed by a gradual increase. Intravenous injection produced effects more rapidly than the other routes of administration. The increase in heart rate induced by 1 mg of atropine sulfate injected intravenously was similar to, but of shorter duration than, that induced by subcutaneous or intramuscular injection of 2 mg. Subcutaneous injection of atropine sulfate induced acceleration of the pulse more rapidly, but less lastingly, than the same dose injected intramuscularly.

All of 44 men given 5 mg of atropine sulfate complained of dryness of the mouth and throat, of dizziness, giddiness, or light-headedness, and of difficulty in reading. Other complaints, in order of diminishing frequency, were of difficult urination; of tiredness, lassitude, or sleepiness; of headache or eyeache; and of nausea. After 3 mg of this drug, the only complaint voiced by 12 men so treated was of dryness of the mouth and throat; after 2 mg of atropine sulfate, only 37 of 45 subjects complained of dryness of the mouth and throat. The most definite objective effect of atropine was an increase in heart rate. Systolic blood pressure tended to decrease slightly and diastolic pressure to increase. There were also slight, but insignificant, changes in the concentrations of hemoglobin, leukocytes, and blood glucose. Some subjects had changes in their electrocardiograms that indicated sinus arrhythmia, P-wave enlargement, premature atrial contractions, and flattened, coved, or inverted T waves. Daily injections of 2 mg of atropine repeated for 5 d decreased either the severity of or the subjective response to the changes in secretion by the glands of the mouth and in accommodation of the eyes to light and to nearness.

Wyant and Dobkin (116) compared atropine, L-hyoscyamine, and scopolamine for ability to stop salivation induced by intravenous injection of a mixture of carbachol and epinephrine. Scopolamine was slightly more active in this regard than L-hyoscyamine, and both were considerably more active than atropine.

Bachrach (117) surveyed the literature related to a group of anticholinergic drugs, including atropine and methylscopolammonium bromide, and reported the results of studies of small groups of patients. Atropine was about 4 times as active a sialolytic drug as methylscopolammonium bromide, but produced more side effects. In a group of 11 subjects given 0.6 mg of atropine orally, nine complained of side effects; blurred vision was the most common of these; one subject complained of dysuria, and another of nervousness, dizziness, and anorexia. Only one of eight patients given about 2.4 mg of methylscopolammonium bromide complained of blurred vision, and one complained of dysphagia.

Herxheimer (118) compared a small group of anticholinergic drugs—including atropine sulfate, scopolamine hydrobromide, and methylscopolammonium bromide—for ability to affect several functional systems of man after subcutaneous injection. Scopolamine hydrobromide (0.2–0.8 mg) was strikingly more active than either methylscopolammonium bromide (0.125–1.0 mg) or atropine (0.5–2.0 mg) in inducing dilatation of the pupil and paralysis of accommodation. Methylscopolammonium bromide was more potent than scopolamine hydrobromide, which was more potent than atropine, in inhibiting secretion of saliva. The two salts of scopolamine were equally potent, and more so than atropine, in slowing urination. Methylscopolammonium bromide was strikingly more potent than atropine sulfate in increasing heart rate. The incidence of drowsiness was 100% with scopolamine hydrobromide, about 25% with methylscopolammonium bromide, and lowest with atropine. The effects of methylscopolammonium bromide and atropine sulfate on heart rate became evident (18–19 min) and reached their greatest intensities (37–40 min) at about the same times, despite the fact that the dose of methylscopolammonium bromide was only 22.5% that of atropine sulfate. When equally effective doses of the compounds were administered, methylscopolammonium bromide was slower than scopolamine hydrobromide and atropine sulfate in exerting its maximal effect on salivation and on the iris. The effects of scopolamine hydrobromide on the iris and on accommodation for near vision lasted considerably longer than those of equal doses of methylscopolammonium bromide and atropine sulfate.

Using salivary secretion as the indicator, Mirakhur (118) found that atropine and scopolamine were about 2 and 4–5 times as active, respectively, after intramuscular injection as after ingestion. When heart rate was used as the indicator, both alkaloids were about twice as active after intramuscular injection as after ingestion. When the secretion of sweat was used as the indicator, however, ingested scopolamine was nearly as active as that injected intramuscularly, whereas atropine was about twice as active when ingested as when injected.

In a combination of literature review and reports of personal experiences with patients, Schweitzer and Mark (120) summarized the effects of atropine on the intracardiac conduction system and the myocardium. The chronotropic effects of atropine are the best-known ones on the heart, consisting of an early and brief (a few minutes) slowing of the heart, most apparent with small doses or with doses administered by routes likely to yield slow entrance of the substance into the body, and a later and predominant increase in heart rate. The predominant action is due largely to blockade of vagal impulses to the heart, but is contributed to by improved sinoatrial conduction, increased frequency of discharges by the atrioventricular node, and facilitation of conduction through that node. Atropine may affect the refractory period of atrial muscle and conduction by the His-Tawara bundle, but these actions are uncertain. Atropine may induce atrial fibrillation, atrial ventricular dissociation, ventricular tachycardia, and even ventricular fibrillation. The last result is particularly likely (122,123) when the work of the heart is increased suddenly under conditions of hypoxemia, as when atropine is administered intravenously to an apneic person who has just been overcome by exposure to an inhibitor of cholinesterases and whose heart still beats.

Despite the reputation of scopolamine for causing amnesia, Hardy and Wakely (124) found that only 13 of 100 patients given subcutaneous injections of hyoscine at about 6.3 μg/kg and morphine at about 157.2 μg/kg had any amnesia of being shown a card just before induction of anesthesia. Only seven of these patients had complete amnesia of preoperative events. When atropine at about 9.4 μg/kg was substituted for scopolamine in the preanesthetic mixture, only one of 100 patients had partial amnesia of being shown a card before induction of anesthesia.

Dervent and Karacan (125) examined the effects of intramuscular injections of atropine sulfate (15–60 μg/kg), scopolamine hydrobromide (1.5–6 μg/kg), and methylscopolammonium bromide (1.5–6 μg/kg) on rapid-eye-movement (REM) sleep and its neurogenic correlate, nocturnal penile tumescence (NPT). The drugs were injected 2 h after onset of sleep. Fifteen healthy male volunteers between the ages of 21 and 35 were used. Scopolamine at 6 μg/kg and atropine at 60 μg/kg suppressed both REM sleep and NPT. Quaternized scopolamine had neither of these effects.

Ostfeld et al. (126) gave oral doses of 10 mg of atropine sulfate in 100 ml of water to 10 subjects and recorded the rate of salivation, the grades in the seven moods of the Clyde Mood Scale, the electroencephalogram, and EEG arousal induced by single and repetitive flashes of light before and after drug administration. Atropine increased heart rate by 60% and decreased the amount of saliva produced during 1 min by 68%. Five subjects complained of mild difficulty in urinating. These effects were maximal at 2 h after atropine was ingested, as were those on EEG arousal by flashes of light. The last effects consisted of decreasing the duration of the arousal induced by single flashes (by 60.6%) and repeated flashes (by 57.1%). There was a slight tendency for arousal by repeated flashes of light to be affected before that by single flashes. In the spontaneous EEG recordings, waves of lower amplitude and longer duration (5–8 per second) replaced the normal waves at 8–12 per second, beginning at about 30–60 min after atropine ingestion. Observation of spontaneous behavior and evaluation of mental status and mood revealed that atropine shifted behavior in the direction of reduced energy, decreased voluntary speech and motion, reduced attention span, impaired memory of contemporaneous events, and drowsiness. The changes in the spontaneous EEG and in behavior appeared at about the same time.

Ostfeld and Aruguete (127) performed a similar study in which 54 volunteers were subjected to subcutaneous injections of 150–800 ug of scopolamine hydrobromide. The lowest dose induced moderate bradycardia, but decreased salivation. The highest dose completely blocked the secretion of saliva and seemed to induce sleep, hallucination, and mental disorientation more frequently than the dose of 10 mg of atropine sulfate used by Ostfeld et al. (126). The larger doses were associated with decreased ability to perform tasks requiring close attention.

Commin et al. (128) reported that intravenous injection of physostigmine sulfate at 40 µg/kg into six patients in coma as a result of administration of atropine was capable of decreasing the depth of the coma, but that the duration and extent of the change were variable. The treatment could be repeated, however. In a later paper, the same group of investigators (129) reported that treatment with physostigmine in six cases of attempted suicide with anticholinergic preparations could be monitored with EEG recordings, using EEG alerting as a signal of effect. When the preparations used included overdoses of drugs other than atropine or other anticholinergic substances, physostigmine had no effect. They pointed out that therapy for anticholinergic coma with physostigmine was limited by the short period of its activity and by its secondary effects, including premature ventricular contractions and convulsant activity.

Hollender et al. (130) reported on a woman who complained of auditory and visual hallucinations after taking two Sominex tablets. It developed that she had also taken chlorpromazine, which has distinct anticholinergic activity, and amitriptyline, which has the greatest anticholinergic activity of any of the common tricyclic antidepressants. The woman's pupils were markedly dilated and fixed and did not constrict when drops of a 1% solution of pilocarpine were applied to the eyes. Eventually, she was found to have been using eyedrops of tropicamide, a potent but nonpersistent mydriatic. Shortly after admission to the hospital, the woman had a seizure. This was thought to have been of psychogenic origin; there were no recurrences. With no drug treatment and prevention of any access to drugs by the patient, her disorientation cleared rapidly. (She claimed, however, that hallucinations persisted during her stay in the hospital of 2 wk, but the investigators considered that she was probably attempting to prolong her hospitalization.)

Aucamp and Meyer (131) reported that well-controlled epileptics may experience sudden exacerbation of their seizures if they begin taking drugs with anticholinergic activity (as in some antacid preparations) or antihistaminic activity (as in preparations for amelioration of colds and influenza).

Moskovitz et al. (132) analyzed the hallucinations experienced by a group of 88 patients, 32–84 yr old, with idiopathic parkinsonism for at least 6 mo. The therapeutic regimens when these patients began to hallucinate had included anticholinergic drugs with or without amantadine (13.9%), alone (23.3%), and with amantadine or anticholinergic drugs or both (62.8%). The hallucinations had been visual for 87.1% of the patients, auditory for 35.5%, and tactile for 4.8%; 25.8% had both visual and auditory hallucinations. The hallucinations were nonthreatening for 72.7% of the patients and threatening for 27.3%. The investigators believed that anticholinergic drugs probably were the principal factors in giving rise to hallucination.

Holland et al. (133,134) gave men intramuscular injections of sterile water, 2 mg of atropine sulfate with 500 mg of N-methyl-2-hydroxyiminomethylpyridinium methyl sulfonate (P2S), 750 mg of P2S, 750 mg of P2S with 2 mg of atropine sulfate, or 2 mg of atropine sulfate. The initial rate of absorption was judged by the time after injection required for attainment of the maximal reduction in heart rate. Five volunteers were used to examine the effects of water, 750 mg of P2S, 750 mg of P2S with atropine sulfate, and atropine sulfate alone; seven were used to determine the effects of 750 mg of P2S with atropine sulfate and atropine sulfate alone; 10 were used to compare the effects of 500 mg of P2S with atropine sulfate and atropine sulfate alone.

EEGs were recorded after the subjects had reclined quietly on beds for an hour. The injection was then given into the upper outer aspect of the thigh, and ECG recording continued for about 2 hr. The air temperature was 21–24°C. All 22 volunteers had control ECGs recorded on day 1. After 2 or 3 d, volunteers 1 to 12 were given 2 mg of atropine sulfate; 5 d later, they received a mixture of 2 mg of atropine sulfate and 750 mg of P2S. Five of these volunteers were given sterile water 2 d after the injection of the mixture of atropine and oxime; a day later, these five received 750 mg of P2S. The 10 remaining volunteers received injections of 2 mg of atropine sulfate and 5 d later of 2 mg of atropine sulfate and 500 mg of P2S.

The heart rates of the volunteers given no injection, injections of sterile water, or injections of 750 mg of P2S had closely similar variations with time. The graphs of heart rate after injections of 2 mg of atropine sulfate, of 2 mg of atropine sulfate and 500 mg of P2S, and of 2 mg of atropine sulfate and 750 mg of P2S followed closely similar courses with time after injection. These were distinctly different from the first set of lines. One can conclude that 750 mg of P2S alone had no significant effect on heart rate and that addition of either 500 or 750 mg of P2S to 2 mg of atropine sulfate had no striking effect on absorption of atropine from a site of intramuscular injection. The initial rate of absorption of atropine seems to have been increased slightly by both doses of P2S (mean time to maximal bradycardia was decreased from 18.7 min to 12.9 min by 500 mg of P2S and from 15.3 min to 12.2 min by 750 mg of P2S).

Martin (135) followed up this work by comparing the effects of intramuscular injections of 2 mg of atropine sulfate alone and mixed with 750 mg of P2S on heart rate and sweating in nine exercising men. These volunteers worked at 25°C and a relative humidity of 65–75% until their heart rates had risen to 110–130 beats/min and then got injections of atropine alone or of atropine and P2S. The exercise was continued for an additional period. The dose of atropine increased the mean heart rate at the end of the period of exercise by 34.4% and decreased the mean rate of sweat production by 36.1%. Addition of P2S to atropine had no important effect on these changes.

The General Practitioner Research Group (136) surveyed the effects of drugs in women in the United Kingdom during the first trimester of pregnancy. Of 661 pregnant women who took drugs of some sort during the first trimester, 57 (8.6%) had either miscarriages, stillbirths, neonatal deaths, or abnormal children. Antihistamines were involved in 38 of these, barbiturates in 10, and female sex hormones in 10. Antihistamines had been used by 76.9% of the pregnant women, barbiturates by 9.2%, and female sex hormones by 9.1%. Thus, women who used barbiturates or female sex hormones during the first trimester of pregnancy were the most likely to have one of the four types of reproductive accidents considered. A pregnancy that resulted in a blind infant had involved the use of scopolamine during the eighth week and of promethazine during the ninth week. Scopolamine was the only tropic acid ester used by a pregnant woman who delivered an abnormal child in this survey.

Hellman et al. (137) studied the reliability of the response of the fetal heart to administration of atropine to a pregnant woman, the fetal heartbeat being recorded and analyzed by a computer as an indicator of transplacental transfer. Intravenous injection of atropine sulfate at 22 ug/kg during a 2-min period to 28 normal pregnant women resulted first in slowing of fetal heart rate and then tachycardia in 26 cases. In two cases, there was no apparent transfer of atropine to the fetus, although both women delivered apparently normal infants.

Mellin (138) studied the consequences of the use of drugs during the first trimester of pregnancy by 3,200 pregnant women seen at the Columbia-Presbyterian Medical Center in New York during 1953–1957. Of these 3,200 pregnancies, 266 were recorded as resulting in malformed infants. Two control groups were established by selecting 266 records immediately preceding and 266 immediately after each record relating to a malformed infant. Drugs were used during the first trimester by 53% of the women who delivered malformed babies, whereas 50.9%±1.9% of the women in the control groups had used drugs during the first trimester. The difference is not significant. However, when fetal and neonatal deaths among the offspring of women who used drugs during the first trimester and of those who did not were summed separately, only 4.9% of the offspring of the latter group died in utero or soon after birth, whereas 9.5% of the offspring of the drug-users died before or soon after birth. Atropine was found to have been taken by one woman whose infant died soon after birth among 13 offspring of women who had used this drug during the critical period of embryogenesis; three of the other 12 babies of these women were malformed. Of three women who had taken scopolamine during the first trimester, two delivered apparently normal children. There is no way of determining from the paper what other drugs the women who used these two anticholinergic substances may have taken, except that one of seven babies with both congenital heart disease and another unspecified malformation was born to a woman who had used chlorpromazine, dimenhydrinate, meperidine, morphine, and secobarbital sodium in addition to scopolamine.

Hellman and Fillisti (139) extended the study by Hellman et al. (137) of placental transmission of atropine in normal women by studying 24 pre-eclamptic women, three with eclampsia, 11 with chronic hypertension, and 16 with diabetes. Sixteen of the 24 pre-eclamptic patients, two of the three eclamptic patients, and four of the 24 records on the 16 diabetic women gave no evidence of transfer of atropine to the fetus. Two of the negative records on diabetic mothers can be paired with one each from the same woman that provided evidence of transplacental transfer of atropine. Another of the negative records pertained to a woman who had yielded three records indicating placental transmission of atropine to the fetus. The general conclusion from both studies (137,139) was that the atropine test of placental function is not sufficiently precise to indicate whether placental function is normal in any given case.

Quilligan (140) pointed out that comparison of blood from the umbilical veins and arteries of placentas of six women who received 1.0–1.5 mg of atropine and of 13 women who received no atropine revealed that the umbilical blood from the atropine-treated women had lower than that from the control patients, although the acid-base relationships of the umbilical blood from the two groups were not particularly different.

Kivalo and Saarikoski (141) studied transplacental transmission of atropine by injecting [^{3}H]atropine intravenously (0.5 µg/kg) into 25 women undergoing delivery by Cesarean section. Samples of blood were taken from the antecubital vein of the mother and from the umbilical vein and artery. The investigators found that the concentration of the label in blood from the umbilical artery was about 50% that in blood from the umbilical vein. At 1 and 5 min after injection, the concentrations of ^{3}H in the blood from the umbilical vein were 12% and 93%, respectively, of those in the maternal venous blood at the same times. Atropine seems to cross the placenta rapidly.

Onnen et al. (142) estimated the concentrations of atropine in blood samples by bioassay on isolated ileum from guinea pigs stimulated by acetylcholine. They gave rapid intravenous injections of atropine sulfate to 28 pregnant women at 12.5 µg/kg and found that tachycardia in the fetus started 5–10 min after injection, at a time when the initial rapid decrease in the atropine concentration in the mother's blood had been replaced by a comparatively low rate of decrease. In another experiment, they gave 45 women in labor atropine injections and took samples of maternal and of mixed cord blood at birth. They found that 5–15 min after injection the ratio of the mean concentration of atropine in cord blood to that in maternal blood was 1.2±0.5. This paper also indicates that atropine is transferred across the placenta within a matter of a few minutes.

In addition to the effects on the reproductive functions of the female, there has been some study of the effects of tropic acid esters and related compounds on sexual function in the male. One paper on this topic (125) has been mentioned above. Horowitz and Goble (143) have reviewed the literature on drug-induced sexual dysfunction in the male and have concluded that any drug with atropine-like effects may interfere with penile erection. (They pointed out that impotence induced by antimuscarinic drugs may occur without reduction of libido and may thus be particularly frustrating to the male.)

Knuutila et al. (144) reported finding an unusually high incidence of chromosomal aberrations (broken chromatids, acentric fragments, dicentric chromosomes, etc.) in lymphocytes from a woman who had been vaccinated against rubella and 3 d later had received a variety of drugs, including atropine, in connection with an operation on varicose veins; after the operation, she had been given indomethacin for 10 d and furosemide for 12 d. The young woman was considered to be in good health. Lymphocytes cultured from the blood of four other women who had been vaccinated against rubella also contained unusually large numbers of broken chromatids, but did not have the other chromosomal abnormalities. The percentages of metaphase cells with broken chromosomes from these four women were less (by 3–11%) than that in the blood from the first patient (15%). The investigators suggested that the major aberrations in the first patient's chromosomes may have resulted from a combined effect of the vaccine and one or more of the 10 drugs that she took during and after her operation. They recommended, therefore, that prescribing drugs should be avoided, if possible, near the time of vaccination for rubella. (In view of the fact that the investigators had no knowledge of the state of the patient's chromosomes before her vaccination and operation, their suggestion and recommendation seem to be on rather shaky ground.)

Minich et al. (145) attempted to detect mutagenic products in the urine of patients who were taking a large variety of drugs, including atropine, as a part of their therapeutic regimens in the hospital. The urine was tested with and without the addition of a preparation of microsomes from the livers of rats that had been given a 1% solution of phenobarbital in place of drinking water for 6 d before removal of the livers. Minich et al. found no evidence of mutagens in the urine of the patients given atropine.

HUMAN DATA FROM EDGEWOOD ARSENAL

Grob et al. (147) compared the effects of atropine administered intravenously and intramuscularly. Four subjects were used. Two received injections of 2 mg of atropine sulfate by the intramuscular route only; one of these subjects received only one injection, and the other, two. A third subject received 1 mg of atropine sulfate intravenously on one occasion, 1 mg of atropine sulfate intramuscularly on another occasion, and 2 mg of atropine sulfate intramuscularly on four other occasions. The fourth subject received 1 mg of atropine sulfate intravenously on two occasions and 2 mg of atropine sulfate intramuscularly on three occasions. Atropine sulfate by either route of administration induced an increase in heart rate, an increase in skin resistance, an increase in pupil size, dryness of the skin, and an increased sensation of dryness of the mouth. In general, the effects appeared earlier after intravenous than after intramuscular injection. Prior administration of sufficient TEPP to increase sweating and salivation and to induce anorexia and mild nausea slightly delayed the onset of the effects of atropine and slightly reduced the extent of those effects.

Marzulli and Cope (148) gave 40 men single injections of 1 mg of atropine intramuscularly, 28 men two doses of 1 mg of atropine 90 min apart, and 20 men single doses of 3 mg of atropine. The last group of subjects contained men who had received the two doses of 1 mg of atropine at least 2 wk previously and the single dose of 1 mg still earlier. The injections were into a deltoid muscle. Testing of the subjects began 30 min after each dose of 1 mg of atropine and 60 min after the dose of 3 mg. A battery of 10 tests, including nine for grading visual acuity, stereopsis, and other properties of the eye and one for grading cognition, was used.

Four of 25 subjects who had received intramuscular injections of saline reported slight dryness of the mouth, two complained of headache, and one who had complained of headache also mentioned a slight numbness of the lips.

The 40 men given intramuscular injections of 1 mg of atropine all reported dryness of the mouth; 17 complained of dizziness, 13 of feeling tired and sleepy, 10 of dryness of the nose, and eight of having difficulty in reading.

The 28 men who received 2 mg of atropine all complained of dryness of the mouth; 17 complained of feeling tired and sleepy; 12 reported either that they had slept during the day or had gone to bed earlier than usual; nine complained of feeling incoordinated.

The 20 subjects given 3 mg of atropine all reported dryness of the mouth; 18 had felt tired or sleepy; 14 recalled either that they had slept during the day or had retired early in the evening; seven reported that their sleep during the night after the injection had been broken by vivid and disturbing dreams. Three men in the latter group reported seeing objects in unusual colors; a fourth had a visual aura. No true hallucinations were described.

The only significant objective changes seen in this study were a decrease in the ability of the eye to accommodate for near vision and tachycardia. The mean magnitude of accommodation by the eyes of the 20 men before they were exposed to atropine was 10.3 diopters (148). After a single dose of 1 mg, the mean magnitude of accommodation was 8.5 diopters. When the same subjects had been given two doses of 1 mg, the mean amount of dioptric change was 7.5. After a single dose of 3 mg, the eyes of the same 20 subjects had a mean magnitude of accommodation of 4.8 diopters.

This report provided no data to enable the reader to judge whether the tachycardia that followed the various doses of atropine was dose-related; data on heart rate are provided only after the single dose of 3 mg. The mean heart rate in the 20 men before exposure to atropine was 75.8 beats/min. At 15 min after injection, the mean was 116.8 beats/min. After another 15 min, the mean was 117.8 beats/min. Thereafter, the heart rate decreased gradually, the mean at 7–8 h after the injection being 66.1 beats/min. The mean was below that during the control period from the third hour to the eighth hour after injection.

Himwich (149) used four subjects to study the effects of intramuscular atropine at 0.07–0.12 mg/kg on the EEG, personality structure, heart rate, breathing rate, and pupil diameter. The pupil diameter increased by a mean of 114.3%, the heart rate by a mean of 51.0%, and the breathing rate by a mean (for only three subjects) of 48.5%. There was an increase in the voltage of the predominant frequency in the EEG, impairment of the alerting reaction on opening the eyes, and increased proportions of slow waves and spindles in the EEG, even though the subjects were awake and responsive to spoken commands. All four subjects found concentration on a single topic difficult, both attention and logical progression of thought being attenuated. Judgment became poor, sensitivity to the environment was lessened, the grasp of reality was weakened, and drowsiness affected all four subjects. Three of the subjects became irritable, although the mood of the fourth was predominantly euphoric. The three irritable subjects had disturbed sleep and fantastic dreams during the night, whereas the fourth slept well. All four subjects had some degree of ataxia; two complained of urgency of urination and congestion of the conjunctivae. The most persistent effect of atropine noticed in this study was paralysis of accommodation, which disappeared gradually during 3–4 d after injection.

Wechsler and Koskoff (150) used nine surgical patients to study the effects of doses of atropine between 1.3 and 5.85 mg—administered by intravenous, intramuscular, or intracisternal injection—on cerebral blood flow and metabolism and on neural and cardiac indexes of normal function. One patient received atropine intracisternally on two occasions, 1.8 mg the first time and 4.2 mg the second time. Three patients were given intravenous doses of 1.3–1.6 mg of atropine, and five were given intramuscular doses of 2.0–5.85 mg. The intracisternal injections induced no changes in heart rate or blood pressure within 30 min after injection. The intravenous injections produced tachycardia in the three patients so treated and small increases in mean arterial blood pressure in two of them, whereas the third had a small decrease in blood pressure within 3 min after injection. All five patients given intramuscular atropine developed tachycardia, and four of them (the only ones whose blood pressures were recorded) developed small increases in mean arterial blood pressure. Atropine by any route of administration in these studies induced no consistent changes in cerebral blood flow, cerebral vascular resistance, cerebral consumption of oxygen, cerebral arteriovenous oxygen difference, cerebral respiratory quotient, or pH of blood from the jugular vein. The only significant neurologic change after administration of atropine was dilatation of the pupil. Only one patient given atropine yielded any indication of an effect on the EEG: a minimal depression of the alpha component. No morbidity was attributable to the atropine. The authors concluded that timidity in the use of atropine is not warranted.

Andrews et al. (151) used nine subjects in a preliminary comparison of the effects of atropine sulfate injected intramuscularly and subcutaneously in warm (35°C, 80% relative humidity) and cool (16°C, ambient relative humidity) environments and 24 subjects in a preliminary comparison of the effects of inhalation of 2 mg of atropine sulfate as a dust and as an aerosolized solution in water. Finally, 10 subjects were used to repeat the comparison of the effects of atropine sulfate administered by the two methods of injection in warm and cool environments and to compare the effects of 2 and 5 mg of atropine sulfate inhaled as a dust and an aerosolized aqueous solution in the warm environment. The report did not state whether the same subjects were used in more than one part of this study.

The results of the study indicated that 2 mg of atropine sulfate had practically identical effects on heart rate and sweating, whether injected intramuscularly or subcutaneously.

The mean increase in heart rate after injection of 2 mg of atropine sulfate in the warm environment was 42.7%, whereas that in the cool environment was 31.3%. The mean increase in heart rate after inhalation of 2 mg of atropine sulfate as a dust or an aerosolized solution was 23.7%; the mean increase after inhalation of 5 mg of the drug was 48.1%. The increases with the two types of dispersion of atropine sulfate were essentially identical for the two doses used. It is apparent that inhalation of atropine sulfate is not as effective a method of delivery, with heart rate as the indicator, as intramuscular or subcutaneous injection.

Although inhalations of 5 mg of atropine sulfate induced an increase in heart rate comparable with that induced by the injection of 2 mg, all subjects reported headaches, giddiness, tiredness, lethargy, and muscular weakness or incoordination after inhaling the 5-mg doses. It took 24 h for all the subjects to feel able to return to their usual activities after inhaling the larger dose of atropine sulfate, and several reported visual hallucinations, euphoria, and dysuria. The authors concluded that the use of inhalators for self-administration of atropine sulfate would be impractical and that intramuscular injection would be the easiest method to teach to nonmedical persons.

White et al. (152) gave 12 subjects intramuscular doses of atropine sulfate at 22.6–134.0 ug/kg (mean, 50.8 μg/kg). The mean blood pressure before administration of atropine sulfate was 123/79; after administration, it was 129/91. The mean rate of the radial pulse was 69 beats/min before administration of atropine; within 30–60 s after injection, it was 61.5 beats/min; it increased later, reaching a mean maximum of 100.5 beats/min. The mean breathing rate before atropine was administered was 15.1/min; after atropine had been administered, it rose to 17.1/min. The mean diameter of the pupil was estimated to be 4.8 mm before administration of atropine; under the influence of that drug, it increased to 6.5 mm. Eight of the 12 subjects had extensions of the near point under the influence of atropine, the subjects given the two largest doses having the greatest increases.

All 12 subjects complained of dryness of the mouth; six became restless; two complained of sensations of heat on their skins, although they were in an air-conditioned room; 10 were observed to become drowsy; 10 found that walking a straight line was difficult; and five had malaise, headache, vertigo, and slight nausea. These complaints had disappeared by the following morning.

Other effects of atropine sulfate seen in the 12 subjects who took part in this experiment, in order of decreasing incidence, were sleepiness, shortened attention span and blurred vision, dreaming, eye irritation, lack of desire for mental work, and urinary urgency. The usual changes in the EEG pattern after administration of atropine were a flattening of the record, a decrease in frequency, and intermittent blocking of the alpha rhythm. There was a decrease in alerting on opening the eyes. Five subjects had notable improvement in photic driving after administration of atropine, and five others had slight improvement. Only one subject had a decrease in response to photic stimulation.

In attempting to devise a battery of tests useful in assessing the effects of chemicals on human capabilities, Elkin et al. (153) gave 11 subjects intramuscular injections of scopolamine at 12 µg/kg after the subjects had spent a day gaining familiarity with the test battery. Four subjects received placebo injections. The only part of the test battery that may have been affected by the placebo was a manual-dexterity test in which the subject moved as many blocks as possible in 30 s from one hole to an adjoining hole. The number of blocks moved 0.75 and 2.25 h after the placebo was about 8.3% greater than the number moved before administration of the placebo and 4.25 h after the placebo. Scopolamine decreased both near and far visual acuities, the effect on near visual acuity being much the greater. In the manual-dexterity test, scopolamine decreased by 38.6% the number of blocks moved, this decrease persisting for 2.25 h after the injection of scopolamine and then diminishing gradually. By 8.75 h after the injection, the number of blocks moved was only slightly below the baseline measurement. Mean grip strength was lowered from about 54 kg to about 48 kg by the dose of scopolamine. Scopolamine lowered the mean duration of balancing on a beam on one foot from about 17 to about 5 s. The mean number of additions performed within 3 min decreased from about 42 to about four. The mean number of digits that could be recalled after auditory presentation decreased from about 7.1 to about 5.1. The mean ability to estimate 10 was altered only slightly by scopolamine in the direction of underestimation. However, the variance of this estimation was increased by about 128% by scopolamine. The mean reaction time (the subject pressed a switch as soon as possible after receiving a visual cue) increased from 0.22 to 0.38. The authors concluded that the most dramatic change was in near visual acuity. Grip strength, simple reaction time, and estimation of elapsed time were affected only slightly by scopolamine.

Kitzes et al. (154) analyzed the records of 18 subjects who had been given intramuscular injections of atropine at 32–125 ug/kg and who weighed 57.2–90.0 kg. The results on the Number Facility Test (completion of as many additions as possible during 3 min) were used to grade each subject's response to the drug. No correlation between body size and decrease in the Number Facility Test was found.

Cummings and Craig (155) exposed 10 men to temperatures up to about 52°C to induce a range of rates of sweating and then subjected them to a variety of doses of atropine sulfate by intravenous injection of various priming doses followed by infusions of various concentrations of the drug for 13–53 min. The total dose of atropine received by a subject varied between zero and 1.6 mg; the report was unclear on whether this value is for atropine alkaloid or atropine sulfate. Higher rates of sweating, induced by exposure to high environmental temperatures, were found to require larger doses of atropine to inhibit sweating. Sweating recovered rapidly (6–74 min) after the end of infusion of atropine sulfate, particularly at the higher temperatures.

Kligman and Copelan (156) reported that 13 subjects had been exposed to atropine. No details or results of the study were given.

Crowell and Ketchum (157) used 33 normal male volunteers to assess the ability of physostigmine to antagonize the deliriant activity of scopolamine. The volunteers received intramuscular injections of scopolamine hydrobromide at 24 µg/kg; subgroups of the intoxicated men received physostigmine salicylate intramuscularly at 50 ug/kg 15, 30, or 90 min after injection of scopolamine. Those treated 15 min after scopolamine did not have any immediate reversal of feelings of fatigue and drowsiness and gradually developed a typical delirium, but at a lower rate than those who had not been given physostigmine. Physostigmine lowered the heart rate in these men and maintained it on a plateau below that in the untreated group until the heart rate in the untreated group decreased to equal that in the treated group 3–4 h after the scopolamine had been administered. Physostigmine had only a minor and brief effect on the diameter of the pupil, but decreased significantly the decrement in the Number Facility Test due to the dose of scopolamine during the 2 h after its administration.

The subjects treated with physostigmine 30 min after the injection of scopolamine improved dramatically and rapidly in their performance of the Number Facility Test. These men relapsed into delirium about 3 h after administration of physostigmine and then recovered at about the same rate as the untreated men. The effects of physostigmine on heart rate and pupil diameter were similar to those in the group treated earlier in the intoxication.

The men treated 90 min after injection of scopolamine, after development of a full-blown intoxication, improved dramatically and rapidly, the change being noticeable within 10 min of injection of physostigmine and becoming maximal within 30 min. At that time, the subjects were alert, well-oriented, and logical. Their performance in the Number Facility Test decreased slightly about 1.5 h after the dose of physostigmine, but did not fall to near the level of performance of the untreated group at that time. The performances in the Number Facility Test of the untreated group and the group treated with physostigmine became essentially identical at about 6 h after the dose of scopolamine. The heart rate decreased within 30 min after the dose of physostigmine, but this drug had little effect on pupil size.

Baker et al. (158) summarized their studies with 82 volunteers given scopolamine. No details of the experiments or of their results were given, but the individual subjects were said to have reacted quite differently to a given dose of scopolamine. After extensive analysis of the data, the authors concluded that research was needed to identify the factors that cause "subject by treatment" interactions and that means for controlling for them were necessary for obtaining consistent effects by a given chemical.

Krieger (64) examined the effect of injected atropine on the circadian variation in the concentration of

17-hydroxysteroids in human plasma, using two healthy male subjects. One subject was given a subcutaneous injection of 3.0 mg of atropine, the other a similar injection of 3.5 mg. Three other subjects took oral doses of 2.5 mg of atropine. None of these subjects gave any evidence of an effect on the usual pattern of variation in plasma 17-hydroxysteroids. The author pointed out that the dose of atropine used in these human studies was only about one-tenth that used in experiments with cats in which atropine had been found to block the nocturnal rise in plasma 17-hydroxysteroid concentration, when administered before the time at which the circadian rise could be expected to occur.

Safer (159) investigated the possible interplay of sleep deprivation and scopolamine intoxication in producing deterioration of human abilities. Men were deprived of sleep for one or two nights and were then given scopolamine hydrobromide intravenously at 5 or 10 μg/kg. Behavior was rated with a checklist. The performances of the subjects in the Number Facility Test, a manual-dexterity test, and a vigilance test were evaluated. Eleven subjects were given scopolamine at 10 μg/kg, 10 were deprived of sleep for a night and given intravenous saline, six were deprived of sleep for a night and were given scopolamine at 10 μg/kg, two were given scopolamine at 5 μg/kg, four were given scopolamine at 5 μg/kg after being deprived of sleep for a night, and four were given scopolamine at 5 μg/kg after being deprived of sleep for two nights.

The combination of loss of sleep for one night and scopolamine at 10 μg/kg had a more than additive effect on performance in the Number Facility Test and considerably more effect on manual dexterity than the scopolamine alone. The combination also produced hallucinations in about 2.7 times as many subjects as the same dose of scopolamine alone. The results with the lower dose of scopolamine were similar to those reported above, but less striking. The men deprived of sleep for two nights became so somnolent after the dose of scopolamine that the tests could not be run.

The effects of large intramuscular doses of atropine on the electrocardiogram were examined by Hayes et al. (160,161), with six healthy male volunteers. The volunteers were given atropine sulfate at 175 μg/kg. By 2 min after injection, there was slowing of the heart without any striking change in the ECG record. At 5 min after the dose, the Pwave was engulfed by the QRS complex, and an A-V nodal rhythm was instituted. This A-V dissociation was temporary, a sinus tachycardia becoming apparent at 6–7 min after the injection and persisting for about 3 h.

Four volunteers who had received scopolamine, three who had been given atropine, and nine who had received no chemicals were interviewed and examined at some unknown time after their service as experimental subjects (162). None of the subjects felt that he had suffered physical or psychic injury as a result of serving as a subject. Blood counts for all the former subjects were within the normal range. Three of the former control subjects and one former subject who had been given both scopolamine and a salt of LSD had up to 10 white cells per high-power field in their urine; that suggested low-grade urinary tract infection. One former subject who had been given atropine had a mildly positive test for urinary glucose (there are so many possible causes of such a situation that no precise meaning can be attached to it). One of the former control subjects had an increased activity of GOT in his serum, and another had a slightly increased concentration of BUN. No abnormalities of serum chemistry were recorded for the former subjects who had been given atropine or scopolamine. This study revealed, therefore, no definite evidence of long-term physical or psychic injury by esters of tropic acid.

Klapper et al. (163) examined the records of eight subjects who had been given intramuscular injections of atropine at 62 μg/kg, six who had been given atropine at 104 μg/kg, and six who had been given scopolamine at 11.8 μg/kg. The performance of each subject on the Number Facility Test after injection was compared with his scores on the three validity scales and 10 standard scales of the Minnesota Multiphasic Personality Index (MMPI). The best correlations between performance in the Number Facility Test and scores on scales in the MMPI for the men given atropine at 62 μg/kg were with those in the hypochondriasis and mania scales. The best correlations for the men given atropine at 104 μg/kg were with scores in the lie and mania scales. For the men given scopolamine at 11.8 μg/kg, the best correlations were with scores in the hypochondriasis and lie scales. If atropine and scopolamine are taken as representative of the group of tropic acid esters, the best indexes of performance in the Number Facility Test under the influence of such esters are the scores in the lie, hypochondriasis, and mania scales of the MMPI. After a similar analysis for five anticholinergic compounds, three other substances with atropine and scopolamine, Klapper et al. (162) decided that scores in the positive test-taking attitude, hypochondriasis, and mania scales of the MMPI were positively related to performance in the Number Facility Test.

Sidell et al. (164) reported that intravenous injection of the potent cholinesterase inhibitor, S-(diisopropylaminoethyl)-ethylmethylphosphonothioate, at 1.5–1.7 μg/kg into men 1.5 h after intramuscular injection of scopolamine hydrobromide at 24 μg/kg was effective in reversing the performance and cognitive decrements produced by the scopolamine. If the anticholinesterase was administered 30 min after scopolamine, the initial therapeutic benefit was less than when it had been given at 90 min. This anticholinesterase had a more durable effect than physostigmine, its antagonistic activity persisting as long as the agonistic activity of scopolamine, whereas that of physostigmine had been found by others to last for only 2.5–3 h.

Ketchum et al. (24,165,166) reported the results of intramuscular injection of atropine sulfate at 32–175 μg/kg, of intravenous injection of scopolamine hydrobromide at 5–24 μg/kg, of intramuscular injection of scopolamine hydrobromide at 5–24 μg/kg, of intramuscular injection of scopolamine methylbromide at 5–30 μg/kg, and of attempts to find antagonists of intoxication with these chemicals. The research involved 127 individual experiments. The ED_{50}s of atropine sulfate to initiate the following effects to recognizable extents were: poorly coordinated, 89 μg/kg; attention span shortened, 95 μg/kg; stumbling gait, 106 μg/kg; restlessness, 126 μg/kg; temporal confusion, 130 μg/kg; inability to obey simple orders, 135 μg/kg; and hallucination, 169 μg/kg. Although the ratios between the ED_{50}s of atropine and of the other anticholinergic compounds for the production of the different effects varied somewhat, intravenously injected scopolamine hydrobromide was about 8.7 times as active as atropine sulfate and, when injected intramuscularly, about 7.5 times as effective as atropine sulfate. Scopolamine methylbromide was about 4 times as active as atropine sulfate, except that no dose of the former substance produced as much as a 90% decrease in performance in the Number Facility Test, so comparative potencies had to be assigned on the basis of less than maximal actions by atropine and scopolamine. Therefore, scopolamine methylbromide was only about 53% as potent as the hydrobromide.

Intramuscular injections of scopolamine hydrobromide at 24 and of atropine sulfate at 175 ug/kg induced approximately equal decrements in performance in the Number Facility Test, except that the maximal effect after scopolamine was attained in about half the time required to reach that after atropine. Intravenously injected scopolamine hydrobromide produced its maximal effect even more rapidly than that injected intramuscularly. Scopolamine methylbromide was slower in exerting its maximal tachycardial effect than scopolamine hydrobromide, but no information about the rapidity of its action on performance in the Number Facility Test was provided in any of the three reports (24,165,166). The increase in heart rate induced by scopolamine methylbromide was greater than those induced by the same amounts of scopolamine hydrobromide or atropine sulfate.

The decreases in performance in the Number Facility Test induced by atropine or scopolamine were antagonized rapidly by intramuscular injections of physostigmine at 15–60 μg/kg, but the antagonistic effect wore off after 3–4 h. Neostigmine had no immediate effect on performance in the Number Facility Test, but may have increased slightly the rate of spontaneous recovery 5–10 h after the intramuscular dose of atropine sulfate. With respect to the tachycardia induced by atropine, neostigmine may have exerted a prompter and more potent bradycardiac action than physostigmine. Physostigmine also antagonized the decrement in performance on the Number Facility Test induced by scopolamine. The organophosphorus anticholinesterase compounds, DFP and Sarin, had some antagonistic effect against the decrement in performance in the Number Facility Test induced by scopolamine, but these effects were less striking, although more persistent, than those of physostigmine. Tetrahydroaminoacridine, also an inhibitor of cholinesterases, had some antagonistic activity against the decrease in performance in the Number Facility Test due to scopolamine. Intramuscular injection of chlorpromazine after a small dose of scopolamine hydrobromide (8 μg/kg) increased the decrement in performance in the Number Facility Test due to the scopolamine. When 50 mg of chlorpromazine was injected intramuscularly after intramuscular scopolamine hydrobromide at 8 μg/kg, the resulting decrease in the score in the Number Facility Test approached that induced by scopolamine hydrobromide at 24 μg/kg alone, although chlorpromazine by itself induced only a slight decrease in performance. Perphenazine, another derivative of phenothiazine, had effects similar to those of chlorpromazine.

Sidell (167) reported that absorption of atropine from an intramuscular site of injection in man was prolonged when it was mixed with a 30% solution, but not when mixed with an 18% solution, of pralidoxime chloride. Because absorption of atropine was also delayed when it was mixed with an 8.5% solution of sodium chloride, Sidell suggested that the delayed absorption with 30% pralidoxime chloride may have been due to osmotic effects, rather than to any specific effect of the oxime. However, as pointed out by Sidell, although absorption of atropine from the mixture with 8.5% sodium chloride was delayed, eventually this mixture caused the same mean increase in heart rate in the six volunteer subjects as atropine alone at the same time after injection. In contrast, neither mixture of atropine with pralidoxime chloride accomplished this; the mixture of atropine with the 18% solution came closer than the mixture with the 30% solution to achieving the same increase in mean heart rate as atropine alone. Therefore, pralidoxime may have had a dose-related effect on the total absorption of atropine. Whether this is due to a simple osmotic effect on the absorption of atropine, with some of the alkaloid held at the site of injection by unabsorbed pralidoxime chloride in solution, or to some more specific effect of the oxime is not clear.

Sidell (168) studied the effectiveness of intravenous administration of physostigmine as an antagonist of the central (Number Facility Test) and peripheral (heart rate) actions of atropine and scopolamine. The report simply reiterated the effectiveness of physostigmine as an antagonist of the central actions of these two alkaloids and demonstrated that repetition of administration of physostigmine after disappearance of the effect of a first dose renewed the antagonism of persistent central actions of the alkaloids, but, like the first dose of physostigmine, had only a minor effect on the tachycardia due to the alkaloids.

SUMMARY

This review of the general literature portrays the tropic acid esters with which this report is concerned as compounds that are not particularly lethal in single or repeated doses. The members of the group that are tertiary amines may affect psychic activity in comparatively small doses, but there is fairly rapid adaptation to repeated doses. Removal of the compounds from the body, so far as it has been studied, seems to be comparatively rapid and to occur by a combination of urinary and fecal excretion and metabolic transformation. The

transport of intact atropine through the tubular epithelium of the chicken is by the cation transport mechanism and is reduced by drugs and chemicals that inhibit that transport process: quinine, choline, cocaine, and cyanine dyes (146).

Symptoms and signs of the action of the tropic acid esters on human subjects include complaints of mouth dryness and decreased secretion of saliva; increase in heart rate, possibly preceded by a short period of decrease in heart rate; dryness and flushing of the skin, particularly in the “blush area”; difficult urination; ataxia; disorientation; hallucination; delirium; dilated pupils, possibly nonreactive to light; prostration; and coma.

The greatest hazard to life arises from suppression of the ability to secrete sweat, which can give rise to fatal hyperthermia if body temperature is not controlled artificially during hot weather or strenuous activity. Another source of hazard to life arises from the effect of the compounds other than scopolamine and its quaternary form in accelerating heart rate and facilitating intramural conduction and transmission of impulses. These actions may result in serious arrhythmias up to and including ventricular fibrillation. The quaternary amine forms of the tropic acid esters in which we are interested are more active in some respects than the tertiary amines, as far as peripheral actions are concerned. This is especially true with respect to actions on nicotinic effectors in ganglia and striated muscles. The quaternary amines penetrate into the central nervous system poorly, but, once there, affect muscarinic effectors in the same way as the tertiary amines.

The anticholinergic compounds that are esters of tropic acid are not known to have any important mutagenic, teratogenic, or carcinogenic actions. There has been little study of long-term toxicity of these compounds, however, so one cannot say categorically that they have no carcinogenic activity. The rapidity with which atropine is removed from the body, although it cannot be taken as proof of noncarcinogenicity, does militate against a high probability of such an action by this compound.

ESTERS OF BENZILIC ACID

The master file of the Board on Toxicology and Environmental Health Hazards lists four compounds that are esters of benzilic acid. One of these compounds seems to be an isomer of another—both are benzilic acid esters of tropine. Of the 12 reports that concern administration of compounds of this group to volunteers, either at Edgewood Arsenal or at contractors' facilities, 11 are related to the results of studies with quinuclidinyl benzilate and three with tropinyl benzilate (these three reports are concerned exclusively with the L isomer of the ester). Accordingly, the literature review in this section centers on these two compounds insofar as possible, and especially on quinuclidinyl benzilate.

The first compound made in this group of esters of benzilic acid seems to have been N-diethylaminoethyl benzilate (benactyzine), whose synthesis was reported in 1938 by

Horenstein and Pahlicke (169). The synthesis of tropinyl benzilate was described in 1952 by Hromatka et a1. (170). In 1952, Sternbach and Kaiser (171) reported methods for producing several bicyclic alcohols, including 3-quinuclidinol. These alcohols were then used to produce esters with organic acids (172), including benzilic acid. The esters were examined for their ability to induce relaxation of isolated rabbit intestine that was made to contract by acetylcholine; the esters of 3-quinuclidinol were found to have greater spasmolytic activity than the corresponding esters of diethylaminoethanol and other bicyclic alcohols. The 3-quinuclidinyl ester of diphenyl acetic acid was prepared in its L and D forms. The levorotatory ester was found to have most of the activity, the dextrorotatory ester having only very slight activity. The toxicities of the isomers were stated to have been identical, but no data on toxicity were reported. A paper by Randall et al. (173) gave the intravenous LD_{50}s for the mouse of the L isomer as 29.5 mg/kg and of the D isomer as 27 mg/kg.

The synthesis of esters of 2-tropanol was described by Archer and Bell (174) in 1961. In 1971, Atkinson and McRitchie (175) reported methods for synthesizing all four of the isomers of 2-tropanol. The chemical literature seems not to reveal when the production of L-2-tropinyl benzilate actually occurred, but it was probably in 1971–1972.

Randall et al. (173) assessed the potencies of 3-quinuclidinyl benzilate hydrochloride and N-methyl-3-quinuclidinium benzilate bromide in several different activities. The LD_{50}s of 3-quinuclidinyl benzilate for the mouse were about 1.46 and 2.04 times those of the quaternized compound after intravenous and intraperitoneal injection, respectively. In producing relaxation of isolated intestinal smooth muscle stimulated by acetylcholine, 3-quinuclidinyl benzilate was somewhat more effective than atropine. The 3-quinuclidinyl benzilate had some activity in relaxing spasm of intestinal smooth muscle induced by histamine and was quite effective in antagonizing that induced by barium. It was also effective in preventing contraction by bronchial smooth muscle on exposure to aerosolized methacholine and in overcoming the bradycardia and hypotension induced by injection of acetylcholine or by electric stimulation of the vagal nerves. A dose of 2 mg/kg prevented the response of the cat nictitating membrane to electric stimulation of the cervical sympathetic nerve.

The chemist who made 3-quinuclidinyl benzilate took about 0.5 mg of the compound and experienced not only dryness of the mouth and mydriasis, but also a sensation of weakness in the knees. Despite having received several subcutaneous doses of neostigmine, he slept fitfully that night and spoke incoherently during waking periods. On the next morning, he was still unsteady on his feet, and his pupils were dilated. By the second morning after he had taken the compound, all symptoms except those due to persistent mydriasis had disappeared. He revealed that he had felt confused and that time had seemed to pass very slowly. Two other employees involved in work with 3-quinuclidinyl benzilate had similar illnesses, with apparent hallucination in one patient (176).

In addition to those three occupational exposures, two of six patients given 0.1 mg of 3-quinuclidinyl benzilate three times a day as a treatment for parkinsonism had nocturnal confusion (176).

STUDIES IN ANIMALS

In an attempt to explain these confusional states, Schallek and Smith (177) studied the effects of five spasmolytic substances, including 3-quinuclidinyl benzilate and its quaternized form, on the electroencephalogram, the electrocardiogram, and the blood pressure of the dog. Intravenous injection of 3-quinuclidinyl benzilate at 0.01 mg/kg decreased the dominant frequency of the EEG of two of five dogs; a dose of 0.1 mg/kg enlarged to three the number of dogs in which the dominant frequency of the EEG was decreased. Larger doses, up to 10 mg/kg, did not further enlarge the proportion of dogs with a decreased dominant frequency. The dose of 0.1 mg/kg induced bradycardia in one of five dogs. A dose of 10 mg/kg induced bradycardia in all five dogs and resulted in death due to cardiac arrest in two of five.

The only dose of quaternized 3-quinuclidinyl benzilate that induced any alteration in the EEG was 10 mg/kg, which resulted in a decrease in the dominant frequency in the EEG of one of five dogs. This dose also induced bradycardia in three of five animals and hypotension in all five. No dogs given this dose of N-methyl-3-quinuclidinyl benzilate bromide died. Intravenous doses of 0.1 and 1.0 mg/kg caused tachycardia in one of five and two of five dogs, respectively.

Before proceeding with a discussion of the effects of 3-quinuclidinyl benzilate, it may be worth while to look at the available information on the lethality of single doses of the benzilic acid esters with which we are concerned. Table I–2 is based on information supplied by Hoffman-LaRoche, Inc., the Sterling-Winthrop Research Institute, and a number of investigators (36,173,176,178–189).

The benzilic acid esters of both diethylaminoethanol (179, 180,185) and tropine (182,183) were found to be capable of overcoming spasms of smooth muscle induced by acetylcholine and of inducing relaxation of smooth muscle, such as the circular muscle of the iris. Both compounds had some local anesthetic activity, both produced changes in the EEGs of experimental animals similar to those induced by atropine, and both had slight antihistaminic activity. Lisunkin (187) reported that tropinyl benzilate increased the effectiveness of other antispasmodic drugs, such as tropinyl diphenylacetate (tropacine), in antagonizing the tremor induced by subcutaneous nicotine.

Larsen (181) fed rats diets containing either 0.01% or 0.05% of the benzilic acid ester of diethylaminoethanol from 28 d of age until spontaneous death. Another group of rats was fed the basal diet. The rats fed benzilic acid ester gained weight slightly faster than those fed the normal diet, but died younger than the controls (means of 24 and 26 mo, compared with one of over 29.5 mo). No striking pathemas in the animals due to the benzilic acid ester were reported.

3-QUINUCLIDINYL BENZILATE (BZ, EA 2277)

McNamara (184) reported that rats placed into atmospheres containing 3-quinuclidinyl benzilate dispersed as a dust, as a thermally generated smoke, or as an aerosol of a 1% solution of the benzilic acid ester in methylene dichloride for concentration-time (Ct) products up to 29,508 mg.min/m^3 experienced no permanent effects from the exposures, except occasional deaths randomly distributed with respect to intensity of exposure. One of four guinea pigs exposed to a Ct product of 10,123 mg.min/m3 and two of four guinea pigs exposed to a Ct product of 14,748 mg.min/m^3 died. Mice exposed to an aerosolized 1% solution of the benzilic acid ester in methylene dichloride at Ct products up to 14,137 mg.min/m^3 did not experience any clearly dose-related mortality, but 69 of 70 mice exposed to thermally generated smoke of 3-quinuclidinyl benzilate at Ct products of 8,213–9,331 mg.min/m^3 died.

McNamara (184) reported that daily subcutaneous doses of 3-quinuclidinyl benzilate at up to 150 µg/kg on 5 days of each week for 3 wk induced no evident signs of toxicity in mice and guinea pigs. Dogs given daily intravenous doses of 100 µg/kg for 14 consecutive days had the same LD_{50} as dogs that had not been pretreated, but the time between injection of the daily dose and the appearance of ataxia increased from 4 min to 14 min during the period of pretreatment. In dogs trained in a conditioned-escape routine and then given graded intravenous doses of 3-quinuclidinyl benzilate at up to 12.5 µg/kg, there was no effect on performance. A dose of 25 µg/kg resulted in failure to escape by four of four dogs.

The same report (184) summarized data on the effect of intraperitoneally injected 3-quinuclidinyl benzilate on spontaneous motor activity of the rat. When the data were plotted and the curve extrapolated to zero change in spontaneous activity, the maximal dose that could be given without altering spontaneous activity seemed to be about 0.12 mg/kg. No rats were given daily injections of saline, however, so a decision on what portion of the observed changes was attributable to the compound, rather than to the procedure, is not possible.

Studies of the absorption of 3-quinuclidinyl benzilate from solutions applied in a mixture of alcohol and cresol to the clipped skin of five species of animals, summarized in the same report (184), found that doses of up to 500 µg/kg produced ataxia in the cat after about 30 min and in the dog after about 55 min. No ataxia was produced in the goat or the monkey (probably rhesus) by the highest dose; the highest dose applied to the skin of the rabbit was 100 µg/kg. Doses of 50 µg/kg applied to the skin of the rabbit, of 100 ug/kg applied to the skin of the cat, of 250 µg/kg applied to the skin of the monkey, and of 500 µg/kg applied to the skin of the dog and the goat were the lowest doses found to have effects on the eyes. In all species except the monkey, recovery from the ocular effects was stated to occur overnight. In the monkey, the ocular effects apparently persisted for 2 d or more.

In the dog, an intravenous dose of 10 μg/kg produced a mean increase in four dogs of about 143% in heart rate, which decreased slowly during an observation period of up to 6 h, but was still evident at the end of that period. By 24 h after the dose, the heart rate was back to normal. A dose of 12.5 μg/kg, but not one of half that amount, decreased by 20% the mean time during which dogs would run on a treadmill. None of the experiments summarized in this report (184) seems to have involved any persistent effect of exposure to 3-quinuclidinyl benzilate in surviving animals. Neither did the report of Wal (186) mention any consistent toxic effects of this compound.

Ketchum et al. (189) reported that rats and dogs with whole-body exposures and rabbits and monkeys with head-only exposures to clouds of 3-quinuclidinyl benzilate dispersed in the field at wind speeds of 8–11 mph developed such effects as ataxia, dyspnea, mydriasis, cycloplegia, tachycardia, sedation, hyperactivity, and nasal stuffiness. The most persistent of these effects was cycloplegia, which persisted in three of five monkeys for more than 7 d. Most other effects disappeared within 48 h after exposures.

No study of long-term administration of 3-quinuclidinyl benzilate to experimental animals has been found. The closest approaches to such a study are 1-yr studies of the results of feeding the methyl bromide of 3-quinuclidinyl benzilate to rats in the diet (190) and of gavaging dogs 5 d/wk with the same quaternary amine salt of 3-quinuclidinyl benzilate (191). Rats were fed diets containing the quaternary amine salt at concentrations calculated to yield daily doses of zero, 5, 25, and 50 mg/kg. Dogs were gavaged with the quaternary salt at zero, 1, 5, and 25 mg/kg. In both experiments, no evidence of dose-related toxicity was seen. The livers of the male dogs that had received daily doses of 5 and 25 mg/kg seemed to be larger than those of the controls (3.0% and 3.2% of body weight, respectively, versus 2.7% for the controls), but were normal in structure on necropsy and in function (glutamic-pyruvic transaminase and alkaline phosphatase activities in serum and Bromsulphalein retention within normal limits). Although the compound used in these two studies was not 3-quinuclidinyl benzilate and probably was absorbed from the intestinal tract less rapidly and less completely than that compound, it contained the same combinations and arrangements of atoms. Therefore, the negative findings with respect to organ and tissue damage in the two studies may indicate that similar experiments with 3-quinuclidinyl benzilate would result in no chemically induced pathemas.

The great avidity of mitochondria from rat brain for 3-quinuclidinyl benzilate has been mentioned previously (56). Larsson et al. (192) recorded the infrared spectra of seven esters of benzilic acid, of eight esters of 3-quinuclidinol, and of atropine and scopolamine and attempted to correlate the relative strengths of the intramolecular hydrogen bond with the threshold doses of the various compounds in producing psychotomimetic effects in dogs. The data of Albanus (42) on the psychotomimetic activities of anticholinergic compounds were used for the comparison. Of the esters of benzilic acid, the most active was 3-quinuclidinyl benzilate. According to Albanus's data, it was 50 times as active as tropinyl benzilate. Four other esters of 3-quinuclidinol were as active as 3-quinuclidinyl benzilate, but none was more active. Larsson et al. were not able to detect any correlation between the strength of the hydrogen bond, as derived from their spectra, and the biologic activities of the compounds estimated by Albanus.

Dogs given subcutaneous doses of tropinyl benzilate at 0.50 mg/kg became ataxic after a mean elapsed time of 16 min and obstinately progressive after a mean of 19 min; ataxia disappeared after a mean elapsed time greater than 283 min, whereas obstinate progression lasted only a mean of 244 min. Dogs given doses of 3-quinuclidinyl benzilate at 50 μg/kg became ataxic after a mean of 36 min and became obstinately progressive after a mean of 42 min. These two effects disappeared at mean times of greater than 313 and greater than 305 min, respectively. Tropinyl benzilate in a dose 10 times that of 3-quinuclidinyl benzilate produced toxic effects somewhat more rapidly than the latter, but these effects were less durable than those of the smaller dose of 3-quinuclidinyl benzilate.

Lavallee (50) reported that doses of 3-quinuclidinyl benzilate at 10 and 18 μg/kg had no progressive effects on performance of a multiple-stimulus conditioned-avoidance response by dogs, but doses of 24 and 32 μg/kg decreased the number of correct responses progressively. Monkeys given doses of 32 and 56 μg/kg may have made a few more errors in a visual-discrimination avoidance task than monkeys given no compound, but the difference was not statistically significant in the absence of control data.

Cats given intravenously injections of 3-quinuclidinyl benzilate labeled with ^{3}H in the 3-position of quinuclidinol were used to determine the distribution of the label in the brain and brain stem (193). Taking 304 counts/min.mg in the nervous tissue as the halfway point between the highest and the lowest concentrations measured, the areas of the brain that retained the greatest concentrations of the label were motor cortex, sensory cortex, caudate nucleus, lateral geniculate, and medial geniculate (167,140,121,107, and 101 counts/min.mg, respectively). The areas that retained smaller concentrations, in order of decreasing concentration of the label, were thalamus, hippocampus, hypothalamus, medulla oblongata, colliculi, cerebellar cortex, pyramids of the medulla, cerebral white matter, and cerebellar white matter.

Both cholinomimetic and anticholinergic compounds were found (193) to reduce the retention of the label in some areas of the brain, the most strongly affected areas varying with the compound. The cholinomimetic compounds used were tetrahydroaminoacridine (THA) and S-diethylaminoethyldiethylphosphorothioate (TETRAM), both inhibitors of cholinesterases. The anticholinergic compounds used were N-methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate (EA 3443) and 302,028 (not identified except as an analogue of 3-quinuclidinyl benzilate). The last compound had a much more general action than any of the others used, displacing the label of 3-quinuclidinyl benzilate from most of the sampled areas of brain and brain stem. In the cases of both EA 3443 and 302,028, displacement of the label was related probably to the substitution of one intoxicant by another, so that neither of these two anticholinergic substances would be practical antagonists of intoxication induced by 3-quinuclidinyl benzilate. Antagonism might reasonably be expected from THA, which partly displaced the label from motor cortex, lateral geniculate, medulla, and cerebellar cortex; antidotal activity by TETRAM—which displaced the label from lateral geniculate, thalamus, and hippocampus—is less likely. No attempts to assess antidotal effectiveness were made in this study.

Zvirblis and Kondritzer (56) found that a mitochondrial fraction prepared from rat brain adsorbed 3–4 times as much 3-quinuclidinyl benzilate as atropine in the physiologic range of pH. The optimal pH for adsorption of 3-quinuclidinyl benzilate was about 8.0, whereas that for atropine was about 9.7; at these pHs, the adsorption of the two anticholinergic compounds was about the same. THA decreased the adsorption of the benzilate by mitochondria, but a ratio of 100:1 (THA:3-quinuclidinyl benzilate) was required to reduce the adsorption by 50%. Both Ca^{2+} and PO_4^{2-} decreased the mitochondrial adsorption of 3-quinuclidinyl benzilate, the effect of Ca^{2+} not being particularly large and requiring an estimated ratio of 30,000:1 (Ca^{2+}:3-quinuclidinyl benzilate) to decrease adsorption of the benzilate by 50%. With phosphate, a ratio of about 21.5:1 (PO_4^{2-}:3-quinuclidinyl benzilate) would be expected to decrease adsorption of the benzilate by 50%.

Yamamura et al. (194) studied the adsorption of ^3H on particles in homogenates of various regions of monkey brain after incubation with [^3H] 3-quinuclidinyl benzilate in solution in phosphate buffer at a pH of 7.4. The regions that were the most avid adsorbers of the benzilate, in order of decreasing uptake, were putamen, caudate nucleus, occipital cortex, cingulate gyrus, postcentral gyrus, hippocampus, amygdala, precentral gyrus, pyriform cortex, frontal cortex, superior colliculi, thalamus, and inferior colliculi. This distribution of adsorptive sites among various parts of monkey brain differs from that for uptake of ^3H from intravenously injected [^3H]3-quinuclidinyl benzilate by cat brain (193). The two studies are in agreement that the caudate nucleus is one of the most important areas of localization of the label in the form of [^3H]3-quinuclidinyl benzilate. Yamamura et al. found that uptake of the label by various areas of brain had a tendency to be correlated with choline uptake and with choline acetylase activity.

Yamamura and Snyder (195) found that interruption of the septal hippocampal tract, and elimination thereby of cholinergic afferents to the hippocampus, reduced by about 70% the activity of choline acetylase in homogenates of the hippocampus, but did not alter the binding of [^3H] 3-quinuclidinyl benzilate by the particles of such homogenates. They proposed, as a possible explanation, that presynaptic muscarinic sites that are innervated by the septal hippocampal nerve fibers do not adsorb the labeled benzilate, but that postsynaptic muscarinic sites just across the synaptic gap from the presynaptic ones do adsorb it. Another possibility mentioned is that there may be no presynaptic muscarinic sites in the hippocampus.

The ATPase in microsomes prepared from whole rat brain, from rat cerebral cortex, and from cerebral cortex of rats that had received 3-quinuclidinyl benzilate intraperitoneally at 1 mg/kg an hour before they were killed was found (196) to be enzymatically competent in all cases and not to be inhibited significantly by the addition of 3-quinuclidinyl benzilate in vitro. Furthermore, slices of guinea pig brain cortex incubated in vitro with 10^{-4} M 3-quinuclidinyl benzilate had the same patterns of loss of K^+ and uptake of Na^+ as slices incubated without the benzilate. Inasmuch as ATPase is related intimately to the active transport of the monovalent cation in nervous tissue, the experiment with the brain slices gave an additional indication that ATPase activity is not altered by 3-quinuclidinyl benzilate.

Jovic and Zupanc (197) measured the effects of 3-quinuclidinyl benzilate on oxygen consumption by rats and oxygen uptake by slices of rat cerebral cortex and medulla oblongata. Subcutaneous injections of the benzilate at 4–15 mg/kg decreased oxygen consumption by a mean of 12.1%; there was no clear dose-effect relationship. Rats whose oxygen consumption had been increased by a mean of 33.5% by a small dose of soman (25 ug injected subcutaneously) had the increase limited to about 2% by a subcutaneous dose of 3-quinuclidinyl benzilate at 15 mg/kg.

Slices of cerebral cortex and medulla oblongata stimulated by KCl at a final concentration of 100 mM increased their oxygen uptake by respective means of 59.2% and 41.7% (197); inclusion of 5×10^{-4} M 3-quinuclidinyl benzilate in the medium bathing the slices reduced the stimulated oxygen uptake to 16.3% for cerebral cortex and to 3.6% for medulla oblongata. When rats were given 3-quinuclidinyl benzilate subcutaneously at 5–20 mg/kg and were killed 1 h later for preparation of slices of cortex and medulla oblongata, there were dose-related decreases in oxygen uptake by both tissues stimulated by inclusion of KCl in the fluid surrounding the slices.

Frances and Jacob (81) reported that intraperitoneal injections of 3-quinuclidinyl benzilate into mice at 0.3–3.0 mg/kg decreased the concentration of acetylcholine that could be measured in the brain. There was a linear relation between the decrease in acetylcholine concentration and the logarithm of the dose of benzilate. The line for this relationship with 3-quinuclidinyl benzilate was close to and nearly parallel with that with scopolamine.

Aquilonius et al. (77) found that intravenous 3-quinuclidinyl benzilate at 50 µg/kg produced about the same loss of acetylcholine from the rat cerebral cortex as atropine at 750 µg/kg. Intravenous 3-quinuclidinyl benzilate at 0.15, 0.2, and 0.5 mg/kg produced greater initial increases in rat locomotor activity than atropine at 1.3, 2, and 10 mg/kg. The immediate responses to the benzilate were not so well maintained as those to atropine. As the benzilate dose was increased from 0.1 to 0.2 mg/kg, locomotor activity increased rapidly to an almost maximal value that was about 9.3 times the control value.

The concentration of acetylcholine in rat hippocampal tissue was increased slightly (10.3%) by intraperitoneal 3-quinuclidinyl benzilate at 0.1 mg/kg (79); after a dose of 5 mg/kg, the acetylcholine concentration was reduced by 44.5%, compared with the mean concentration found in control rats. When septal lesions were created, the mean concentration of acetylcholine in the hippocampus increased by about 47.4%. As in intact rats, administration of 3-quinuclidinyl benzilate at 0.1 mg/kg increased the mean concentration of acetylcholine in the hippocampus, in this case by 5.3%. Unlike the result in intact rats, administration of 5 mg/kg resulted in a further increase in the mean concentration of acetylcholine in the hippocampus, to 16.6% above that in control rats. This last result was the converse of that found with atropine, but was similar to that found with scopolamine.

Meyerhöffer (198) found that L-3-quinuclidinyl benzilate was about 20 times as active as the Disomer in dogs in producing ataxia, tachycardia, and obstinate progression and in reducing salivation.

Kabes et al. (199) reported that rats given doses of 0.8, 2.0, and 5.0 mg/kg of 3-quinuclidinyl benzilate (by an unidentified route) had dose-related increases in spontaneous motor activity. Physostigmine (0.2 mg/kg), amobarbital (20 mg/kg), and chlorpromazine (2 mg/kg), all given by unidentified routes before the benzilate, held the spontaneous motor activity even below the control values.

Kabes (200) examined the effects of 3-quinuclidinyl benzilate on the abilities of rats to learn a conditioned-escape task and, once trained, on performance of the task. Doses of 0.5, 1, and 2 mg/kg of the benzilate hastened acquisition of the conditioned response in a dose-related manner, but a dose of 1 mg/kg had different effects on performance of the task in relation to the proficiency of the animal in its performance before the benzilate was administered. Rats that were poor performers (more than 20% errors in control sessions) and some that were good performers (less than 20% errors in control sessions) had their performance improved by the benzilate, whereas other good performers had their performances worsened.

Kabes (201) examined the effects of 3-quinuclidinyl benzilate on beagles. There were dose-related responses to doses of 10 and 50 µg/kg in autonomic functions (heart rate, pupil diameter, flow of saliva, etc.), motor functions (motor coordination, tremor, ataxia, etc.), and central nervous system functions (alertness, orientation, reaction to external stimuli, etc.). Physostigmine (1 mg/kg) aborted all these types of action. Routes of administration were not stated; physostigmine was said to have been injected, but the site was not stated.

Fusek et al. (202) used isolated rat jejunum and atria to examine the effects of 3-quinuclidinyl benzilate on tissue response to cholinergic agonists. The benzilate alone had no effect on isolated jejunum, but, when applied before a stimulant concentration of furtrethonium (10^{-7}–10^{-4} M), it shifted the dose-response curve for furtrethonium to the right, i.e., a higher concentration of furtrethonium was required to produce a given response in the presence of 3-quinuclidinyl benzilate than in its absence.

The application of 3-quinuclidinyl benzilate at a range of concentrations ($3^{-30}\times10^{-9}$ M) to isolated atria had no effect on the contractions of either spontaneously beating or electrically driven atria (202). When the atria were subjected to the influences of both furtrethonium and 3-quinuclidinyl benzilate, the dose-effect curve for the former was shifted to the right. This was similar to the result with isolated jejunum, the molar ratio of furtrethonium to 3-quinuclidinyl benzilate being about 3.3×10^{6}:1.

Herink et al. (82) injected 3-quinuclidinyl benzilate at 1 mg/kg into the peritoneal cavities of normal rats and rats with electrolytic septal lesions. In normal rats, there was no significant effect on aggressiveness, but defecation was decreased. In the rats with septal lesions, both aggressiveness and defecation were reduced by 3-quinuclidinyl benzilate (the latter being affected to the greater extent).

Poel (203) trained female rats to run in a circular runway for a reward of a drop of chocolate paste. After completion of training, the rats were given intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 0.3, 1, 3, or 10 mg/kg and were placed in the runway 20 min later. The benzilate induced a marked increase in the time required for the rat to leave one reward area and begin to seek a second reward (latency); the increase in latency was dose-related. There was a dose-related increase in the time required for the rat to traverse the circular runway (running time), but the slope of the regression line for the relationship of running time to the dose of 3-quinuclidinyl benzilate was much smaller than that for the relationship of latency to dose. These results were interpreted as indicating that the benzilate intensified a conflict between a desire to remain in the vicinity of a prior reward and a desire to obtain a later reward, coupled with some interference with motor function similar to that found by Meyerhöffer (198) and Kabes et al. (199,200).

Bell and Gershon (86) found that intravenous injection of 3-quinuclidinyl benzilate at 50 ug/kg into dogs induced intoxication that persisted for more than 5 h. Intravenous injection of 1,2,3,4-tetrahydro-9-aminoacridine (Tacrine or THA) at 1 mg/kg within 30–50 min after administration of the benzilate induced recovery in only two of nine dogs, but similar treatment 2–3 h after the benzilate had been injected was successfully antidotal in seven of seven dogs. Yohimbine, which has cholinomimetic and sympatholytic actions (204), injected intravenously at 0.5 mg/kg within 30–50 min after the benzilate had antagonistic activity in four of nine dogs. When yohimbine was not administered until 2–3 h after the benzilate, it antagonized the intoxication in only two of seven dogs. Piperoxan, also an adrenergic blocking agent, was mentioned as being capable of antagonizing intoxication with 3-quinuclidinyl benzilate, but no details of study of its action were given.

Fusek et al. (205) reported that Tacrine inhibited both acetylcholinesterase and butyrylcholinesterase in dog lungs and heart and slightly inhibited acetylcholinesterase in the brain, especially in basal ganglia. Most of the change in the acetylcholinesterase activity of the brain was found to be in the microsomal fraction. Butyrylcholinesterase in the bodies of ganglion cells was said to be completely inhibited in all regions of the brain. These authors found that an intramuscular dose of tacrine at 10 mg/kg completely abolished within 2 h the signs of intoxication induced by an intramuscular injection of 3-quinuclidinyl benzilate at 50

Sram et al. (206) reported the results of cytogenetic analyses of spermatozoa from mice that had been given intraperitoneal 3-quinuclidinyl benzilate in single injections at 40 mg/kg or 15 daily doses at 10 mg/kg , of bone marrow cells from mice that had received 15 daily doses at 2 or 10 mg/kg , and of human lymphocytes that had been exposed in vitro at 10^{-4}–10^{-3} M. They looked also for reverse mutations in yeast cells exposed in vitro at 5×10^{-4} M and 5×10^{-2} M, for host-mediated transformations in yeast cells injected into the peritoneal cavities of mice that were then given 50 or 100 mg/kg subcutaneously, and for dominant-lethal effects in mice after single intraperitoneal injections at 10 or 40 mg/kg or repeated injections—15 at 2 mg/kg, 15 at 10 mg/kg, or 5 at 20 mg / kg. The yeast cells exposed to 3-quinuclidinyl benzilate in vitro gave evidence of weak mutagenic activity. The only other positive results were in bone marrow cells of mice given doses of 10 mg/kg or more. Sram et al. (206) concluded that, because the frequency of chromosomal breaks was not dose-related above the threshold dose for the occurrence of breaks, the breaks were probably due to systemic toxic activity by the benzilate, rather than to any specific mutagenic effect, and that the compound has no serious genetic risk for man.

In another paper, Sram (207) examined the production of chromosomal abnormalities in bone marrow from male Chinese hamsters given single intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 1, 10, 20, 40, or 60 mg/kg, dissolved in dimethyl sulfoxide. Control hamsters received dimethyl sulfoxide alone. Doses of 1 mg/kg or more induced more chromosomal gaps and breaks per cell than the solvent. Doses of 1–40 mg/kg yielded apparently dose-related increases in the incidence of chromosomal abnormalities: 0.012 per cell with 1 mg/kg, 0.064 per cell with 10 mg/kg, 0.224 per cell with 20 mg/kg, and 0.292 per cell with 40 mg/kg. The highest dose, 60 mg/kg, produced only 0.076 per cell. Sram considered, on the basis of the count with the highest dose, that the incidence of chromosomal gaps and breaks was not dose-related, but that is questionable for the doses between 1 and 40 mg/kg. However, as Sram pointed out, chromosomal gaps and breaks seem to be evidence of toxic damage to the individual, rather than of heritable change, so they probably do not indicate a serious genetic risk for man.

Hughes et al. (208) gave 10 beagles intravenous 3-quinuclidinyl benzilate at 3 mg/kg twice a week for 3 wk. Five control dogs were given 0.1 N HCl, corresponding in volume and pH to the solution of benzilate at 0.1 ml/kg. Some animals from each group were killed 72 h after the last dose of benzilate, and the others were killed 60 d after the last dose. No lesions attributable to benzilate were found in the testes of the dogs that had been given that compound.

DIETHYLAMINOETHYL BENZILATE

The only other member of the group of esters of benzilic acid on whose effects there is a considerable body of information is diethylaminoethyl benzilate. One review of its pharmacology has been mentioned previously (181); it concerned the general pharmacology of the drug as a peripherally effective muscarinic blocking agent. In addition to having cholinergic spasmolytic activity, diethylaminoethyl benzilate also has mydriatic effectiveness, is able to antagonize barium-induced contraction of intestinal smooth muscle, has local anesthetic properties, and has an antiarrhythmic action on the heart similar to that of quinidine. In 1955, a series of papers established that diethylaminoethyl benzilate affects the functioning of the nervous system in the cat (209), the rat (210), and man (211).

Berger et al. (212) studied the toxicity of single doses of diethylaminoethyl benzilate in mice and monkeys and its general actions on several functional systems. In the monkey, an intravenous dose of 1 mg/kg resulted in mydriasis and some decrease in voluntary movement. A dose of 2 mg/kg induced ataxia with occasional convulsive jerks. A dose of 6 mg/kg induced intermittent clonic convulsions in one monkey; the convulsive state lasted for about 10 min and was followed by a depressed state lasting for over an hour. Pupillary dilatation, the effect of longest duration, sometimes persisted for as long as 20 h.

Diethylaminoethyl benzilate injected intraperitoneally into mice with hexolbarbital at 100 mg/kg (212) prolonged anesthesia due to the hexolbarbital: benzilate at 10 mg/kg increased the duration of anesthesia by 35.9 min (124%) and at 20 mg/kg increased it by 68.3 min (236%). When diethylaminoethyl benzilate was administered to mice by mouth at 24 mg/kg and the mice were then subjected to what was usually a nonlethal convulsigenic electric shock, 50% of the mice died. The only antimuscarinic effect detectable in these mice was mydriasis. This dose of diethylaminoethyl benzilate did not prolong the latency to onset of convulsion after delivery of the electric shock; a larger dose, 37 mg/kg, did prolong the latency significantly.

When different agonists (acetylcholine, 5-hydroxytryptamine, and histamine) were used (212) to stimulate the smooth muscle in isolated rat colon, diethylaminoethyl benzilate had some ability to antagonize the stimulant action of all three agonists. Its ability to block the action of acetylcholine was about 20 and 100 times its activity against the stimulant actions of 5-hydroxytryptamine and histamine, respectively. Diethylaminoethyl benzilate did not increase the urinary excretion by the rat of 5-hydroxyindoleacetic acid, but did magnify the pressor action of epinephrine and reduced its LD_{50}. The compound appears, therefore, not to affect the metabolism of serotonin, but to alter in some way the relation between epinephrine and its receptors.

Diethylaminoethyl benzilate had no significant effect on the knee-jerk or flexor reflex in the cat (212); when injected into curarized cats, it altered the predominant frequency of the EEG from 40 per second to 8–15 per second and induced enlarged discharges. The changes in the potentials recorded from the brain were seen in records from both cortical and subcortical regions. Electric arousal consequent to peripheral or thalamic stimulation of cerebral afferent fibers was blocked by diethylaminoethyl benzilate, but recruitment of cortical neurons by stimulation of the thalamus was not affected.

Giarman and Pepeu (75) reported in 1962 that diethylaminoethyl benzilate differed from atropine in that a dose equimolar with atropine at 50 mg/kg (which reduced the concentration of acetylcholine in rat brain by 33%) caused no significant change in the brain's concentration of acetylcholine. Both atropine and diethylaminoethyl benzilate were said to induce mild excitation, however. In contradiction of this report, Frances and Jacob (81) published a graph indicating that intraperitoneal atropine at 1 to 7 or 8 mg/kg had a greater effect in lowering the brain acetylcholine concentration in the mouse than doses of diethylaminoethyl benzilate in the same range, but that the benzilate at 25–30 mg/kg induced a greater lowering of the acetylcholine concentration than atropine.

McColl and Rice (213) found that intraperitoneal injection of mice with diethylaminoethyl benzilate at 10 mg/kg 15 min before similar administration of tremorine increased the ED_{50} of tremorine from 5.8 to 38.0 mg/kg. Such an effect can be explained as being due to depletion by the benzilate of the store of acetylcholine in the brain cells.

In 1964, Jacobsen published a review of the literature through 1962 on the actions of diethylaminoethyl benzilate (214). It reported that oral doses of this benzilate of up to 80 mg had been tolerated by man, but that daily doses of about 90 mg had given rise to mild arrhythmias and cyanosis. The compound had been found to enter the rat brain in unaltered form. When ^{14}C-labeled diethylaminoethyl benzilate had been administered to rats, 85% of the label had been recovered in the urine within 24 h. Nothing was known at that time about the nature of the metabolites. The compound had been found to inhibit oxidative phosphorylations in brain cells in vitro, and, at high concentrations (10^{-3} M), to inhibit monoamine oxidase. In man, doses of 40–200 mg of this benzilate had been found to reduce excretion of 5-hydroxyindoleacetic acid to zero. Thus, the compound interferes in some way with the utilization or metabolism of serotonin, and presumably with the function of serotonergic synapses. In addition to having peripheral antimuscarinic activity, graded by various investigators as between 0.01 and 0.33 that of atropine, diethylaminoethyl benzilate had been found to be particularly potent in blocking transmission through the ascending reticular system; it had also been found to affect the descending reticular system, but not to be useful in treating parkinsonism, because of its other central effects. Diethylaminoethyl benzilate inhibits the metabolism of barbiturates and potentiates their central depressant activity. In addition to inducing hyperactivity in mice and rats and reducing the reactions to stress in rats and cats, diethylaminoethyl benzilate had been found not to affect sham rage, to inhibit fighting between rats exposed to electric shocks, and to decrease the rate of learning of complicated tasks (tasks requiring discrimination between stimuli and the making of different responses to different stimuli) in mice, rats, and cats.

Berger et al. (80) reported that diethylaminoethyl benzilate was particularly effective in increasing the duration of after-discharges by the cat hippocampus after a weak, brief electric shock, being 40–100 times as active as atropine in this regard. As to its antispasmodic action in vitro and its ability to block salivation induced by intracranial injection of 80 ug of acetylcholine chloride into a mouse, diethylaminoethyl benzilate was 4% and 77%, respectively, as potent as atropine.

Shitov (215) found that benactyzine and two other anticholinergic compounds increased the action of magnesium sulfate on the EEG of the rabbit in proportion to the activity of the compounds in activating cholinesterase.

Banshchikov and Stoliarov (216) reviewed a group of substances that had been found to be capable of evoking mental disturbances. All the substances have marked anticholinergic activity, and diethylaminoethyl benzilate was among them. The others are Biel's compounds, JB-318, JB-329 (Ditran), and JB-336.

The authors (216) stated that usual medicinal doses of diethylaminoethyl benzilate not infrequently produce mental changes—retardation or stoppage of the current of thought, a feeling of emptiness, forgetfulness, shortened attention span, sensations of heaviness and altered shape of the limbs, apathy, sluggishness, impression of isolation from the environment, hostility, apprehension, horror, anxiety, and hypochondriasis. A relatively brief period of these effects might be followed by a period of euphoria if more than 5 mg of the benzilate had been taken.

The first report of a psychotomimetic episode due to diethylaminoethyl benzilate in a human being was credited to Vojtechovsky (217) by Banshchikov and Stoliarov (216). This investigator had published in 1958 a case report on a female physician who had accidentally taken about 1.4 g of the benzilate. The woman experienced visual hallucinations and brief delirium. Intentional administrations of diethylaminoethyl benzilate to human subjects had shown (218) that the psychotomimetic state lasted for 4–12 h and was accompanied by a decrease in urinary excretion of 5-hydroxyindoleacetic acid. These authors stated that diethylaminoethyl benzilate had been found to inhibit monoamine oxidase only slightly less strongly than iproniazid. As the psychotomimetic state waned, the urinary excretion of 5-hydroxyindoleacetic acid increased to such an extent that the 24-h excretion of this degradation product of serotonin was within normal limits. It had been found in the same experiment that the urinary excretion of 17-ketosteroids was markedly reduced during the period of psychosis and that the activity rebounded at about the same time as the urinary excretion of 5-hydroxyindoleacetic acid. In the case of the 17-ketosteroids, however, the rebound increased the total daily urinary excretion of 17-ketosteroids by nearly 50%.

Edelson et a1. (219) used [^{3}H]diethylaminoethyl benzilate to study the absorption, distribution, and metabolic fate of this benzilate in the rat. The substance used was randomly labeled. After intraperitoneal injection of benzilate at about 82.5 mg/kg, ^{3}H was detectable in the blood at a fairly high concentration about 12 min after injection; the blood concentration was highest about 36 min after injection. At 5 h after injection, the ^{3}H concentration was about 28% of its peak. The authors estimated the half-time of the label in the blood to be about 80 min. If one calculates (from data in the paper) the time required for removal of half the peak ^{3}H concentration from the blood, that time is found to be about 114 min after the injection. The authors' value of 80 min for the half-time would require the concentration of label in the blood 5 h after injection to be about 6.7% of the peak concentration, whereas it was actually about 28% of the peak. The estimate of 114 min is believed to be a better value for the half-time.

Within 24 h after injection, 44.8% of the label was recovered in the urine and 41.6% in the feces and the gastrointestinal tract and its contents. The carcass contained 14.2% of the label, of which the greatest amount was in the liver. The expired air contained about 0.3% of the ^{3}H, in the form of water. The urine contained the unchanged compound, ethylaminoethyl benzilate, and benzilic acid. Apparently, a considerable portion of the diethylaminoethyl benzilate had been hydrolyzed to release benzilic acid, which accounted for 54.4% of the label in the urine. A smaller portion of the original compound had been monodealkylated to ethylaminoethyl benzilate, which accounted for 13.8% of the label in the urine. This dealkylation may have contributed the small amount of the label found in the water of the expired air. The fate of the diethylaminoethanol that must have been released by hydrolysis of the original compound remains unknown. A study of the hydrolysis in vitro of the original benzilate at a pH of 7.4 during incubation at 37°C revealed that approximately 50% of the compound underwent spontaneous hydrolysis within 5 h.

Sram (207) gave Chinese hamsters diethylaminoethyl benzilate intraperitoneally at 0.1, 1, and 10 mg/kg. The animals were killed 24 h later, and the cells of their bone marrow were examined for chromosomal abnormalities. The lowest dose of the benzilate did not give rise to any detectable chromosomal abnormalities; 1 mg/kg resulted in a 50% increase in the occurrence of chromosomal gaps and breaks in the bone marrow cells. The highest dose (10 mg/kg) induced a 325% increase in the incidence of gaps and breaks in chromosomes of bone marrow cells. The same dose of 3-quinuclidinyl benzilate had resulted in only a 107% increase in the incidence of chromosomal gaps and breaks; thus, diethylaminoethyl benzilate, despite its lower anticholinergic activity, was more toxic cytogenetically than 3-quinuclidinyl benzilate.

EFFECTS ON MAN

Davies (220) summarized the effects of diethylaminoethyl benzilate at various doses on human beings. Moderate doses—1–4 mg—characteristically produced a sense of divorce between outer reality and emotional reactions. Larger doses—4–8 mg—produced a more distinct detachment: muscles were felt to be relaxed and limbs were sensed as awkward, enlarged appendages with blunted sensations. The ability to concentrate was felt to be diminished, and thought processes were slowed. Mental vacuity was common, and reaction to external stimuli was slow. Continuity in thinking was lost, so that people who had taken doses in this range frequently said something like: “I have forgotten what I wanted to say.” Complaints of mild palpitation and of dryness of the mouth might be made. The most common complaint among 110 patients treated with diethylaminoethyl benzilate was of a loss of ability to concentrate and a feeling of detachment from the people and objects in the immediate environment. All doses were given by mouth, the highest dose administered to this group of people being 16 mg/d for several months. No delayed effects or effects that persisted after benzilate administration was stopped were reported.

Raymond and Lucas (221) studied the effects of diethylaminoethyl benzilate on a group of 43 outpatients and 10 normal subjects. Patients given daily oral doses of the drug of 1 mg three times a day (t.i.d.) complained of no side effects. About half the patients who took 2 mg t.i.d. experienced side effects, particularly a sensation of limb heaviness. Most patients given 3 mg t.i.d. complained of side effects, including a feeling of heaviness or rubberiness of the legs, ataxia and clumsiness, difficulty in reading small print, poor ability to maintain continuity of thought or attention, giddiness, diarrhea, anxiety, and drowsiness. The 10 normal subjects were given single subcutaneous injections of 5 mg and then had their EEGs recorded. Five had markedly reduced alpha rhythms in their EEGs. Complaints made by these subjects after they had received the drug, in order of decreasing frequency, were weakness; headache, lightheadedness, and a feeling of heaviness of the limbs; drowsiness; and increased complexity and coloration of photic pattern and facial numbness. Complaints expressed only by some of the 10 subjects were dry mouth, difficulty in expressing thoughts, fear approaching panic, sensation that the floor was distant or seemed to slope, huskiness of voice, heaviness of the shoes, slight nausea, and sweating.

Kinross-Wright and Moyer (222) reported the results of giving diethylaminoethyl benzilate to 42 patients. They agreed with others that daily doses of 1 mg produced no effects that could be distinguished from those of a placebo. Side effects were infrequent with daily doses of less than 4 mg. With daily doses of 4–8 mg, there were complaints of dryness of the mouth, pupillary dilatation, slight palpitations, and difficulty in concentration. Daily doses of more than 8 mg resulted in such complaints by half the patients, with the addition in some of nausea, anorexia, and constipation. Patients given daily doses of 12 mg or more were affected also by feelings of heaviness of the limbs and of mental confusion, emotional lability and feelings of unreality, drowsiness, dizziness, and ataxia. One patient developed a maculopapular rash.

Vojtechovsky et al. (223) summarized their trials of administering doses of up to 75 mg of diethylaminoethyl benzilate to 17 healthy volunteers. The state of toxic psychosis—resembling a combination of psychoses due to atropine intoxication, to chronic alcoholic damage of the brain stem (Korsakov's syndrome), and to chronic alcoholic delirium—lasted for 4–6 h. They found no somatic complications after doses of up to 75 mg. These investigators suggested that the combination of anticholinergic activity and ability to inhibit monoamine oxidase (resulting in the demonstrated interference with the metabolism of serotonin and possibly of catecholamine transmitters as well) explains the phenomena of intoxication by this benzilate.

Rickels et al. (224) reported the findings in an experiment in which 52 patients were given tablets containing 1 mg of diethylaminoethyl benzilate and were instructed to take five tablets a day, but were allowed to decrease that to three tablets a day if it seemed desirable and, for short times, to two a day. The group of patients studied consisted of approximately equal numbers of psychiatric patients (mildly to moderately depressed neurotic outpatients) and patients seen in general medical practice. A placebo group (45 people) of similar composition received tablets of identical appearance and with the same instructions. The side effects experienced by the subjects were recorded 2 and 4 wk after beginning ingestion of the tablets. The six subjects who had taken a daily average of two or fewer tablets for a 2-wk period were excluded from the final compilations of data. Mean intakes of the two types of tablets were not stated, but would be expected to be about 4.5 tablets a day on the basis of reported intakes of similar tablets containing other drugs involved in the same experiment.

Of the 45 persons who took the placebo for 2 wk, 23 (51%) complained of side effects; 15 (33%) complained of sedation. Of the 52 persons who took the benzilate for 2 wk, 26 (50%) complained of side effects; 15 (29%) complained of sedation. Of the 30 persons who took the placebo for 4 wk, 17 (57%) reported side effects; 11 (37%) complained of sedation. Of the 26 persons who took the benzilate for 4 wk, 10 (38%) mentioned side effects; 4 (15%) complained of sedation. It is apparent that the persons who ingested diethylaminoethyl benzilate had no greater incidence of side effects than those who ingested the placebo.

Sidell (225) surveyed studies of the effects of 3-quinuclidinyl benzilate on human subjects carried out by Edgewood Arsenal or its contractors between 1960 and 1969. The initial studies were by and on four members of the research staff, who took the compound to become familiar with its effects, so that they could describe to potential volunteers the experiences that they might have as experimental subjects and could devise appropriate means for measuring functional impairment and safeguarding the subjects from physical harm. Sidell was able to identify 314 other subjects who had been given 3-quinuclidinyl benzilate at the Biomedical Laboratory at Edgewood Arsenal or at other sites. In addition, he reported that there had been 24 cases of accidental exposure to 3-quinuclidinyl benzilate among employees of Edgewood Arsenal. Of the 24, 14 had no effects other than mydriasis; four others complained of dry mouth, fatigue or sleepiness, photophobia, and inability to accommodate for near vision; six had difficulty in concentrating on a topic, slowed thought processes, and mild confusion. No long-term effects were reported.

In 1964 and 1969, field tests had been conducted with 3-quinuclidinyl benzilate. As a part of each test, eight volunteers were exposed to monitored aerosolized benzilate and were then assigned model military tasks to perform. A contractor had performed a study of the effect of exposure to this benzilate on the ability of trained pilots to fly airplanes, using the Link flight simulator and other appropriate measures of performance. The procedures used in 23 studies with 3-quinuclidinyl benzilate were outlined by Sidell (225), who also summarized the effects of the compound on man and stated that it is about 20 and 3 times as potent as atropine and scopolamine, respectively, in altering functions of the central nervous system. When equipotent doses were used, the effects of 3-quinuclidinyl benzilate lasted about 6 and 9 times as long as those of atropine and scopolamine, respectively.

Sidell's description of the effects induced by 3-quinuclidinyl benzilate is as follows:

> At low doses, the effects include a dry mouth, decreased gastric mobility, inhibition of sweating, an increase in heart rate, pupillary dilatation and loss of accommodation, mild sedation and mental slowing.
>
> At high doses these effects are intensified. There are marked disturbances of function at all levels of the central nervous system: motor coordination, attentiveness and control of thought and learning processes all decline. Confusion, restlessness, impairment in perception, interpretation, and memory span, poor judgment and deficient insight are all features of this syndrome. True hallucinations are present. If the dose is quite high the subject may become stuporous or even comatose for several hours.

Freedman (226) reported the findings of a research project designed to determine whether small amounts of 3-quinuclidinyl benzilate modify undesirably the ability of experienced pilots to perform the tasks involved in flying an airplane. The 18 pilots chosen to take part in the study, from a panel of 41 potential subjects, were 25–45 yr old (mean, 38 yr) and had flying times of 200–5,000 h (mean, 1,800 h).

A Link flight simulator and 14 tasks, including the Number Facility Test, were used to assess the competence of the subjects before and at intervals after intramuscular injection of 3-quinuclidinyl benzilate at 1–4 µg/kg. These tasks yielded a total of 35 items that were graded in cycles during each test period. Practically no effects followed doses of 0.5 and 1.0 ug/kg. After doses of 2 µg/kg, there were slight decrements in performance in some of the tasks, a definite increase in heart rate, a decrease in systolic blood pressure, and a slight increase in diastolic blood pressure. The subjects given 4 µg/kg all made lower scores on the performance tests and exhibited definite functional changes, including increased heart rate, dizziness, drowsiness, mydriasis, and difficulty in accommodating the eyes for near vision. Most of the subjects had increased diastolic blood pressure and complained of dryness of the mouth.

The tests associated with the Link flight simulator were the first to demonstrate effects of the 4-µg/kg dose; especially striking changes occurred in vigilance and in judgments of altitude, headings, air speed, and glide paths. These decrements appeared between 1 and 1.5 h after injection. By 3 h, half the subjects could not have completed a mission involving simply taking off and landing an airplane. None could have completed a moderately involved mission. The abilities of the pilots to perform the standard tasks presented to them decreased further up to the end of the observation period, 6 h 40 min after the benzilate had been administered.

In July 1962, it was estimated (227) that exposure of a human population to a Ct product of 3-quinuclidinyl benzilate of 118 mg.min/m^3 would result in incapacitation of 30% of the population, a Ct product of 170 mg.min/m^3 50% of the population, and a Ct product of 347 mg.min/m^3 84% of the population.

Ketchum (228) summarized the experiments with 3-quinuclidinyl benzilate performed between August 1960 and July 1963 with human volunteers. The report included an appendix, prepared by Claude McClure, Jr., on the chemistry and biochemistry of 3-quinuclidinyl benzilate. The outstanding information derived from this appendix is that the brain was the only organ of mice found to contain ^{3}H 48 h after intraperitoneal injection of [^{3}H]3-quinuclidinyl benzilate. Perfusion of this benzilate through the livers of rats had indicated that the liver destroyed the compound quickly, 90% of the compound having disappeared from the perfusate within 30 min and less than 0.2% of the compound being found unaltered in the bile. The products were not known. Several hypotheses of biochemical mechanisms of action of 3-quinuclidinyl benzilate were presented, but no firm support for any of them was available.

The main report concerned 290 exposures of 215 subjects other than staff members to 3-quinuclidinyl benzilate by intravenous injection (11.3%), intramuscular injection (39.3%), ingestion (12.4%), inhalation (21.7%), and application to the skin (14.8%).

Intravenous and intramuscular injections were considered to be equal in effectiveness, although changes occurred more rapidly after intravenous than after intramuscular injection. By ingestion, 3-quinuclidinyl benzilate was about 0.9 times as effective as it was by injection. Inhaled 3-quinuclidinyl benzilate was about 0.6 times as effective as it was by injection. Application to the skin in solution in benzyl alcohol resulted eventually (after 24–36 h) in a group of effects representative of the responses to an injection of about one-seventh the amount.

Estimates of the doses required to be inhaled (expressed as Ct, mg.min/m^3) to produce various effects were as follows: mild incapacitation (moderate dilatation of pupils and slight blurring of vision, minimal incoordination, some slowing of thought), 66–124; moderate incapacitation (hallucination, confusion, unorganized hyperactivity, incoherent speech, shortening of memory and attention span), 102–152; and severe incapacitation (stupor or coma, possibly preceded by a period of agitation, followed after 10–15 h by prolonged hallucination and inappropriate behavior), 110–165. The LD_{50} for a man was estimated to be 5.7–6.7 μg/kg.

Repeated daily exposures of volunteers to intramuscularly injected 3-quinuclidinyl benzilate at 1 μg/kg yielded no evidence of cumulative effects, but 2 μg/kg induced clear signs of cumulation. One of 12 volunteers given daily doses of 1–2 μg/kg appeared to develop tolerance.

Repetition of a second administration of 3-quinuclidinyl benzilate 2–3 wk after an initial dose induced a more rapid and more pronounced development of intoxication than the first dose; that suggested a sensitization mechanism in the response to the second dose.

Tetrahydroaminoacridine (THA) had been found to decrease heart rate when tachycardia was present, to increase secretion of saliva, to increase sweating and lower core temperature if it had become high, to reduce muscular rigidity, and to improve, at least temporarily, the mental status of an intoxicated subject. THA seemed not to antagonize sleepiness and might have promoted it. Physostigmine, given orally at 2–6 mg/h, caused nearly total remission of toxic effects within about 8 h after administration of the benzilate. Physostigmine seemed to be a more complete antagonist of the effects of 3-quinuclidinyl benzilate than was THA.

In 1965, the Directorate of Medical Research of the Chemical Research and Development Laboratories, Edgewood Arsenal, issued a booklet (229) on the management of casualties from 3-quinuclidinyl benzilate. The booklet gave the following sequence of effects to be expected in persons who had received large doses: in 1–4 h, tachycardia, dizziness, ataxia, vomiting, dry mouth, blurred vision, confusion, and sedation progressing to stupor; in 4–12 h, inability to respond effectively to the environment or to move about; and in 12–96 h, increasing activity, random unpredictable behavior, and gradual return to normal 48–96 h after exposure. The booklet recommended treatment of casualties with intramuscular injection of 3 mg of physostigmine salicylate, followed by another such dose 40 min later, if necessary. Maintenance by oral doses of 2–5 mg of physostigmine salicylate every 1–2 h was suggested. With improvement in mental status, the frequency and size of the maintenance doses can be reduced gradually. Heart rate was recommended as an indicator of the adequacy of therapy. The pulse should be between 70 and 80 beats/min when good control has been achieved. If the heart rate falls below 70 beats/min, the frequency or magnitude, or both, of the maintenance doses of physostigmine salicylate should be reduced, but the patient should be observed carefully for possible reversion to toxic delirium. Peripheral effects of physostigmine overdoses (profuse sweating, clammy skin, abdominal cramps, vomiting, muscular twitching, tremor, and weakness) may be antagonized by small doses of methylatropinium nitrate or, if that is not available, of atropine itself.

Kitzes and Vancil (230) reported the results of a study to determine the intramuscular dose of 3-quinuclidinyl benzilate that constitutes the minimal effective dose (MED) for a man, here defined as the smallest dose that produces a decrease of at least 25% in performance in the Number Facility Test. The effects on heart rate were also recorded. Thirteen male volunteers were given intramuscular injections of 3-quinuclidinyl benzilate hydrochloride at 2.3 or 2.7 μg/kg. One man given 2.7 μg/kg became confused and developed hallucinations. The most common complaints of the other volunteers were of dry mouth, blurred vision, and drowsiness. Several developed restlessness, an inability to sleep despite drowsiness, and anorexia. Of six men given 2.3 μg/kg, one had a decrease in performance in the Number Facility Test of more than 25%, and three had dilated pupils. This dose induced no tachycardia or hyperthermia. Of seven men given 2.7 μg/kg, five had decreased performance in the Number Facility Test of more than 25%, three had dilated pupils, two had heart rates greater than 100 beats/min, and one had a blood pressure above 140/90. There were no cases of hyperthermia (the report did not state the environmental conditions of the study). The mean heart rate after the larger dose was about 92 beats/min, whereas that after the smaller dose was about 79 beats/min.

Kitzes and Vancil estimated that the MED related to performance in the Number Facility Test was 2.54 μg/kg, with 95% confidence limits of 2.31 and 2.80 μg/kg, and that the MED for inducing changes in somatic functions (vision, heart rate, and blood pressure) was about 2.7 μg/kg.

Crowell (231) described experiments in which 27 male volunteers were given intramuscular L-2-alpha-tropinyl benzilate at 1.9–8.6 μg/kg after a previous unpublished estimate that the intravenous MED was about 3.8 μg/kg. This benzilate was found to have a quicker onset of effects after intramuscular injection and a briefer duration of action than 3-quinuclidinyl benzilate. Every subject who received a dose of 2.8 μg/kg or more experienced dryness of the mouth, blurred vision, and dizziness. Nearly all the subjects given 4.1 μg/kg or more complained of anorexia, nausea, weakness, confusion, hallucinations, and ataxia. Most expressed the illusion that their hands and feet were as red as blood. About one-third suffered headaches, and a few vomited. The intramuscular ED_{50} for inducing a decrement in performance in the Number Facility Test of at least 25% was about 3.1 μg/kg, that for increasing heart rate to at least 100 beats/min was about 5.6 μg/kg, and that for reducing performance in the Number Facility Test by at least 90% was about 5.9 μg/kg. For men given L-2-alpha-tropinyl benzilate at a mean dose of 6.1 μg/kg, the mean duration of severe effects of the compound was 2.5–3.1 h, and the mean time of onset for reduction of performance in the Number Facility Test by at least 90% was about 1 h. At 10 d after intramuscular injection of L-2-alpha-tropinyl benzilate, no evidence of any organic damage attributable to the compound could be found. Crowell's estimate of the incapacitating dose by intramuscular injection was 6.0±0.75 ug/kg.

Kitzes et a1. (154) reported the results of 40 experiments in which volunteers with body weights between 59.1 and 96.3 kg were given 3-quinuclidinyl benzilate at 2.0–8.0 μg/kg. These experiments revealed no clear relation between body weight or benzilate dose and performance in the Number Facility Test. As part of the same study, 10 experiments were performed with volunteers with body weights between 65.4 and 87.7 kg who were given L-2-alpha-tropinyl benzilate at 1.9–8.6 μg/kg. With this compound, there seemed to be no clear relation between dose and performance in the Number Facility Test, but there may have been a positive relationship between body weight and performance. When the range of weights of these subjects was divided in half, the upper half of the range included four experiments with men given a mean dose of 5.4 μg/kg and having a mean body weight of 85.3 kg, and the lower half included six experiments with men given a mean dose of 4.3 μg/kg and having a mean body weight of 69.7 kg. Despite the smaller mean dose of the benzilate received by the men in the lower half of the weight range, that half included all the men rated as being the most severely affected in performance in the Number Facility Test. With 3-quinuclidinyl benzilate, the two halves of the weight range contained close to the same proportions of severely affected men: 45.4% in the upper half and 48.3% in the lower half. Here, also, the mean doses for the two halves of the weight range were similar: 4.3 μg/kg for the upper half and 4.7 μg/kg for the lower half.

Ketchum et al. (189) reported the results of field tests in which two groups of four men each were exposed on different days to clouds of an aerosolized solution of 3-quinuclidinyl benzilate in chloroform. After exposure to the cloud, the subjects were rated on their performances in a variety of tests of physiologic and psychologic properties. Baseline performances in these tests had been recorded after 12–20 practice runs before exposure; the five best scores in the practice runs were averaged as the baseline scores. A dry run before the actual experiment incorporated all features of the experiment except the exposure; the scores on the test battery during the dry run did not deviate significantly from the baseline scores. The conclusions from the study were that the ED_{50} for incapacitation by 3-quinuclidinyl benzilate under field conditions is about 60 mg.min/m^3 for a man with a body weight of 75 kg and a minute volume of respiration of 15 L. Intramuscular injections of 2 or 3 mg of physostigmine salicylate were found to reverse the toxic delirium rapidly; repeated injections every 2 or 3 h or oral doses about one-third larger than the intramuscular doses, on the same schedule, restored and maintained acceptable performance in the test battery and in carrying out simulated guard duty of a post perimeter or simulated sentry duty in a foxhole under attack by an aggressor.

Craig et al. (232) reported the results of exposing 24 volunteers to high environmental temperatures at various times after they had been given intramuscular 3-quinuclidinyl benzilate at 3, 4, 5, or 6 μg/kg by intramuscular injection. Two series of exposure to heat were performed. In the first, four subjects were exposed to 41°C for 2 h on each of two days and six subjects were exposed to the same temperature during two 2-h periods on one day; on the third day after the first day of exposure to heat, all 10 subjects were given intramuscular injections of the benzilate during the first of two 2-h exposures to 41°C, separated by 3 h, followed by two similar exposures on the same schedule on the following day and by a single 2-h exposure of six men on the morning of the sixth day of the experiment (four of these men had another 2-h exposure to heat on the afternoon of the same day). In the second series, 14 men were exposed to 41°C for 8 h on day 1, to 52°C for 8 h on day 3, and to 41°C for 8 h on day 7, beginning 4 h after an injection of 3-quinuclidinyl benzilate.

The effects of the benzilate reached their maximum about 6 h after injection. At that time, the heart rate, skin temperature, rectal temperature, and inhibition of sweat secretion were increased in relation to the dose of benzilate administered. The highest rectal temperature was 38.3°C, an increase of less than 1°C above the mean normal temperature. The disturbance of regulation of body temperature by 3-quinuclidinyl benzilate under the condition of recumbent rest used in these experiments should not be hazardous to a healthy person. Physical activity during the exposure to heat might alter this general picture, although a previous study had found that the combination of 3-quinuclidinyl benzilate at 4 μg/kg, exercise, and an environmental temperature of 46°C produced only a slightly greater increase in rectal temperature than the exercise and environmental temperature without the benzilate. The inhibition of sweating by this benzilate regressed at about half the rate at which that induced by atropine regressed.

Klapper et al. (162) reported the results of followup studies on two men who had been given 3-quinuclidinyl benzilate and one who had been given L-2-alpha-tropinylbenzilate earlier. The first two had histories of intermittent hematuria before their service as volunteer subjects; at the times of the followup examinations, they had fewer than 10 red blood cells per high-power field in their urine. One of them reported that he had had a flashback of his experiences under the influence of the benzilate when drinking alcoholic beverages about 3 wk after exposure. There were no remarks about the man who had received the other benzilate.

Klapper et al. (163) examined the records of 24 volunteer subjects who had taken the MMPI test and then had been given intramuscular injections of 3-quinuclidinyl benzilate at 2.1 (six men), 2.4 (11 men), or 4.5 (seven men) µg/kg in an attempt to determine the factors of the MMPI that are best correlated with the response to the benzilate represented by performance in the Number Facility Test. The dose of 2.1 µg/kg produced a mean decrease in performance in the Number Facility Test of 27%, 2.4 µg/kg produced a mean decrease of 26%, and 4.5 µg/kg produced a mean decrease of 74%. The scales of the MMPI that were best correlated with the individual scores in the Number Facility Test were that for positive test-taking attitude for 2.4 µg/kg and that for hysteria for 4.5 µg/kg. There were no significant positive correlations for the lowest dose. There were significant negative correlations with the schizophrenia and the hypochondriasis scales after that dose, but these negative correlations were not reported for either of the larger doses.

Sidell et al. (164) found that S-(2-diisopropylaminoethyl)-ethylmethylphosphonothioate injected intravenously at 1.5–1.7 µg/kg after intramuscular injection of 3-quinuclidinyl benzilate at 6 µg/kg was effective in inducing rapid improvement in the scores of three volunteers in the Number Facility Test. This finding was similar to that reported earlier in subjects exposed to scopolamine.

Sidell (168) reported that four volunteer subjects given intramuscular injections of 3-quinuclidinyl benzilate at 7 ug/kg had their scores in the Number Facility Test improved dramatically by intramuscular injection of 7 or 11 mg of physostigmine salicylate followed by oral administrations of 24 or 12 mg, respectively, of the same drug during total periods of treatment of 43 and 42 h, respectively. Two other subjects given the same dose of the benzilate were treated entirely with oral administration of physostigmine in total doses of 80 and 211 mg during periods of treatment of 37 and 71 h, respectively. The scores in the Number Facility Test of the last two subjects were better maintained than those of the first two.

SUMMARY

3-Quinuclidinyl benzilate, the benzilic acid ester that has been studied the most by Edgewood Arsenal, has effects similar in general to those of the tropic acid esters, but more prolonged. Two other benzilic acid esters have similar actions. In part, the particularly long duration of the central action of 3-quinuclidinyl benzilate may be related to its greater affinity for nervous tissue, and especially for mitochondria within neuronal cells. In agreement with its especially strong adsorption on mitochondria, the subcellular organelles concerned principally with the supply of energy to the cell, this benzilate has been found to reduce the oxygen consumption by nerve cells stimulated in various ways. This

and the other two benzilates on which some information was found have some activity as local anesthetic agents and as antihistaminic compounds, in addition to their activity as peripherally and centrally effective antagonists of cholinergic agonists. The only long-term toxicity study of a benzilic acid ester that was found was of the ester with diethylaminoethanol; no significant pathemas were found in rats during this lifetime feeding study, but the lifetimes of the rats fed the benzilate may have been shortened somewhat. Feeding of the ester with quaternized 3-quinuclidinol to rats for a year and gavaging dogs with this compound 5 d/wk for a year produced no significant pathemas.

Both cholinergic and anticholinesterase compounds antagonized the toxic actions of the benzilates. Although 3-quinuclidinyl benzilate has been found to have weak mutagenic activity for yeast cells in culture and to produce gaps and breaks in chromosomes of bone marrow cells, it has not been proved to produce heritable changes in mammals. Diethylaminoethyl benzilate was more toxic cytogenetically than 3-quinuclidinyl benzilate, despite the lower anticholinergic activity of the former compound.

ESTERS OF PHENYLCYCLOPENTYLGLYCOLIC ACID

This group of anticholinergic esters contains five compounds, the subject acid being esterified with the following alchohols: tropine, 3-quinuclidinol, 2-methyl-3-quinuclidinol, N-methyl-4-piperidinol, and a mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol. Of 20 reports on these compounds by personnel of Edgewood Arsenal and its contractors, 10 are on the ester with N-methyl-4-piperidinol, four on the ester with the mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol, and two each on the other three esters. The literature on the ester with N-methyl-4-piperidinol is entirely from Edgewood Arsenal, whereas more has been written about the ester with N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol (Ditran) by investigators other than those at Edgewood Arsenal than by the ones at that site. Most of the information about the other esters in this group originated at Edgewood Arsenal. The following discussion concentrates on the two most popular esters in this group.

The information on the lethal activities of the members of this group of esters is sparse. Even when reports dealing with lethality according to their titles were furnished by the Board on Toxicology and Environmental Health Hazards, the texts provided were brief and uninformative. An example is a report (233) entitled A Review of the Pharmacology and Toxicology of CAR 301,060. This compound is the ester of phenylcyclopentylglycolic acid and 2-methyl-3-quinuclidinol. The entire text reads:

> CAR 301,060, a BZ-like glycolate, is, on the basis of extensive pharmacological evaluation, a potent anticholinergic compound that displays marked behavioral

> and CNS effects. The compound, inmost instances, is equal to or more potent than BZ, and has similar temporal parameters. It is exceptionally unique in regard to profound potency in the ACRBL-HAZLETON visual discrimination test. Extensive acute and subacute toxicological evaluation failed to reveal any extremely profound toxic or pathological effect in any major organ system at the doses tested. CAR 301,060 has a very substantial safety margin in all species tested.

Such pap is a waste of paper and time.

Other reports of that ilk (234–236) give a little useful information. The first of these (234) stated that repeated daily intravenous doses of the phenylcyclopentylglycolic acid ester of N-methyl-4-piperidinol produced mydriasis in monkeys, that doses of 0.1 mg/kg or more resulted in above-normal ratios of adrenal:body and testes:body weights, and that the highest dose administered, 1.0 mg/kg, resulted in unusually small livers. None of the organs mentioned was considered to have been harmed histologically. The second report (235) concerned the effects of the same compound in the dog. The lowest daily intravenous dose, 0.01 mg/kg, induced mydriasis; the two higher doses, 0.1 and 1.0 mg/kg, resulted in ataxia, decreased activity, and dryness of the mouth, in additon to mydriasis. They also induced significant effects on the performance of the dogs in a multiple-stimulus conditioned-avoidance test. The highest dose induced a statistically significant decrease in body weight. At necropsy, no significant effects that could be attributed to the agent were found. The third report in this group (236) described the effects of the phenylcyclopentylglycolic acid ester of 3-quinuclidinol on dogs. Repeated daily intravenous doses of 0.1 mg/kg produced maximal mydriasis that persisted throughout the study, decreased activity and ataxia, decreased concentration of the urine, and significant changes in the weights of the gonads and kidneys. The lower dose, 0.01 mg/kg, was not reported to have produced any effects other than mydriasis and decreased activity. The dogs that had been trained in the multiple-stimulus conditioned-avoidance test displayed a rapid development of tolerance to a daily dose of 0.1 mg/kg.

N-METHYL-4-PIPERIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3443)

N-Methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate, also designated EA 3443, was found (193) to be effective both prophylactically and therapeutically in reducing the adsorption of 3-quinuclidinyl benzilate by the cat's motor cortex, caudate nucleus, and sensory cortex after intravenous injections of the benzilate and the glycolate. When given after the benzilate, EA 3443 reduced the retention of that compound by the medial and lateral geniculates. When it was injected first, EA 3443 reduced the adsorption of 3-quinuclidinyl benzilate by the hypothalamus and thalamus, in addition to the three parts of the brain first mentioned. Prophylactically administered EA 3443 decreased the adsorption of the benzilate by the hypophysis, liver, lungs, skeletal muscles, spleen, and sciatic nerve. When it was administered to cats after the benzilate, it reduced retention of the benzilate by skeletal muscles, lungs, sciatic nerve, pancreas, liver, spleen, and heart. When added to the fluids surrounding a segment of isolated guinea pig ileum, EA 3443 reduced almost to zero the response to acetylcholine. This glycolate had about the same activity in this regard as 3-quinuclidinyl benzilate, but the anticholinergic effect of the glycolate was the one more readily reversed by tetrahydroaminoacridine.

Kitzes and Ketchum (237) reported giving 39 volunteers intramuscular EA 3443 at 1.0–2.7 μg/kg. These doses induced no hallucinations or abnormal neurologic signs. Dry mouth, blurred vision, and drowsiness were the most common complaints. Mydriasis occurred to a mild degree. There was no consistent dose-response relationship for the heart rate, but that rate may have been decreased, rather than increased. The minimal effective dose for reducing performance in the Number Facility Test of half the subjects by at least 25% (MED_{50}) in this study was 1.21 μg/kg, with 95% confidence limits of 1.00 and 1.48 μg/kg. The time for onset of these minimal effects was about 8 h, and the duration of effects was about 4 h.

Kitzes et al. (154) found that 80% of a group with a mean body weight of 87.2 kg who had been given EA 3443 at a mean dose of 2.2 μg/kg suffered degradation of their performances in the Number Facility Test. Only 46.7% of a group with a mean body weight of 71.6 kg who had received a mean dose of 2.4 μg/kg suffered degradation of their performances in the same test. In the case of this compound, therefore, body size may have conditioned the response.

Hart and Balter (238) exposed an unstated number of volunteers to EA 3443 by application to an unidentified area of the skin at 60–120 μg/kg (mean, 68.5 μg/kg). The volunteers were tested with a modification of the Army General Classification Test before and at 7 wk after EA 3443 exposure. During the exposure, the Number Facility Test was used to determine whether the subjects experienced any degradation of their mental abilities. The criterion for an MED (two of five consecutive scores below 75% of the baseline score) was met by 71% of the exposed volunteers. A control group of 15 volunteers were tested and retested 20 d later without any known intervening exposure to a psychotoxic substance. The mean score of the control group at the second testing was 3.2% below that at the first testing. The second testing of the exposed group yielded a mean score for that group that was 2.4% above that at the first testing. Neither of these differences is significant.

Lavallee (50) reported that the minimal intravenous dose of EA 3443 that decreased performance by at least 25% in the Number Facility Test by half the members of a group of volunteers was 1.2 μg/kg. The corresponding incapacitating dose, the ID_{50}, was 3.1 μg/kg. That for 3-quinuclidinyl benzilate was 6.6 μg/kg. When EA 3443 was injected intravenously into dogs trained in a multiple-stimulus conditioned-avoidance test, a dose of 100 μg/kg decreased the number of correct responses to visual stimuli by about 28.2%. This decrement had been surpassed by that induced by 3-quinuclidinyl benzilate at 32 µg/kg. In monkeys trained in a visual-discrimination avoidance task, intravenous EA 3443 at up to 56 µg/kg had no significant effects on performance of the task. The same doses of 3-quinuclidinyl benzilate also had no significant effects on the responses of monkeys in this test. Lavallee concluded that the screening tests with dogs and monkeys were not satisfactory for predicting the relative potencies in man of the anticholinergic compounds tested.

Klapper et al. (163) found that the scale of the MMPI that was the best correlated with the degradation of performance of volunteers in the Number Facility Test after intramuscular doses of EA 3443 (six volunteers, 1.8 µg/kg; five volunteers, 2.4 µg/kg) was that for paranoia. The lowest negative correlation was that between the social introversion scale and the degradation of performance in the Number Facility Test. These statements were true for both doses of EA 3443; that is different from the situations with some of the other compounds studied in this way. With both atropine and 3-quinuclidinyl benzilate, the larger dose produced a degree of degradation of performance in the Number Facility Test that was best correlated with the score in a different scale of the MMPI from the one that yielded the best correlation with the lower dose of the same agent.

Armstrong et al. (239) used a test that required water-deprived rats to press four levers in a preset sequence to obtain water. After administration of EA 3443 by an unstated route, the ED_{50} for a minimal mydriatic effect was 37 µg/kg; that for reduction of the number of drops of water obtained within a given period was 53.2 µg/kg.

DITRAN (CS 4297)

In 1958, Abood et al. (240) reported initial studies of a group of esters of alkylated derivatives of piperidine, including a material that was thought to be N-ethyl-3-piperidyl-(phenylcyclopentyl)-glycolate. This substance was given the trivial name Ditran; it is known now to be a mixture of two compounds: the phenylcyclopentyl glycolates of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol in the proportion 3:7. This substance was not as centrally active (producing hyperactivity in rats) as the methyl homologue, but was less lethal and more potent as a peripheral anticholinergic. Ditran was administered to more than 40 human volunteers by Ostfeld et al. (241) and was found to be actively hallucinogenic. Esters of 3-piperidyl alcohols were found to be much more hallucinogenic than corresponding esters of 4-piperidyl alcohols.

Abood et al. (242) compared 14 related esters of substituted piperidyl alcohols. They found that the phenylcyclopentyl and phenylcyclohexyl glycolates were more actively hallucinogenic in human subjects than diphenyl, phenylpropyl, or phenyl 2-thienyl glycolates. Esters of quaternized piperidinium alcohols were not hallucinogenic. A hydroxyl group on the carbon atom alpha to the carboxyl group of the acid was found to be essential for hallucinogenic activity. Ditran was found to have an intravenous LD_{50} for the rat of 19 mg/kg and one for the mouse of 44 mg/kg. The latter value may have been erroneous, inasmuch as Usdin and Efron (243) later stated that the intravenous LD_{50} for the mouse was 10 mg/kg. However, Usdin and Efron may have confused the dose given to human subjects by Abood et al., which was 10 mg/man, with the figure for the LD_{50} for the mouse after intravenous injection. The value reported by Abood et al. by this route accords better with the LD_{50} after intraperitoneal injection in the compilation of Usdin and Efron than that stated by the latter authors. Usdin and Efron gave also intravenous LD_{50}s for the guinea pig and the rabbit of 45 and 12 mg/kg, respectively. Intraperitoneal injections of Ditran into mice and rats yielded LD_{50} estimates of 60 and 25 mg/kg, respectively. Little correlation between peripheral anticholinergic activity and psychotogenic potency was found by Abood et al. They reported that intraperitoneal injection of physostigmine at 0.5 mg/kg into rats 5 min before injection by the same route of Ditran at 25 mg/kg prevented mydriasis and lessened tachycardia and hyperemia of the tail and limbs, but had no visible effect on hyperactivity or on weakness and poor coordination of muscles.

Gershon and Olariu (244) assessed the ability of Tacrine to antagonize the actions of Ditran on human subjects and compared the effects of Ditran with those of mescaline, Sernyl, and LSD. The most common complaints of subjects given doses of Ditran by intramuscular injection or by mouth, or by both routes, were of hallucination, confusion, and impaired contact. Gershon and Olariu found that Tacrine was able to antagonize both the peripheral and CNS effects of Ditran in man, but was incapable of preventing those due to Sernyl. Succinate, which effectively reverses both the peripheral and central effects of Sernyl, had no activity against intoxication with Ditran. The authors gave the following typical sequence of signs and symptoms after intramuscular injection of 10–15 mg of Ditran: in 20–30 min, autonomic reactions (dryness of the mouth, slight tachycardia, flushed face, and muscular relaxation); in 45–60 min, central effects (some confusion, speech difficulty, decreased ability to concentrate, slight disorientation, occasional vertigo, and hallucinations); in 2–4 h, maximal central effects (hyperreflexia and ataxia); and in 5–8 h, abatement of symptoms and signs.

Biel et al. (245) reviewed the effects produced by a large group of anticholinergic compounds in an attempt to develop guidelines for the development of new psychotropic drugs and reached the following general conclusions: only the piperidyl and pyrrolidyl glycolates that were potent anticholinergic compounds exerted profound effects on CNS functions. However, peripheral anticholinergic potency did not endow a compound automatically with brain activity. Over 95% of a dose of Ditran is excreted in the urine within 2 h after its administration. The largest brain concentrations of several of these substances were in the hypothalamus and caudate nucleus, areas related intimately with emotion and mood. Psychotropic anticholinergic compounds had little effect on several enzyme systems. Ditran is a clinically effective antidepressant agent. An inhibitor of cholinesterases, tetrahydroaminoacridine, is capable of ameliorating the psychotic episode induced by anticholinergic psychotogens. Piperidine, trifluoperazine, and several other tranquilizers were effective in overcoming hyperactivity induced by anticholinergic compounds in rats.

Bell et al. (85) used dogs to study the psychotropic activity of Ditran and its antagonism. After developing a rating scale including six indexes of autonomically evoked activity, six of motor activity, and eight of central nervous system activity, they were able to present a graph demonstrating that the time courses of each of the major types of effect induced by intravenous Ditran at 0.5 mg/kg had a somewhat individual time course. Mydriasis, tachycardia, and decreased secretion of saliva were apparent within 3 min after injection. Between 3 and 6 min after injection, the pupil response to light became sluggish, and alertness and responsiveness to commands began to decrease. Within 12–15 min after injection, incoordination and ataxia had reached a plateau, and the pupil response to light usually had disappeared. Obstinate progression and disturbed behavior (whining or barking, pawing at the floor, and apparently chasing imaginary objects) became evident at 30–40 min after injection. By an hour after the injection, most of the dogs had collapsed. Prostration, from which the dogs could be roused temporarily by noise or handling, lasted for 15–120 min. The integrated total effect (obtained by summation of scores on the various parts of the rating scale) reached an approximate plateau at about 0.5 h after injection and remained there till about 2.5 h after injection. Approximately complete recovery from the effects of this dose on dogs did not occur until about 5 h after injection. Mydriasis was the last sign of effect by Ditran to disappear, often being evident for up to 8 h after injection.

Bell et al. (85) found that doses of physostigmine or neostigmine injected intravenously at 20 μg/kg 30 min after Ditran antagonized the effects on autonomic functions, but did not alter the central actions. Arecoline (150 μg/kg) restored salivation and heart rate to normal, but had no other detectable antagonistic actions when injected intravenously 30 min after Ditran. Tacrine at 1 mg/kg, injected intravenously 30 min after Ditran, abolished all effects of Ditran except mydriasis and decreased secretion of saliva. About 40% of the dogs so treated had relapses about 10 min after the dose of Tacrine; repetition of the dose of Tacrine induced complete and permanent recovery, except for persistent mydriasis. Some derivatives of glycolic acid (phenanthrylglycolic acids), p-phenylmandelic acid, and benzilic acid injected 30 or 60 min before Ditran shortened by up to 55% the duration of the intoxication due to Ditran, but did not alter the initial severity of its effects. These organic acids exerted less antagonism to intoxication with Ditran when they were administered therapeutically than when they were used prophylactically.

Bell and Gershon (86) reported that yohimbine antagonized the intoxicating actions of Ditran, but differed from Tacrine in this sort of activity, in that its antagonistic activity was less time-dependent than that of Tacrine. The latter had only slight antagonistic activity early in the intoxication and had greater activity when it was administered later after the Ditran, whereas yohimbine had more nearly equal antagonistic activities at various times after the dose of Ditran.

Brown et al. (246) gave 48 dogs intravenous Ditran at 0.5 mg/kg and 30 min later injections of saline, chlorpromazine, imipramine, or two unidentified substances, IN 1060 and W2045. IN 1060 is known also as cyprolidol, an antidepressant, but an equivalent for W2045 could not be found. Chlorpromazine and imipramine deepened the intoxication induced by Ditran, whereas IN 1060 and especially W2045 lightened the intoxication. When chlorpromazine and the two other compounds were fed to dogs daily for a month before the animals were given Ditran, chlorpromazine accentuated the psychotogenic action of Ditran, whereas IN 1060 and W2045 both attenuated the psychotogenic action of Ditran. After the month-long dosing with the last two antagonists, IN 1060 had a much greater effect on the central actions of Ditran than W2045, whereas the reverse had been true after single therapeutic administrations of the two drugs. For both compounds with both schedules of administration, the effects on the central actions of Ditran were greater than those on the motor and autonomic actions.

Banshchikov and Stoliarov (216) compared the effects of three of Biel's compounds—JB-318, JB-329 (Ditran), and JB-336—among themselves and with those of benactyzine and atropine. They also discussed the use of these compounds in the practice of medicine. Ditran had a much less prolonged action than JB-318, which was described as causing loquacity and hyperactivity with an elevated mood that persisted for days, weeks, or even months. In the few people who had been persuaded to take repeat doses of this compound, resistance to the hallucinogenic action developed, but the mydriatic effect persisted. The effects of all three of Biel's compounds were qualitatively similar and resembled those induced by atropine and benactyzine. Daily doses of Ditran were stated to lead to development of resistance to its actions: after 3 wk of daily intake, a dose of 20 mg/kg no longer resulted in mental disturbance. The posthallucinatory euphoriant action of Ditran has been applied to the treatment of patients in depressed states of mind, apparently with some success. Ditran has also been used in treating patients with neuroses and psychoses.

Albanus (42) reported that subcutaneous injection of Ditran at 0.5 mg/kg into 11 dogs resulted in the development of ataxia after a mean of 22 min, of obstinate progression after a mean of 31 min, and of complete loss of contact with the environment after a mean of 45 min. Obstinate progression disappeared about 230 min after the injection of Ditran, and ataxia after about 280 min.

Gershon and Angrist (247) summarized the research that had been done with Ditran and other anticholinergic psychotomimetic compounds and with possible antagonists of their psychotomimetic activities. Nothing particularly new was reported in this paper. Tacrine is regarded as the best overall antagonist.

Sayers and Burki (71) compared the effectivenesses of 13 psychoactive compounds, including Ditran, in four assays of anticholinergic potency: displacement of 3-quinuclidinyl benzilate from the particles in a homogenate of rat brain, antagonism of stimulation of isolated guinea pig ileum by acetylcholine, antagonism of induction of tremor by oxytremorine in mice, and production of pupil dilatation in mice. Ditran was graded first in the first two tests, third in the oxytremorine test, and second in the mydriasis test. For comparison, atropine was as good as Ditran in the second test, first in the last two tests, and second in the first test. Overall, therefore, Ditran was judged to be slightly less anticholinergic than atropine. The general conclusion was that none of these tests is satisfactory for predicting in vivo effects on brain functions.

Kligman and Copelan (156) reported that 16 volunteers had been used for studies with Ditran, but gave no information about the studies.

Ketchum et al. (24,165,166) gave 22 male volunteers intramuscular Ditran at 50–170 µg/kg and concluded that 150 ug/kg produced a syndrome of peripheral parasympatholytic effects, disturbances of basic neuroregulatory functions, and disruption of higher integrative functions that culminated in delirium characterized by restlessness, confused speech, hallucinations, and moment-to-moment variability. Twelve of these volunteers were treated with physostigmine and three with Tacrine after development of toxic psychosis. Both inhibitors of cholinesterases were found to be effective in overcoming changes in the Number Facility Test induced by Ditran.

3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3167)

The phenylcyclopentylglycolic acid ester of 3-quinuclidinol was included in the group of glycolic esters studied by Larsson et al. (192). They recorded its threshold dose for producing a psychotomimetic effect in the dog as 50 ug/kg. Five substances with threshold doses of 10 ug/kg had hydrogen bond strengths (expressed as log K, where K is the constant for the equilibrium between free and bonded CO groups in infrared spectra of the glycolates) of 0.8–1.2 (mean, 1.01). Two substances with threshold doses of 50 µg/kg, one being Ditran, had log K values of 0.80 and 0.93; two other substances with threshold doses of 0.5 mg/kg, one being tropinyl benzilate, had log K values of 0.92 and 1.2. It is evident that there is no correlation between the strength of the intramolecular hydrogen bond and the threshold dose for psychotomimetic effect.

Ketchum et al. (248) gave six volunteers intramuscular 3-quinuclidinyl-(phenylcyclopentyl)-glycolate by intramuscular injection at 2.4 µg/kg, seven at 2.9 µg/kg, and six at 3.4

μg/kg (the hydrochloride salt was used). Measurements and observations of the subjects were made hourly for 6 h, bihourly for another 6 h, and every 4 h thereafter until recovery was judged to be virtually complete. The MED_{50} was found to be 2.5 μg/kg, and the ID_{50} was estimated to be 4.1 μg/kg. With the latter dose, mild effects were to be expected after 2 h and severe ones after 5 h. Severe effects would be expected to last for about 96 h, and mild ones for about 240 h. Eighteen of the subjects were examined for evidence of organic changes, and 13 were given psychologic evaluations about 6 mo after they had received the compound.

Most subjects displayed some change from baseline performance and behavior when examined 2 wk after injection of the glycolate; these changes usually disappeared by the fourth week after the injection. The effects detected in these early followup examinations included increased irritability; mild impairment of memory, judgment, or abstraction; mental sluggishness with occasional confusion; nervousness; and tenseness. No positive evidence of organic change was found at 6 mo after the doses; there may have been a small increase in IQ at that time. The profiles in the MMPI of seven subjects who were tested 6 mo after exposure, as well as before exposure and a month after exposure, were close to the baseline profiles, although a month after exposure they had contained significant increases in the scores on the hypochondriasis, depression, hysteria, psychasthenia, schizophrenia, and mania scales. Intoxication with the glycolate was found to be treatable with physostigmine or Tacrine; because of the long duration of the effects in some subjects, doses of antagonists may have to be repeated for a rather long time.

L-2-ALPHA-TROPINYL-L-(PHENYLCYCLOPENTYL)-GLYCOLATE (226,086)

The actions of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate on experimental animals have been described (188). This was found to be the most active and the safest (largest ratio of LD_{50} to MED_{50}) of the six possible isomers of tropinyl-(phenylcyclopentyl)glycolate by intravenous injection into mice. LD_{50}s by intravenous injections into male mice and rats were 61±3 and 65±3 mg/kg, respectively; the LD_{50}s by gavage in the same two species were 98±21 and 170±53 mg/kg. Depression of general motor activity and depression of respiratory activity were the principal effects observed in the two test species during the estimations of LD_{50}s. A range-finding test with intravenous injection of the glycolate into monkeys yielded an LD_{50} probably between 10 and 20 mg/kg. In the cat, the LD_{50} probably is greater than 20 mg/kg.

L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate elicited mydriasis in the mouse after intravenous doses of 10 μg/kg or more. One estimate of the MED_{50} was 5.6±1.5 mg/kg. Mydriasis became maximal after intravenous doses of about 0.3 mg/kg. Other effects that were evident after doses of 10–30 ug/kg were hyperactivity, hyperreactivity to touch, and motor deficits. Respiratory depression became evident after intravenous doses of 0.3–3.0 mg/kg. General activity became lessened and ptosis of eyelids appeared after doses of about 30–50 mg/kg. Mydriasis was the most persistent effect, which might last for 3–4 d after the highest doses mentioned above. With mydriasis as the indicator, this glycolate was about one-third as active when applied to the skin of the mouse as when injected subcutaneously (benzyl alcohol was a suitable solvent for application of the glycolate to the skin). Intravenous doses of about 50–100 µg/kg depressed a polysynaptic reflex (ipsilateral flexor reflex) in the anesthetized cat, but did not affect the patellar reflex. This glycolate was effective in antagonizing tremor in mice (induced by subcutaneous injection of tremorine at 10 mg/kg), in subcutaneous doses of 10–30 µg/kg whereas, by the same route of administration, doses of 12–16 µg/kg were effective in inducing mydriasis. The glycolate had a dose-related negative inotropic effect on the isolated, perfused rabbit heart, with only a slight negative chronotropic effect. In the intact anesthetized dog, it had dose-related negative effects on both heart and mean blood pressure. L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate was active prophylactically against 2 LD_{50}s of TEPP (2.3 mg/kg intraperitoneally) in mice pretreated by intraperitoneal injection of benzoquinonium at 0.3 mg/kg (to prevent neuromuscular paralysis by TEPP), its intraperitoneal ED_{50} being 40 ug/kg.

Rats given five daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 50 mg/kg during 8 d and rats given intravenous injections 5 d/wk for 1 mo at 10 mg/kg had no changes observable at necropsy, except decreases in the sizes of the spleen and the thymus in the animals given 50 mg/kg. Histologically, both groups of rats had increased numbers of granules in the Paneth cells in the crypts of Lieberkühn in the small intestine. Two of five rats given the largest dose were thought to have slight depletion of thymocytes.

Monkeys given daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 0.4, 2.0, or 10 mg/kg 5 d/wk for a month had mydriasis with all doses. Tremors occurred in the animals given the two larger doses, and convulsions in those given the largest. Various extents of involution of the thymus and of increased granule formation in the Paneth cells were seen in animals given each of the doses. Fatty changes were seen in the livers of some of the monkeys given the two larger doses. One of five monkeys given 0.4 mg/kg died on the twelfth day, and one of five given 10 mg/kg died on the twentieth day. No specific cause of death was identified in either instance, but both animals had lost more weight than the means of the living members of their groups.

The actions of this glycolate in unanesthetized beagles were studied (249). Groups of four dogs were given daily intravenous injections at 0, 0.8, 4.0, and 20.0 mg/kg 5 d/wk for a month, receiving 21 doses during 29 d. Each group contained two males and two females. Three of the dogs given the highest dose were found dead on the fourteenth, seventeenth, and twenty-sixth days; the fourth dog in that group was moribund and was killed on the twentieth day. No animals from the other groups died during this study.

All dogs that received the glycolate had mydriasis and reduced activity, the mydriasis persisting even over weekends. In the high-dose group, panting, persistent anorexia, tremor, ataxia, prostration, fits of running movements of the legs (possibly accompanied by barking) in prostrate animals, and convulsions were seen commonly. These dogs lost an average of at least 23.5% of their body weight before death. The dogs of the groups given the low and medium doses of the glycolate lost 4.4% and 10.8%, respectively, of their original body weight during the administration of the glycolate. The heart rates of the dogs given the glycolate all increased during the first 15 min after administration of the first doses, whereas those of the control animals, given intravenous injections of saline, decreased slightly on the average. After the first weekend, after three injections during the previous week and before injection of another dose of the benzilate, the heart rates of the animals given the two higher doses were still above their baseline values. Baseline heart rates were not available for the dogs given the lowest dose, but the heart rates of two of the four dogs in this group on day 6 were above the highest baseline rate recorded for the other 12 dogs in the experiment. On the twenty-ninth day of the experiment, at 2 h after the last doses of saline or glycolate, the mean heart rates, in comparison with those measured at the same time after the first doses, for the three groups of dogs then alive were as follows: control, 9 beats/min below that on the first day; low dose, 29 beats/min below that on the first day; and medium dose, 43 beats/min below that on the first day.

The composition of the blood (blood chemistry and hematology) changed significantly only in the group of dogs given the largest dose of the glycolate. Final samples of blood were obtained on only two of the four dogs in this group; in both, the prothrombin time was prolonged, the total serum cholesterol concentration was very low, and the alkaline phosphatase activity in the serum was very high (70 and 198 K-A units/100 ml vs. 4–12 in baseline measurements). Both dogs had other evidence of interference with liver function: increases in the activity of glutamic-oxaloacetic transaminase and in the concentration of serum bilirubin in one, and decreases in the concentrations of albumin and total protein and in the ratio of albumin to globulin in the serum of the other. The first of these two animals had a concentration of serum urea nitrogen that was above the normal range, so there seemed to be some interference with renal function also. The most striking change in the hematologic picture in these two dogs was the complete eradication of eosinophilic polymorphonuclear cells from the blood. The total leukocyte counts and hematocrits were decreased in both dogs; one also had a moderate decrease in erythrocyte count.

Terminal samples of urine were obtained from the bladders of all four of the dogs in the group given the highest dose of the glycolate. Two were bloody, and three contained bilirubin, bile salts, and urobilin. All four samples contained albumin. The urine of the dog mentioned above as having increased serum urea nitrogen contained hyaline casts.

Electrocardiographic recordings from the dogs given the medium dose of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate contained suggestions of deepening of the S wave and of elevation of the T wave. Because baseline recordings of the ECG had not been made for these animals, the significance of the apparent changes cannot be assessed.

The only significant pathemas found in the dogs at necropsy at the end of the experiment were in those given the highest doses of the glycolate. The livers of these dogs were pale yellow to orange and were coarsely lobular or swollen. Three of these dogs had bile staining of the sclerae and aortas. Two of the dogs had-tarry material in their gastrointestinal tracts, reddish urine in their urinary bladders, and fatty bone marrow. Hemorrhages into the renal cortices were found in these two dogs. Microscopic examination of organs from these dogs revealed decreases in the size and number of the islets of Langerhans in one dog from each of the low-dose and high-dose groups and in two dogs from the medium-dose group. In the high-dose group, irritative and degenerative or necrotic changes were also found in the livers, kidneys, bone marrow, adrenals, testes, epididymides, and gastrointestinal tracts. The livers of all the dogs in this group were markedly fatty, the hepatocytes containing numerous small cytoplasmic vacuoles. Widespread vacuolation of the tubular epithelium in the cortical region of the kidney was seen in three dogs; epithelial crescents were seen in Bowman's spaces in the kidneys of two of these dogs. The bone marrow of all four dogs had shifted to an immature type, with increased numbers of blast forms of the granulocytic series.

2-METHYL-3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (301,060)

Hayes (250) reported the results of a study in which 29 men were given single intravenous doses of CAR 301,060, also called cis-2-methyl-3-quinuclidinyl-(phenylcyclopentyl)-glycolate, at 0.5–5.3 ug/kg . They were graded in performance in the Number Facility Test. Two subjects given 5.3 ug/kg became incapacitated (two consecutive scores in the Number Facility Test below 10% of baseline); the mean duration of significant effect was 73 h. These two subjects were disoriented, confused, restless, and less coordinated than normal, spoke disjointedly and in a slurred manner, had lapses of memory, and experienced visual hallucinations. One was episodically paranoid and mildly hostile to the medical personnel in attendance. Both were drowsy and lethargic. Their heart rates were increased markedly for more than 24 h after injection, but the peak of the tachycardia (+85%) occurred about 12 h after injection. This dose was the only one that resulted in pupil dilatation, by about 2 mm.

Doses of 2.0 and 2.7 ug/kg produced only slight and irregular increases in heart rate. The heart rate was increased in a dose-related manner when doses of 3.2 ug/kg and more were administered. The lowest dose that produced a significant decrease in the performance of one of four subjects in the Number Facility Test was 2.7 μg/kg; 3.2 μg/kg produced significant decrements in the performances of five of seven men, and 3.8 μg/kg caused significant decrements in the performances of six of eight subjects. Hayes concluded that the intravenous MED_{50} of this glycolate for man is about 3.0 μg/kg and that, after a dose of 3.2 μg/kg, a significant decrease in cognitive ability occurs about 5 h after injection and lasts for about 7 h.

ESTERS OF PHENYLISOPROPYLGLYCOLIC ACID

Two esters of phenylisopropylglycolic acid are among 26 anticholinergic substances studied by Edgewood Arsenal: N-methyl-4-piperidinyl-(phenylisopropyl)-glycolate (EA 3834) and 4-(1-methyl-1,2,3,6-tetrahydropyridinyl)-phenyl-isopropylglycolate (302,668).

N-METHYL-4-PIPERIDINYL-(PHENYLISOPROPYL)-GLYCOLATE (EA 3834)

Copelan (251) gave 16 volunteers EA 3834 at 1.0–2.7 μg/kg intravenously and administered the Number Facility Test to them at intervals thereafter. Two groups of two men given 1.0 or 1.4 μg/kg had their performances in the Number Facility Test unchanged. Some members of each of two groups of four men given 1.6 or 2.0 μg/kg had their performances reduced to below 75% of their baseline performances. Three of four men given 2.7 μg/kg had their performances reduced to below 75% of the baseline. The estimate of the MED_{50} was 1.96 μg/kg, with 95% confidence limits of 1.45 and 2.66 μg/kg. Significant change in performance in the Number Facility Test usually began at about 1.5 h at the highest dose administered (2.7 μg/kg), the mean time of onset of significant effect on performance was 1.9 h, and the duration of significant effect was 2.8 h.

Copelan described the effects of this agent as follows. At 3–15 min after injection, the subjects reported that they felt “high,” “light-headed,” or “dazed.” At about this time, they were unsteady in walking and particularly in turning about. Talking among the subjects became quieter, and their speech was slightly muffled, but without significant slurring. A feeling of heaviness of the eyelids and a tendency to diplopia became evident at 10–15 min after injection. Sedation, with drowsiness and dozing, were noticeable at 15–30 min. One subject (dose group not stated) reported an illusion of undulating movement in the glass of a window. Two subjects (dose groups not identified) reported hallucinations of crawling ants and of moving strings or hairs on the floor, respectively. The same two subjects recognized that they were hallucinating. There was no confusion, significant disorientation, or delirium among the subjects in this study, dryness of the mouth, blurred vision, tachycardia, and mild reddening of the conjunctivae were noted occasionally and might appear within 15–30 min after injection. No abnormal changes in blood and urine were detected 24 and 48 h and 8 and 28 d after injection.

Averill et al. (252) summarized studies of the toxicity of EA 3834 in mice, rats, rabbits, cats, dogs, and monkeys. Table 3 gives the available single-dose LD_{50}s for both EA 3834 and 302,668. It is apparent that the latter is less lethal than the former in the species for which values on both compounds are available. The values for 302,668 come from Sim (253).

In addition to the LD_{50} values stated above, Averill et al. summarized the results of studies in which graded doses of EA 3834 had been administered to various animals by a variety of routes. In mice, intravenous injections at less than 10 ug/kg induced mydriasis and those of 32–100 μg/kg induced unusual sensitivity to being touched, restlessness, and ataxia. Intramuscular doses of 100 mg/kg in the rat induced paralysis of the leg into which the compound was injected within 5 min after injection and jerking movements of the head, hyperpnea, and exophthalmos within about 15 min after injection. Intravenous doses of 0.7 μg/kg induced mydriasis in rabbits, and tachycardia followed doses of 10–100 μg/kg. Ataxia in this species appeared after intravenous doses of 3 mg/kg, and prostration and convulsion occurred after doses of 11–14 mg/kg. In the cat, mydriasis, decreased activity, increased heart rate, and ataxia all occurred after intravenous doses of 10–100 μg/kg.

The heart rate of the dog was increased and the performance of a conditioned-avoidance task was degraded by intravenous doses of 10–28 μg/kg. Doses of 12–37 μg/kg reduced physical activity, and those of 25–30 μg/kg induced ataxia. Prostration and convulsions occurred after doses of 10–20 mg/kg. Inhalation exposure of dogs to a Ct product of EA 3834 of 36 $mg.min/m^3$ resulted in increases in heart rate. Physical activity was reduced by exposure to Ct products of 40–50 $mg.min/m^3$. Mydriasis and ataxia followed exposure to a Ct product of 90 $mg.min/m^3$. A Ct product of 102 $mg.min/m^3$, with 95% confidence limits of 82 and 126 $mg.min/m^3$, caused 50% of the exposed dogs to fail in performance of the conditioned-avoidance task. In the monkey (species not stated), intravenous doses of 6 μg/kg induced mydriasis. Visual discrimination was reduced by doses of 7–31 μg/kg. Tachycardia and decreased activity followed doses of about 130 μg/kg. Prostration and convulsions were produced by doses of 14–16 mg/kg.

Sidell and Braun (254) discussed the results of an experiment in which two men were given EA 3834 by mouth at 7 μg/kg, four were given 10 μg/kg, three were given 14 μg/kg, and two were given 20 μg/kg. One man given 7 μg/kg and two given 10 μg/kg suffered severe effects (reduction of score in the Number Facility Test to below 25% of the baseline score). All the men given 14 and 20 ug/kg experienced severe effects.

Of those who experienced severe effects, the three with the lower range of doses had a mean dose of 9 μg/kg and experienced severe effects at about 2.3 h after drinking a solution of the agent in water. The five men with the higher range of doses had a mean dose of 16.4 μg/kg and experienced severe effects at about 1.4 h after ingestion. The two men given 7 μg/kg had a mean score of 47% of their baseline scores. The four men given 10 μg/kg had a mean score of 40.4%. The five men given a mean dose of 16.4 μg/kg had a mean score of 2.6%. The eight severely affected men had recovery times of 12–16 h (mean, 12.5 h). The two men given 10 μg/kg who did not suffer severe effects had recovery times of 6 and 10 h. The severe effects lasted 0.5–9.0 h; the mean duration of severe effects for the three men who took a mean dose of 9 μg/kg was 3.3 h, whereas that of the five men who took a mean dose of 16.4 μg/kg was 6.4 h.

Mild effects (reduction of score in the Number Facility Test by at least 25% of the baseline score) for all the severely affected subjects lasted 10–14.5 h (mean, 11.2 h), whereas those in the two men who took 10 μg/kg without developing severe effects lasted for 3 and 7.5 h. Mild effects in the two men given 10 μg/kg who never developed severe effects began 2.5 and 3 h after ingestion. In the eight men who developed severe effects, the mean onset time for mild effects was 1.3 h (95% confidence limits, 1.0 and 2.0 h).

McCarroll et al. (255) described a study with EA 3834 in which eight enlisted volunteers from the U.S. Army were divided into two four-man teams, with a ninth serving as the leader of both groups. The ninth man had been given intramuscular scopolamine at 23.5 μg/kg several weeks earlier to familiarize him with the effects that the others would experience later, but he was not given an anticholinergic substance during the actual study. The members of the two teams were given directions for performing a simulated military mission in the field and then practiced the mission for 3d. On the fourth day, the teams went through the entire mission under the observation of two judges, who graded their performances on a number of items incorporated into the exercise. The exercise included capture and interrogation by a simulated aggressor. On the day of the test, intramuscular injections were given at 9 a.m., and the exercise was started immediately. It ended at 4 p.m. A battery of tests (heart rate, blood pressure, rectal temperature, Number Facility Test, and behavioral checklist) was administered to each volunteer at 9:30, 10, and 11 a.m., noon, and 1 and 4 p.m. The performance of volunteers on this day was graded by the same judges who had been present on the control day.

One four-man team received EA 3834 at 2 μg/kg (one man), 4 μg/kg (two men), and 8 μg/kg (one man), for a mean dose of 4.5 μg/kg. The second four-man team received 4 μg/kg (one man), 8 μg/kg (two men), and 12 μg/kg (one man), for a mean dose of 8 μg/kg. Both teams were considered to be composed of capable, effective soldiers on the control day.

On the test day, the team with the lower mean dose was judged to be marginally effective. The man given 8 μg/kg had to be removed from the exercise about 2 h after injection. The other members of that team stumbled and fell over minor impediments in the terrain, lost some of their gear, made serious errors in calculating an azimuth with compasses, were unable to remember simple directions or to repeat instructions given just before questioning, and were unable to operate a field telephone. The man given 8 μg/kg cooperated with the "enemy" interrogator and tried to follow his instructions without intellectual or physical resistance. The team that received the larger mean dose became totally ineffective within about an hour. The man given 12 μg/kg became incapacitated within about 30 min and was removed from the exercise. One of the men given 8 μg/kg became incapacitated about 75 min after injection, and the other became incapacitated about 141 min after injection. The man in this group given 4 μg/kg was not incapacitated, but the team leader had to prod him constantly to get him to accomplish the simplest tasks. For both groups, the correlation between performance in the Number Facility Test and in the various military tasks included in the scenario for the field exercise was good.

The volunteers evacuated from the field exercise were flushed and had hot, dry skins, parched lips and tongues, dilated pupils, tachycardia, and exaggerated deep tendon reflexes. After treatment with physostigmine salicylate (55 μg/kg intramuscularly), these volunteers were restored to lucidity within 20 min. After institution of maintenance doses of physostigmine salicylate by mouth, the subjects were able to return to the field exercise and to perform their duties capably. When the maintenance doses of physostigmine salicylate to the volunteer who had been given EA 3834 at 12 μg/kg were discontinued after four such doses, he became delirious again within about 3 h and remained in that state for 5 or 6 h. No abnormalities of renal and hepatic functions were detectable in any subject 24 h and 7 d after the injections of EA 3834.

Cucinell et al. (256) applied EA 3834 in a solution in ethanol to the skins of 11 volunteers on single occasions, of three volunteers on two occasions, and of two volunteers on three occasions. In all instances, the area of skin to which the solution was applied was about 9 cm^2 of the neck at the angle of the jaw. When the solvent had evaporated, the area of application was covered with a plastic cup or with Teflon taped to the skin. The contaminated skin was washed with alcohol 4 h after the application. Some of the volunteers were placed in a hot room at 40.5°C and 24% relative humidity with a wind speed of 0.5 m/s (slightly more than 1 mph); others remained under unspecified room conditions. Three of the volunteers who received EA 3834 more than once were exposed to it both in the hot room and in the regular laboratory rooms; the other two in this category were exposed only in the hot room. The amounts of the agent applied to the skin ranged from 0.1 to 5.0 mg in the hot room and from 2.0 to 4.0 mg in the regular laboratory rooms.

The hydrochloride of EA 3834 had no measurable effect on rate of sweating or performance in the Number Facility Test. When EA 3834 base was applied to the skin, the time to onset of inhibition of sweating (38–170 min) in the hot room was related inversely to the amount of the agent placed on the skin within the range 0.1–3.0 mg. The rate of change of the rate of sweat secretion once inhibition of that rate had begun was somewhat less variable (13–42 $mg/m^2.min^2$) within the same range of amounts of the agent, but was randomly distributed throughout the range. The two extreme values of the rate of change of the rate of sweating came from experiments in which sequential amounts of the agent near the middle of the range were applied to skin, the larger of the two amounts producing the smaller rate of change. Because the earliest time after application of EA 3834 to the skin at which the Number Facility Test was administered to the volunteers was 4 h, there is no factual basis for the assumption by Cucinell et al. that there is a constant relationship between the time of onset of inhibition of sweating and the extent of decrease in performance in the Number Facility Test. Their proposed expression for estimation of the systemic effect of contamination of skin with this agent depends on this spurious relationship and cannot be considered reliable.

4-(1-METHYL-1,2,3,6-TETRAHYDROPYRIDINYL)-PHENYLISOPROPYLGLYCOLATE (302,668)

Copelan (257) summarized experiments in which 20 volunteers were given 302,668 intravenously at 1.0–4.6 µg/kg and were tested at intervals for ability to perform in the Number Facility Test. Men given doses of 1.0, 1.4, and 2.0 µg/kg lost less than 25% of their baseline abilities to perform the additions. Of four men given 2.7 µg/kg, two lost at least 25% of their abilities. Of four men given 3.2 µg/kg, one lost 29% and another lost 24%. Four men given 3.8 µg/kg and two given 4.6 µg/kg had mean decrements of 41% and 50%, respectively, and all lost at least 25% of their abilities.

Karger (258) estimated the ID_{50} of 302,668 by giving 14 volunteers intravenous doses of 5.0–11.0 µg/kg and administering the Number Facility Test at intervals thereafter. Incapacitation was defined as the making of two consecutive scores below 10% of the baseline score. A clinical evaluation of incapacitation based on observation of the subjects was also made. The scores on the Number Facility Test yielded an ID_{50} of 10.1 µg/kg, with 95% confidence limits of 9.2 and 11.1 µg/kg. The clinical evaluations yielded an estimated ID_{50} of 9.5 µg/kg, with 95% confidence limits of 8.5 and 10.6 µg/kg.

As a second part of the same study, Karger gave six volunteers 302,668 intravenously at 10 µg/kg and administered physostigmine at various times. An intramuscular dose of 3 mg of physostigmine injected 10 min before administration of 302,668 attenuated the decrement in performance in the Number Facility Test for about 4.5 h after injection. At that time the performance of two unprotected subjects and of the two prophylactically protected subjects became essentially identical. When a dose of 3 mg of physostigmine was injected intramuscularly 10 min after intravenous injection of 302,668, there was rapid recovery from about 35% of baseline performance to about 92%, but performance in the Number Facility Test was down to about 20% of baseline by 2 h after the injection of 302,668. When the physostigmine was not injected until 45 min after the agent, performance in the Number Facility Test rose from zero to about 85% within about 45 min and to a peak of about 93% within 75 min. Performance in the test oscillated between 93% and 70% of baseline until about 3.75 h after injection of the agent and then drifted down to about 40% at 8 h. Thereafter, it improved gradually without further administration of physostigmine; thus, performance in the test had improved to about 60% of the baseline value by 10 h after initiation of intoxication. Karger concluded that the best results in maximally incapacitated persons would be obtained by giving repeated intramuscular doses of 2 or 3 mg of physostigmine at intervals of about 2 h.

Sidell et al. (259) estimated the incapacitating dose of 302,668 inhaled as an aerosol. Performance in the Number Facility Test was the criterion of incapacitation. Nineteen volunteers inhaled aerosols of the agent while seated and wearing an oronasal mask, so that the expired air could be collected and analyzed for unretained agent. Four ranges of retained dose of the agent were recognized: 1.99–7.60 µg/kg, five volunteers; 8.3–11.54 µg/kg, four volunteers; 12.36–12.95 µg/kg, six volunteers; and 13.05–18.77 µg/kg, four volunteers. The mean performances in the Number Facility Test of all groups fell below the level for minimal effect (score less than 75% of baseline score), but that for the last group was the only one that fell below the level for incapacitation (score less than 10% of baseline score). The mean performance of the last group was below the criterion for incapacitation from about 0.25 h to about 5.5 h after exposure. That group also underwent marked decreases in its scores on an Orthorator for near and far vision, in its ability to move pegs in a pegboard, and in its performance of a bend-twist-touch task, whereas the other three groups had comparatively minor changes in these four measurements.

The initial signs and symptoms induced in these volunteers consisted of dryness of the mouth, drowsiness, and ataxia; these were followed by hallucinations, marked confusion, poor coordination, restlessness, and prolonged impairment of accommodation for both near and far vision with the larger retained amounts. Retention of large amounts (15 µg/kg) sometimes resulted in fine fasciculations of muscles. One subject in each of the two highest ranges of retained agent vomited, and eight others, including subjects from all ranges of retained agent, complained of nausea. The heart rate was not altered significantly among the volunteers in the three groups with the lowest ranges of retention, but three of four subjects in the other group had heart rates above 100 beats/min. No consistent effects on blood pressure were observed. Pupil dilatation occurred in all groups; in some subjects among those with the higher retentions, mydriasis persisted for 72 h.

From the results of these exposures, Sidell et al. (259) calculated that the ICt_{50} for a man breathing 10 L/min would be 181 $mg.min/m^3$, with 95% confidence limits of 159 and 206 $mg.min/m^3$. For a man exercising moderately and breathing 15 L/min, the ICt_{50} would be 121 $mg.min/m^3$, with 95% confidence limits of 106 and 137 $mg.min/m^3$. The incapacitating dose of retained agent for 50% of exposed men, the IRD_{50}, was calculated to be 13.1 μg/kg, with 95% confidence limits of 11.1 and 15.3 μg/kg. With this retained dose, the time to onset and the duration of mild effects were expected to be 25 min and 12 h, respectively; the times for severe effects were predicted to be 30 min and 5 h, respectively.

Lavallee (50) collected and graphed data from human experiments that indicated that 302,668 at comparatively low doses (below 4 μg/kg) approached 3-quinuclidinyl benzilate in activity in decreasing performance in the Number Facility Test, but at higher doses (above 9 μg/kg) was only slightly more active than scopolamine. Extrapolation of Lavallee's graphs suggests that the doses of 302,668 and of scopolamine that would be able to lower performance in the Number Facility Test to zero would be identical.

Sim (253) summarized the body of research with 302,668, printed or unpublished, performed in 1964–1971. The first one-third of the report discussed the chemical and physical properties of 302,668. The last two-thirds summarized the studies performed to determine the actions of 302,668 and several other anticholinergic substances in animal and human subjects. As shown in Table I–3, 302,668 was most lethal to the rabbit and least to the monkey after intravenous injection. It was most mydriatic in the rat and least in the monkey. The species with the safest relation between the lethal dose and the mydriatic dose was the rat, followed by the dog. Species with much smaller values of the ratio of LD_{50} to ED_{50} were the mouse, the rabbit, the cat, and the monkey. Although 302,668 had approximately the same actions as other anticholinergic compounds, the only sort of activity at which it excelled was the inception of increased motor activity in the mouse. The ICt_{50} in humans had been found to be 121 mg.min/m^3, which was larger than those for 3-quinuclidinyl benzilate, EA 3580, and EA 3834. Amiton, diethyl-S-(diethylamino)-ethylphosphorothiolate, was mentioned as having been found to be a more potent and longer-lasting antagonist of the autonomic effects of 302,668 in cats than physostigmine.

ESTERS OF 1-PROPYNYLCYCLOPENTYLGLYCOLIC ACID

This group of glycolates includes two compounds: the esters of the subject acid with 3-quinuclidinol (302,212) and with N-methyl-4-piperidinol (302,196).

Copelan (260) gave 25 volunteers 302,212 intravenously at 1.0–4.6 ug/kg. The initial effect experienced by the subjects was a feeling of "light-headedness" or of mild "dizziness" that started 15–30 min after injection. Although this symptom was reported by the subjects to be especially noticeable when they were standing, it was not associated with orthostatic hypotension. Between 30 and 60 min after injection, the subjects became drowsy. They often fell asleep; when walking, they staggered mildly and often brushed against corridor walls. Speech became muffled and slow, but was not slurred. Slight impairment of logical thought and of performance in the Number Facility Test became evident about an hour after injection. The doses used in this study did not produce incapacitation. Peripheral effects were limited usually to dryness of the mouth and, after the higher doses, mild blurring of near vision. The compositions of blood and urine and the removal of Bromsulphalein from the blood were within normal limits at 24 and 48 h, 8 d, and 4 wk. The MED_{50} for inducing at least a 25% reduction in the score in the Number Facility Test was estimated to be 3.3 ug/kg. The latency of the effect with this dose was expected to be about 4 h, and the duration of the effect was estimated to be about 4 h. The latency of the effect changed little, if at all, as the dose was increased, but the duration of the effect increased irregularly, but rather definitely, after the larger doses.

Kligman and Copelan (156) stated that 25 subjects had been exposed to 302,212, but gave no information about the experiments. The 25 subjects probably were those used by Copelan (260).

Lavallee (50) collected data from studies of the effects of 302,196 on human subjects that enabled him to draw a line relating the effects on the score in the Number Facility Test to the logarithm of the dose. This ester was less effective than scopolamine in decreasing the ability to perform this test.

Sidell et al. (261) injected 302,196 into 10 volunteers intramuscularly at 14–54 µg/kg, bracketing the previously determined intravenous ID_{50} of 22.5 µg/kg. The ID_{50} after intramuscular injection (reduction in score in the Number Facility Test to not more than 10% of the baseline score) was calculated to be 28.9 ug/kg, with 95% confidence limits of 12.0 and 65.5 µg/kg. Therefore, this ester was nearly 80% as active by intramuscular as by intravenous injection. At the ID_{50}, the duration of severe effects was expected to be about 101 min and the total duration of effects to be about 263 min. The latency of onset of severe effects was expected to be only about 12 min after intramuscular injection. The ED_{50} of 302,196 to increase the heart rate by 30 beats/min was calculated to be 23.5 µg/kg. The maximal increase occurred at about 15 min, and the heart rate was close to the baseline value by 4–6 h after intramuscular injection. The mean blood pressure was increased slightly, with a time course similar to that of heart rate. Pupil dilatation was slower both in developing and in disappearing than other changes that followed intramuscular injection. No abnormalities of blood and urine were found at 24 h and 7 d after injection.

N-METHYL-4-PIPERIDYL-(PHENYLCYCLOBUTYL)-GLYCOLATE (EA 3580)

Mongrel dogs unselected for sex were given 20 daily intravenous injections of the hydrochloride of N-methyl-4-piperidyl-(phenylcyclobutyl)-glycolate (EA 3580) at 0.01, 0.1, or 1.0 mg/kg during a 28-d period (262). No detectable effects were observed with the lowest dose. Mydriasis, ptosis, dry mouth, decreased activity, ataxia, and weakness of limbs were observed after the two larger doses. Tolerance to the compound developed during the study.

A similar experiment was performed (263) with male rats. Mydriasis occurred at the low dose (0.01 mg/kg.d). Tremors occurred at the two higher doses. The rats given the two higher doses had their ratios of adrenal weight to body weight increased significantly. No quantitative information has been made available.

A similar experiment was performed with the free base in place of the hydrochloride (264). The same doses used in the two preceding experiments were administered daily, 5 d/wk, for about a month. All doses induced mydriasis. Fine body tremors also occurred. These two observable effects were the only ones mentioned. Their appearance was rapid after injection, their durations were "relatively short," and they were reproduced each day. The groups given the two higher doses had liver weights and ratios of liver weight to body weight that were significantly larger than those of the control rats.

These three estimations of subacute toxicity indicated that a modest number of repeated doses of EA 3580 in the form of the free base or the hydrochloride and in amounts of not more than 10 µg/kg should be safe, although possibly disturbing. Typical anticholinergic effects would be expected.

Aghajanian et al. (265) told of giving four volunteers EA 3580 intramuscularly at 1 µg/kg, seven at 1.4 µg/kg, four at 1.7 µg/kg, and five at 2.0 µg/kg. With the production of at least a 25% decrement in performance in the Number Facility Test as the criterion of a significant effect, the MED_{50} was calculated to be 1.47 µg/kg. After a dose of 2 ug/kg, significant decrement of performance extended from about 3 to about 12 h after injection; the maximal decrement after this dose (mean of four cases) was to about 60% of baseline performance. All subjects experienced drowsiness beginning about 1–2 h after injection and lasting for at least 6 h. Some of the subjects had mildly impaired intellectual functions, but no delirium or hallucinations. None of the subjects had tachycardia, but two subjects developed distinct bradycardia after unstated doses. There was no complete blockage of sweating, although sweating was reduced. Decreased ability to accommodate for near vision was observed in some subjects, and four subjects (with unstated doses) had spasms of accommodation.

A report (266) that presents the results of administering two unstated doses of EA 3580 by an unstated route to groups of four men has been made available. The subjects were familiarized with the problem-solving task, which involved deciding whether an array of seven letter and number symbols was the same as an array seen previously and then pressing one of two buttons to indicate whether the two arrays were the same or were different. Six days after a baseline session following the familiarization session, the subjects were given EA 3580 and were tested for ability to perform the task with the duration of presentation that had been found to yield a stable accuracy of 65–75% in the familiarization session and that had been used in the baseline session. About all that can be derived from the data presented is that EA 3580 produced a more marked decrease in the rate of responding than in the accuracy of the responses and that the effect was more marked under conditions of extinction (no reward for a correct response) than under those of reinforcement. One group of subjects was affected by EA 3580 more than the other, but the doses are unknown.

Crowell (267) presented an estimate of the incapacitating dose of EA 3580, in the form of the hydrochloride, for man, based on the results of intramuscular injections into 22 volunteers at 2.0–5.3 µg/kg. The criterion of incapacitation was reduction of the score in the Number Facility Test to not more than 10% of the baseline score for three consecutive scores. Doses of 2.0 and 2.7 µg/kg incapacitated none of nine subjects. One of five subjects given 3.8 µg/kg was incapacitated. All eight subjects given 4.5 and 5.3 µg/kg were incapacitated. The ID_{50} was calculated to be 3.9 µg/kg, with 95% confidence limits of 3.6 and 4.3 µg/kg. The duration of incapacitation after the ID_{50} was about 90 min; the duration of severe effects after that dose was estimated to be about 5 h. The time to onset of severe effects was about 2.5 h. No adverse effects of single doses of EA 3580 of the magnitude used in this study on the liver, kidneys, or blood-forming organs were detected. The effects on the volunteers were similar to those of other anticholinergic substances. By 24 h after administration of the largest dose (5.3 µg/kg), the subjects were able to care for themselves. Complete recovery did not occur until 28–52 h after injection, without apparent relation to the dose.

Kitzes et al. (154) found that two groups of men with average body weights of 83.1 and 66.7 kg that had received similar doses of EA 3580 (3.1 and 3.2 µg/kg, respectively) suffered significant decrements in their scores in the Number Facility Test in 45.5% and 53.8% of the cases, respectively. It seems probable that body weight played no important role in conditioning the response. This puts EA 3580 in a class with 3-quinuclidinyl benzilate and distinguishes it from EA 3443 and tropinyl benzilate. EA 3443 seemed to have a positive correlation between body weight and response, whereas tropinyl benzilate seemed to have a negative correlation between body weight and response.

Hart and Balter (238) found that volunteers exposed by the respiratory route to EA 3580 at 4.9–18.6 µg/kg and tested before and at intervals up to 5 wk after the exposure with a modified Army General Classification Test gave no evidence of persistent effects from the exposures.

Baker (268) compared the responses of volunteers to EA 3580 administered as a dose per man or as a dose per unit of body weight. Four indicators of effect were used: accommodation for near vision, arm-hand steadiness, dynamic flexibility, and manual dexterity. The general conclusion was that the use of the dose per unit of body weight may increase variance, rather than control for extraneous sources of variation, when the purpose of a study is to establish the effects of a substance itself. The use of multiple-regression equations was suggested as an approach to the establishment of definitive information on effects of chemicals.

Kitzes et al. (269) exposed 16 volunteers to an aerosolized 2% solution in acetone of EA 3580 in the form of the free base and subjected them to a battery of tests before and after exposure. The men were exposed to three ranges of concentration of the ester: seven were exposed to concentrations corresponding to doses of 4.9–5.9 μg/kg (mean, 5.4 μg/kg), six to doses of 6.4–13.4 μg/kg (mean, 9.1 μg/kg), and three to doses of 17.0–18.6 μg/kg (mean, 17.9 μg/kg). The lowest range of doses produced no hallucinations or hostile or irritable behavior. The middle range of doses produced hallucinations in four of the six subjects and mild hostile behavior in one. The highest range of doses produced hallucinations and hostile or irritable behavior in all three subjects. In one of these subjects, the degree of hostility induced was considered severe, whereas it was mild in the other two. All the subjects in the last group were incapacitated within 30 min after exposure. All became delirious, and one semicomatose. All the subjects in this group and two of those exposed to the middle range of doses were thought by physicians who were not privy to the details of the experiment to require treatment by injection and oral administration of physostigmine. The toxic delirium disappeared within about 15 min after the beginning of therapy.

The ID_{50} of aerosolized EA 3580 was calculated to be 8.4 μg/kg, with 95% confidence limits of 5.8 and 12.2 μg/kg. The time to onset of incapacitation was about 2 h, and spontaneous recovery from severe effects required about 9 h from their inception. The effects induced by inhalation of aerosolized EA 3580 were the same as those induced by intramuscular injection of this agent. To judge by comparison of the retained ID_{50} for the aerosolized material and the ID_{50} estimated by Crowell (267) for the intramuscularly injected compound, the inhaled compound seems to have only 46.4% of the activity of the injected compound.

Baker (270) added to the previously mentioned volunteers given fixed doses of EA 3580 in the form of its hydrochloride (268) 16 volunteers given intramuscular injections of the estimated ID_{50} for a subject of average weight; 16 other volunteers were control subjects. Nine measures of performance were used. Each volunteer was tested five times during a 25-h period after injection of agent or placebo. The indicators of effect of EA 3580, in order of decreasing sensitivity, were manual dexterity, accommodation for near vision, accommodation for distant vision, arm-hand steadiness, estimation of elapsed time, short-term memory, dynamic flexibility, and static strength.

Baker et al. (158), in a summary report, stated that performance in a series of tests had been shown to be correlated with various personal traits and that changes in these performances caused by EA 3580 were clearly differentially related to the particular subject being tested. If the personal traits of a subject were taken into account, a significant portion of the subject-related variance could be compensated. The opinion was expressed that in this way generalizations about effects of substances that would be independent of the special group of subjects used in the research could be generated.

Allen and Safer (271) compared the temporal characteristics of intoxication induced by intramuscular injections of 0.25 mg of EA 3580 in the hydrochloride form in men resting in the laboratory and in men considerably more active in connection with field testing. The subjects in both situations developed their initial deficits in performance in the Number Facility Test at the same rate, but the subjects in the field test experienced a somewhat smaller deficit and returned toward normal more rapidly. The duration of marked mental incapacitation among the subjects in the field test was about six-tenths that among those in the laboratory.

Lavallee (50) used log dose-effect graphs to demonstrate that EA 3580 was nearly as active as EA 3443 and considerably more active than 3-quinuclidinyl benzilate and four other anticholinergic substances in bringing about decrements in performance in the Number Facility Test. In contrast with the sequence of decreasing potency in human subjects of EA 3443, EA 3580, and 3-quinuclidinyl benzilate, the sequence in dogs performing a multiple-stimulus conditioned-avoidance task was 3-quinuclidinyl benzilate, EA 3443, and EA 3580. In monkeys performing a visual-discrimination and avoidance task, the sequence was EA 3580, EA 3443, and 3-quinuclidinyl benzilate. In a similar task involving visual discrimination and escape by monkeys, the sequence of effectiveness of the two numbered compounds was EA 3580 and EA 3443. It is clear that none of the screening tests with experimental animals had reproduced the sequence of potencies found in the human test. The author of the report suggested that groups of animals larger than the two of each species required for the screening tests might increase the reliability of the results obtained with laboratory animals.

Klapper et al. (163) found that, for six men given a mean EA 3580 dose of 1.8 μg/kg by intramuscular injection, the scale of the MMPI yielding the greatest correlation with their performances in the Number Facility Test was that for hysteria. For seven men given a mean dose of 3.5 ug/kg, the scale with the best correlation was that for mania. For the lower dose, the most negative correlation was with the paranoia scale; for the larger dose, the most negative correlation was with the depression scale.

Sidell et al. (164) found that intravenous injection of S-(diisopropylaminoethyl)-ethylmethylphosphonothioate into men intoxicated with sufficient EA 3580 to decrease performance in the Number Facility Test to 15% or less of their baseline values removed the decreases promptly and nearly completely. Oral administration of the same phosphonothioate was ineffective. The doses of the theraupetic compound were not stated; oral administration of a different dose of the phosphonothioate might be effective.

Armstrong et al. (239) stated that the ED_{50} for EA 3580 in decreasing the number of rewards obtained by rats trained in a sequential-response task was 37.1 μg/kg. The ED_{50} for producing the least detectable mydriasis in rats was 16–30 μg/kg.

MISCELLANEOUS ESTERS

302,282

Kligman and Copelan (156) reported that the 1-methyl-4-piperidyl ester of phenyl-(3-methylbut-1-yn-3-enyl)-glycolic acid (302,282) had been administered to 20 men. The results were reported by Copelan (272), who gave 24 volunteers intravenous doses of 1.0–6.5 µg/kg. Doses of 3.8 µg/kg and less of this agent had only slight effects on performance in the Number Facility Test by the 12 men given these doses. A dose of 4.3 µg/kg decreased the test scores to below 75% of the baseline scores for three of four men. A similar result followed doses of 5.4 µg/kg in four men. Doses of 6.5 µg/kg reduced the scores of all four men given them to below 75% of the baseline scores and reduced the score of one man to 9%. The mean score for this group was 30.5% of the baseline scores. The time to onset of a minimal effect after the MED_{50} of 4.4 µg/kg was estimated to be about 30 min, and the duration of the minimal effect was estimated to be about 30 h. The peak effect usually occurred about 2 h after injection.

The initial symptom was mild "dizziness." Heaviness of the eyelids was a part of the initial experience in many subjects. Mild unsteadiness on standing and walking began between 5 and 15 min after injection. At the same time, speech became muffled and slowed. Sedation, with drowsiness and dozing, started about 15–30 min after injection. About 30 min after injection, degradation of performance in the Number Facility Test began. A few subjects had visual hallucinations, and one had auditory hallucinations also.

Dryness of the mouth or throat was reported by some subjects given the lowest doses and by all subjects given doses of 3.8 µg/kg or more. Mild blurring of near vision and occasional urinary frequency began after doses in the upper part of the MED_{50} range, but never affected all men in a dosage group. Mydriasis was not apparent. Nausea was rare. Conjunctival reddening frequently became evident within the first hour after injection. No significant alterations in blood and urine were found at 24 and 48 h, 8 d, and 4 wk after injection.

Safer (273) gave eight volunteers intravenous doses of 302,282 at 7.5–13.5 µg/kg. Three of the subjects became anxious after injection and two of them became agitated, restless, and passively resistant to experimental procedures. One subject vomited and later developed mild myoclonus, hyperactive reflexes, and indications of effects on the pyramidal tracts (positive Chaddock's reflex and possibly positive Babinski's sign). One subject had a tactually detected arrhythmia, which was not detectable in a later recording of the ECG. All the subjects had increased alkaline phosphatase in their serum 24 h after injection; one subject whose baseline activity of alkaline phosphatase (12 K-A units) was at the upper limit of normal had that activity increase to 18.2 K-A units at 24 h after injection of 13.5 µg/kg. On the seventh day after injection, the activity of alkaline phosphatase in his serum was 11.5 K-A units. Alkaline phosphatase activity in one other subject rose from 7.9 to 12.4 K-A units at 24 h after injection and was 8.4 K-A units on the seventh day after injection. This subject also received a dose of 13.5 μg/kg. Two of the subjects had 5–10 white blood cells per high-power field on the seventh day after injection. No other abnormalities in blood and urine were reported.

Safer (273) concluded that the intravenous ID_{50} of 302,282 is 12.5 μg/kg, but that the compound should not be administered to human subjects at doses greater than 11.5 μg/kg because of the untoward changes mentioned above.

302,537

Brown (274) stated that the ester of 2-propenylcyclopentylglycolic acid with 3-quinuclidinol (302,537) is a potent antimuscarinic compound with profound effects on the central nervous system and with a potency comparable with that of EA 3580 and somewhat greater than that of 3-quinuclidinyl benzilate. The times to onset and extinction of effects were unusually short with this agent. It was judged to have no marked toxic or damage-producing effects in any organism or organ system studied. The doses and organ systems used were not stated. The organisms studied were the rat, the dog, and the monkey. The blood-forming organs, liver, and kidneys were said to have been normal after administration of the substance. Presumably, other organs also were examined for pathemas.

Leib (275) stated that he gave 302,537 intravenously at 1.0–7.5 μg/kg to 18 volunteers. However, Figure 1 of his report contains 21 points at doses of 1.0–5.6 μg/kg; no explanation for this discrepancy was given. Eight men given doses of 2.0 μg/kg or less had scores in the Number Facility Test greater than 75% of their baseline scores. One of three men given 2.8 μg/kg had his score in that test decreased to slightly below 75% of his baseline score. The 10 men given 4.0–5.6 μg/kg had their scores in the Number Facility Test reduced to below 75% of their baseline scores, but the lowest score represented in Figure 1 of the paper was 25%, for a subject given 4.8 μg/kg. The intravenous MED_{50} of 302,537 was estimated to be 3.3 μg/kg, with time to onset of minimal effects of 3 h. After a dose of 4.0 μg/kg, the time to onset of minimal effects was 2.6 h; but after a dose of 7.8 μg/kg, the time to onset of minimal effects was only 12 min. The duration of minimal effects after the largest dose was 43 h. Doses of 5.6 μg/kg and more were reported to cause hallucination and delirium accompanied by restlessness and anxiety. In the one graph for a man given a dose of 7.8 μg/kg, incapacitation, indicated by reduction of score in the Number Facility Test to below 10% of his baseline score, occurred about 90 min after the injection. Intramuscular injection of 3 mg of physostigmine about 7 h after the injection of 302,537 induced a marked, but temporary, improvement in the subject's performance in the Number Facility Test. Improvement lasted for about 2 h. Repetition of this dose hours later, after some spontaneous improvement had occurred, had much the same sort of effect as the first dose, but may have increased the rate of spontaneous recovery.

WIN2299

Fine et al. (276) found that the ester of cyclopentyl-2-thienylglycolic acid and diethylaminoethanol (WIN 2299) antagonized the muscarinic (lowering of blood pressure of the dog by intravenous injection of acetylcholine at 5–10 µg/kg), the nicotinic (increase in blood pressure by intravenous injection of acetylcholine at 300–400 µg/kg into an atropinized dog), and the central nervous system (production of large-voltage cortical discharges in unanesthetized curarized cats by Sarin) effects originated by cholinergic or anticholinesterase compounds. WIN2299 had intravenous and subcutaneous LD_{50}s for the mouse of 80 µg/kg and 460 mg/kg, respectively, according to information supplied by the Sterling-Winthrop Research Institute. Daily intramuscular doses of 29.3 µg/mouse, beginning on the day before irradiation was started and continuing until death, prolonged slightly (and more than atropine) survival of mice subjected to whole-body irradiation with 550 R of xradiation, according to Haley and Rhodes (277). The mortality was still 100%. These authors attributed the prolongation of life to diminution in activity of intestinal smooth muscle by the anticholinergic compound, with consequently slower leakage of infective and toxic entities into the body of the mouse from the intestinal lumen.

Pennes and Hoch (278) administered tablets of WIN 2299 to seven patients. Two patients given doses of 2 mg exhibited principally sedative effects. In addition, one of these people described visual illusions and "hypersensitivity to light and sound." Four patients given 6 mg experienced illusions, delusions, and feelings of unreality. The single patient given 10 mg had full-blown delirium, with complete disorientation and visual and auditory hallucinations. No information was supplied on the time courses of these effects.

Fink (279) supplied some information about the time course of effects of WIN 2299. He gave 2.0–3.2 mg intravenously and found that the subjects began to feel restless and excited by about 10 min after the injection. These initial effects might develop into tense fearfulness with visual illusions and delusions. Complaints of dryness of the mouth were elicited by questioning, but were not made spontaneously. The heart rate did not change unless or until excitement and hyperactivity developed. The effects of these doses of WIN 2299 lasted for 2–3 h.

Grob et al. (147) reported the effects of oral doses of 0.5–2.0 mg of WIN 2299 administered on seven occasions to two volunteers who weighed 46 kg (volunteer 1) and 77 kg (volunteer 2). A dose of 0.5 mg to volunteer 1 produced feelings of fullness and heaviness of the head, of being far away from the immediate environment, and of walking unsteadily with subjective and objective impressions of mental depression, with spells of crying. The same dose given to volunteer 2 produced only a sensation of heaviness of the head that began about 22 min after ingestion of the dose and that lasted for about an hour.

A dose of 1 mg to volunteer 1 produced the same effects as the smaller dose, but to a greater degree. There was no effect on heart rate, pupil diameter, or muscular strength. Effects began about 45 min after ingestion and ended about 5.5 h after ingestion. This dose had marked effects on volunteer 2, beginning 20–40 min after ingestion with feelings of slowed movement of the arms and the legs and of slowed speech and thought. These effects were followed by sensations of swelling of the tongue and face, dryness of the mouth, weakness of the limbs, mild giddiness, and marked mental depression. Objectively, there was obvious mental depression, with listlessness, apathy, asthenia, weeping, poor attention, slowed speech, thought, and response, impaired memory (particularly for events in the immediate past), mental clouding and confusion, inability to perform simple calculations, and drowsiness. The subject's grip strength did not change, but the ability to hold arms or hands raised from a position of rest decreased. There was slight objective unsteadiness in walking and slight asteriotaxic effect. There were visual illusions and a slightly increased activity of tendon reflexes. The heart rate and pupil diameter did not change.

A dose of 1 mg of WIN 2299 to volunteer 1 during a period of taking daily oral doses of 8 mg of TEPP produced the same effects as before, but time to onset was longer (90 min) and the effects were a little less severe than when TEPP was not being taken. On the basis of this experience, two additional volunteers (weights, 66 and 53 kg) who were receiving doses of 13–20 mg of TEPP during 5–10 h were given doses of 1 mg of WIN 2299. Ingestion of WIN 2299 had a slight inhibitory effect on mild symptoms induced by the TEPP, beginning after 20–40 min, but little or no effect on moderately severe symptoms. A second dose of WIN 2299 of the same size as the first taken 45 min after the first dose, or the ingestion of a single dose of 2 mg, had slight to moderate inhibitory activity on moderately severe symptoms induced by TEPP. These changes began about 30 min after ingestion of WIN 2299. The effects of WIN 2299 on the central nervous system were less severe in these subjects than in those who had not received TEPP. Atropine was a better antagonist of the effects induced by TEPP than WIN Z299.

NONESTER ANTICHOLINERGIC COMPOUNDS

Table I-4 contains the information that could be obtained on the single-dose lethal activities of the two substances in this group, benzetimide and mepiperphenidol. Information on the toxicities of these compounds was supplied by Janssen Pharmaceutica and Sharp and Dohme Institute for Medical Research, respectively. Benzetimide is 2-(N-benzyl-4-piperidyl)-2-phenylglutarimide, and mepiperphenidol is 1-(3-hydroxy-4-phenyl-5-methylhexyl)-1-methylpiperidinium bromide.

BENZETIMIDE

In addition to the information on single-dose LD_{50}s for benzetimide, Janssen Pharmaceutica supplied extensive information on subacute and chronic toxicities of this compound. Rats fed diets containing benzetimide or given daily subcutaneous doses 5 d/wk for 14 wk gained weight at a lower rate than control animals when they were fed a diet containing 10 mg/100 g, but not when they were fed one containing 1 mg/100 g or were given subcutaneous doses of 10 or 80 mg/kg. Daily subcutaneous injections of 1.25 mg/kg did not affect the rate of growth of the rats. In general, organ weights in these animals were below those in control rats. Brain weight was not reduced, however, so its relative weight increased. No pathemas or histopathologic changes were found consistently. Rats fed diets containing benzetimide at up to 10 mg/100 g for 21 mo had no unusual gross mortality due to the compound. Among the rats that died in the control group, males accounted for 37.5% of the deaths; among the animals fed diets containing benzetimide, males accounted for 52.2% of the deaths. Among those fed the diet containing the largest concentration of benzetimide, males accounted for 62.5% of the deaths.

Pregnant rats fed diets containing benzetimide at up to 20 mg/100 g on days 6–15 of pregnancy for one or three generations gave birth to litters of the same size as those borne by control rats and produced no abnormal offspring. Pregnant rats given daily subcutaneous doses of 0.63–160 mg/kg during the first 21 d of pregnancy experienced no striking effects at doses below 10 mg/kg. At 10 mg/kg, but not at 5 mg/kg, there was a decrease in the incidence of implantations to 60% of the females, compared with 90–92% in the controls. With daily doses of 40 mg/kg, one of 20 dams died before parturition, and the incidence of implantations decreased to 10% of the females. At the highest dose (160 mg/kg), three of 20 dams died, and no implantations were found. The weights of the offspring at birth were reduced slightly, but no abnormal offspring were reported.

Pregnant rabbits given benzetimide by gavage at 0.31 or 2.5 mg/kg on days 6–18 of pregnancy had increased numbers of dead and resorbed fetuses (28.5% vs. 7.7% in the control group), and one of 59 offspring born to the rabbits given the largest dose of benzetimide was deformed. These various experiments have demonstrated that benzetimide in the doses used gave no evidence of mutagenic or carcinogenic activity and only questionable evidence of teratogenic activity. High doses of the compound did interfere with reproductive processes.

Janssen and Niemegeers (280), on the basis of tests with prophylactic administration of benzetimide to rats given pilocarpine hydrochloride intravenously at 80 mg/kg, concluded that benzetimide had sufficient action on the central nervous system to justify its consideration as a theraupeutic agent in parkinsonism. They considered also that it was one of only three compounds, in a total of 23 anticholinergic substances examined, that were able at submydriatic doses to prevent lacrimation and salivation induced by pilocarpine.

Soudijn et al. (281) examined the binding of enzetimide to subcellular fractions of the rat caudate nucleus and found both in vitro and in vivo that the (+) isomer was bound to the particles in a fraction composed of membranes and nerve endings to the extent of 4–7 times the (−) isomer. Fractions composed more nearly exclusively of membranes or of nerve endings bound the labeled material less extensively than the fraction that contained both structures. Binding of benzetimide in vitro was reduced markedly by atropine and by carbachol, but only slightly, if at all, by d-tubocurarine.

Beld and Ariens (282) measured the binding of benzetimide to microsomes from smooth muscle isolated from bovine tracheae and to particles in homogenates of bovine caudate nuclei. Plotting the amount of [^{3}H]benzetimide bound to the microsomes as a function of the concentration of [^{3}H]benzetimide in contact with them yielded a biphasic curve for (+)benzetimide, whereas that for (−)benzetimide was rectilinear. At a benzetimide concentration of about 6.3 nM, 9 times as much of the (+) isomer was bound to microsomes from vascular smooth muscle as of the (−) isomer. The particles in a homogenate of caudate nucleus bound about 5 times as much of the (+) isomer as of the (−) isomer. The saturable component of the material that bound (+)benzetimide had a higher affinity for the isomer than the nonsaturable component, which bound (−)benzetimide about as well as it bound the (+) isomer. When the microsomes were exposed to a large concentration of unlabeled (+)benzetimide before addition of [^{3}H]-labeled (+)benzetimide, binding of the labeled isomer seemed to be only to the unsaturable component. Atropine was found to bind to the same entities as benzetimide and to block binding of [^{3}H] (+)benzetimide by the saturable component of the microsomal preparation.

Karger (283) gave 17 volunteers benzetimide intravenously at 1.0–10.0 μg/kg. He estimated that the MED_{50} for lowering the score in the Number Facility Test to 75% of the baseline value for 50% of a group of subjects was 8.9 μg/kg. All men who met this criterion for a minimal central action had difficulty in focusing, which lasted in one case for 24 h.

Klapper et al. (162) re-examined one subject who had received benzetimide at some unstated time in the past. No evidence of long-term effects was found.

MEPIPERPHENIDOL

White et al. (152) gave six volunteers intramuscular injections of 100–300 mg of mepiperphenidol (1.17–3.63 mg/kg) and found that all six had blurred vision and receding of the near point of vision. These effects had nearly disappeared by the following morning. Urinary urgency and an increase of 10.8 mm Hg in mean diastolic pressure were particularly striking effects. The mean systolic pressure increased by 5.9 mm Hg, and the mean heart rate by 45.3 beats/min. The mean breathing rate increased slightly. All the subjects complained of dryness of the mouth, and all but one of becoming drowsy and of feeling unsteady in walking. The dose of mepiperphenidol increased the effectiveness of photic driving of the EEG in half the subjects and reduced the alerting reaction in one of the six subjects. Two subjects complained of eye irritation.

Nine subjects given intramuscular injections of 1.95–6.5 mg of atropine sulfate (0.031–0.064 mg/kg) and 150–300 mg of mepiperphenidol (2.21–3.99 mg/kg) all had blurring of vision and marked receding of the near point of vision, which was still somewhat farther away than normal on the following morning (mean, 10.1 in. vs. 5.3 in.). The mean diastolic and systolic blood pressures increased by 10.0 and 5.6 mm Hg, respectively. The mean heart rate increased by 37.9 beats/min. All nine subjects complained of dryness of the mouth, and eight of drowsiness and ataxia. Four of these subjects experienced slight nausea. Three complained of eye irritation.

According to the data supplied by the Sharp and Dohme Institute for Medical Research, mepiperphenidol is one-tenth as active as atropine in antagonizing the actions of methacholine chloride on the dog. If this value is used to calculate an atropine-equivalent dose for the subjects who received both atropine and mepiperphenidol, those who received sufficient doses of the two substances to yield atropine-equivalent doses of more than 0.320 mg/kg had a four-in-seven chance of developing nausea. The four persons who experienced nausea in this group were three men and one woman; the remainder of these subjects given atropine-equivalent doses above the threshold dose were two men and one women. In the group given atropine alone, the man who was the only subject who complained of nausea had received atropine sulfate at 0.037 mg/kg. Eight other subjects (six men and two women) had been given larger doses of atropine; two of these subjects complained of headache (accompanied by dysmetria in one), and a third complained of vertigo. In the group given mepiperphenidol alone, a woman who had received an atropine-equivalent dose of 0.131 mg/kg complained of slight nausea. Four subjects in this group (three men and one woman) received larger doses of mepiperphenidol than the nauseated subject, without making any complaints.

On comparing the changes in the mean values for the objectively measured properties induced by the three drug regimens used by White et al. (152), those for the subjects given both atropine and mepiperphenidol were below those for the subjects given either compound alone for systolic and diastolic blood pressures and were above those for the subjects given either compound alone for the rate of respiration and the near point of vision. The change in the mean pupil diameter for the subjects given both compounds was the same as that due to mepiperphenidol alone and greater than that due to atropine alone, whereas the change in mean heart rate for the subjects given both agents was almost precisely halfway between those due to the individual compounds. On the basis of this analysis, atropine and mepiperphenidol appear to be mutually antagonistic in relation to the regulation of blood pressure, to be somewhat antagonistic in their actions on heart rate, to be slightly mutually antagonistic with respect to breathing rate and activity of the ciliary bodies in the eye, and to be neither antagonistic nor additive in action on the circular muscle of the iris.

Jager (94) found, in in vitro experiments, that the atropinase in rabbit serum was inhibited strongly by mepiperphenidol, whereas the atropinases in rabbit and guinea pig liver were comparatively resistant to inhibition by mepiperphenidol.

"TAB" MIXTURE

This mixture contained 50 mg of atropine sulfate, 210 mg of N-diethylaminoethyl benzilate (benactyzine), and 2 g of trimethylenebis(4-hydroxyiminomethylpyridinium)dibromide (TMB-4) in 100 ml of solution. Wiles and Ford (284) summarized the results of experiments in which rats, rabbits, dogs, and monkeys were given intramuscular injections of graded single doses of TAB. Rats of the two sexes were studied separately. All the rabbits used were males. Dogs and monkeys were unselected as to sex, but the groups of animals of these two species contained approximately equal numbers of the two sexes. The first sign of toxic actions seen in the rat, the rabbit, and the dog was ataxia; the first one seen in the monkey was ptosis of the eyelids.

Table I-5 summarizes the information obtained from the studies summarized by Wiles and Ford (284) and by Lee et al. (285). The intramuscular doses that resulted in the first signs of an effect in 50% of intact animals of the various species and in death of 50% are given. TAB appeared to be more lethal (by a factor of 1.67) to female rats than to male ones, although the threshold toxic doses for the two sexes were identical. The rat apparently was more resistant to intramuscular TAB than the other species tested, the male rat having an intramuscular LD_{50} nearly 4 times the mean of those for mice, rabbits, dogs, and monkeys. Dogs given 22.6 mg/kg had the activity of their serum glutamic-oxaloacetic transaminase (SGOT) increased during 5 h after injection by a mean of nearly 85%. Thereafter, SGOT fell; at 7 d after injection, it was not significantly different from the control value. Monkeys that died 2–3 d after injection had liver necrosis and deposition of collagen in the renal glomeruli. Monkeys had a series of alterations of the activity of SGOT similar to that reported for the dog, the mean activity 5 h after injection being increased by more than 118% above the baseline activity and decreasing slowly thereafter. Lee et al. reported that all mice that died did so within 2 d after a single injection of TAB, which these investigators concluded was no more lethal than the TMB-4 in the mixture.

Wiles and Ford (286) recorded the results of an experiment in which animals were given subcutaneous (rats) or intramuscular (dogs and monkeys) doses of TAB at 1.2–41.2 mg/kg 5 d/wk for 4 wk. Rats were hyperactive for about 1 h after each injection, whereas the dogs (beagles) and monkeys were less active after each injection than they had been before it. The response of the iris to illumination of the retina in the dog and the monkey became sluggish to absent, depending on the dose of TAB. Three rats, all from a group given doses of 12.9 mg/kg, died during the experiment; a male died after the fifth dose, and two females died after the last (twentieth) dose, but before they were to be subjected to necropsy. No definite cause for these deaths was found. No other animals used as subjects in the experiment died during the 4-wk study.

Holgate and Sidell (287) reported the results of an experiment in which three volunteers were given intramuscular injections of 2 ml of TAB and three other volunteers were given ones of 4 ml. By 9 min after injection, the mean performance in the Number Facility Test had been reduced to 81% (2 ml) or 59% (4 ml) of the baseline performance; by 5.5 h, performance was 98–99% of the baseline performance. The heart rate increased slightly (by 6.6% after 2 ml; by 44% after 4 ml) within the 0.5 h after injection; 6.5 h after either injection, it was below the original value. Systolic and diastolic blood pressures increased slightly after injection and then slowly fell to below the original values within 4.5–6.5 h after injection. The pupil diameter increased after both doses, and both near and far visual acuities decreased.

The most common complaints voiced by the subjects of the experiment were of dryness of the mouth, muffled and indistinct speech, dizziness, weakness of the arms and legs, diminution of the sense of balance, and slowing of reflex responses. Other complaints made less frequently included those of thirstiness, sleepiness, disorganized activity, distraction by noise, restlessness, uncoordinated mental activity, inability to focus the eyes, blurred vision, muscular twitching, weariness, discomfort in the throat during swallowing, malaise, inappropriate desire to laugh, nausea, weakness of the voice, and rubbery legs.

Sidell (288) described an experiment similar to that of Holgate and Sidell (287) in which four volunteers were given intramuscular injections of 2 ml of TAB solution and another four were given 4 ml. The smaller dose resulted in a decrease in the mean score in the Number Facility Test of 40% during the 15 min after injection, whereas the larger dose resulted in a decrease of 78% during the same time. The score returned to its preinjection value at a mean of 2.25 h after injection of the smaller dose, whereas 2–5 h were required for a return to the preinjection value after the larger dose (no mean time for the four volunteers given this dose was stated). Three of the four men given the larger dose experienced hallucinations.

The smaller of the doses administered by Sidell resulted in a slight increase in supine systolic blood pressure for 1–1.5 h after injection and a more marked and prolonged increase in supine diastolic blood pressure. Standing systolic blood pressure was 5–8 mm Hg below supine systolic blood pressure, whereas standing diastolic blood pressure was about the same as that in the supine position. Mean pupil diameter at 5 h after injection was about 0.5 mm greater than that before injection. After the larger dose of TAB, supine and standing systolic pressures were increased for about 0.75 h after the injection, and supine diastolic pressure was increased for about 3.5 h. Standing diastolic pressure was about the same as that before injection. At 5 h after injection, the mean diameter of the pupil was 2 mm greater than that before injection.

Sidell reported that the subjects who received the smaller dose of TAB had xerostomia, blurred vision, “dizziness” or light-headedness, drowsiness, slightly impaired coordination, and some difficulty with concentration and memory. These appeared 5–10 min after injection, and the subjects generally were free of symptoms by about 1 h after injection. One subject was markedly nauseated, vomited, and complained of a severe headache. The subjects who received the larger dose were grossly uncoordinated, one being unable to stand without assistance, in addition to having xerostomia and blurred vision. Three of the four men had hallucinations, poor attention spans, impaired ability to concentrate, and poor memory. The effects started within 5 min after injection, peaked at about 30 min, and had largely subsided by 2 h after injection. Two of the subjects, one from each dosage group, who had been made quite uncomfortable by the effects of TAB were treated with physostigmine 18 min after receiving TAB. In both cases, the toxic symptoms were ameliorated within 10–15 min after administration of the physostigmine.

Holgate and Sidell (289) summarized the effects of intramuscular injection of 2 ml of the TAB solution into three subjects who were exposed in a hot room at 35°C and a relative humidity of 90% for 1 h before the dose of TAB and for 2 h after the injection. Merely increasing the temperature and the humidity was not found to be sufficient to produce an increase in heart rate, so long as the subjects remained at rest. From the dose-response data reported earlier, a single dose of TAB failed to have much effect on this rate. Yet the combination of TAB and increased temperature and humidity greatly increased heart rate. Cognitive function was not significantly altered under the conditions of the control trials, although there was a tendency for the exercising subjects to have lower scores in the Number Facility Test than the group at rest. Comparison of the effects of a single dose of TAB in the dose-response study with those in this study indicates a synergistic relationship between TAB and thermal stress.

Holgate and Sidell (290) described an experiment in which eight volunteers were given intramuscular injections of 2 ml of the TAB solution after having trained in the performance of a lever-pressing task that involved both estimation of time and vigilance; four other volunteers were given intramuscular injections of 4 ml of the TAB solution after training in the same task. The 2-ml and the 4-ml doses induced increases in the mean heart rate of 30% and 90%, respectively. The maximal change in heart rate occurred 10–20 min after injection and lasted for about 4 h after the larger dose. After the smaller dose, the subjects continued to be able to estimate time reasonably well and to respond effectively to visual signals in the vigilance portion of the task. The larger dose of TAB resulted in failure by the subjects to initiate timing responses and to respond to visual signals in the vigilance part of the task. Auditory signals of the erroneous estimation of time and of failure to respond to the visual signals still evoked intermittent, but usually late, responses by these subjects. Holgate and Sidell concluded that the subjects could continue to function at a reduced level after the lower dose of TAB, but were virtually incapacitated after the larger dose.

It is apparent from the signs and symptoms of intoxication by TAB that the sublethal effects are due primarily to the anticholinergic components of the mixture. This impression is strengthened by the reported beneficial effects of administration of physostigmine to seriously intoxicated subjects. However, the data in Table 5 on the lethal effectiveness of the TAB mixture, in comparison with data in Tables 1 and 2, show that the LD_{50} of TAB is closer to that of TMB-4 than to that of either of its anticholinergic constituents. Lee et al. (285) estimated that the intramuscular single-dose LD_{50} of TMB-4 for male mice was 53.5 mg/kg, whereas that of TAB was 64.5 mg/kg. The corresponding figures for benactyzine hydrochloride and for atropine sulfate were 92.1 and 604 mg/kg, respectively. Mice that died after doses of TAB, of TMB-4, or of benactyzine hydrochloride did so after bouts of clonic and tonic convulsions, whereas those which died after doses of atropine sulfate were severely depressed and ataxic. Mice given benactyzine hydrochloride were very sensitive to tactile or auditory stimuli, which often originated seizures. This triggering effect was not reported for the mice given either TAB or TMB-4. The hindleg paralysis described as affecting only those mice given TAB may have resulted from a combination of the ataxic action of atropine and the blocking activity of TMB-4 at the neuromuscular junction.

DISCUSSION

The initial interest of the chemical defense establishment in anticholinergic compounds was related to their use in antagonizing the toxic actions of the nerve gases. The early research on the biologic actions of this group of compounds performed by Edgewood Arsenal and its contractors was directed toward identification of the limits within which atropine might be used advantageously as a prophylactic and therapeutic agent against intoxication by inhibitors of cholinesterases and toward determination of whether any synthetic anticholinergic compound, which might be more readily available than atropine during a conflict, had safety and efficacy at least equal to those of atropine. For example, the group of investigators at the Johns Hopkins Medical School (Abner McGehee Harvey, David Grob, Joseph Lilienthal, Jr., Richard Johns, John Harvey, etc.) studied several synthetic anticholinergic compounds that had seemed in preliminary tests with animals to have some superiority over atropine in antagonizing the toxic effects of inhibitors of cholinesterases. This was done in part by treating cholinergic crises, in patients with myasthenia gravis who were being given inhibitors of cholinesterase (DFP, TEPP, Sarin, and neostigmine) to increase their muscular strength, with the synthetic anticholinergic compounds and comparing the results of such treatment with those of similar administrations of atropine. Some normal volunteers also were used in this sort of research.

Unfortunately, none of the anticholinergic compounds tested as outlined above was found to have any superiority over atropine. The research with WIN 2299 summarized in one of the reports (147) included in the group submitted for this survey is an example of the work of the group at the Hopkins. Other groups doing research on anticholinergic compounds in the same era were those at Galesburg State Research Hospital (Harold Himwich and associates), Galesburg, I11. (144,157), at the Montefiore Hospital (Yale Koskoff and associates), Pittsburgh, Pa. (158), and at the University of Illinois School of Medicine (Archer Gordon and associates), Chicago, I11, (27,151).

After a decision had been made to seek a new type of chemical agent among compounds that had disruptive actions on the normal functions of the central nervous system, anticholinergic compounds became of interest again, because of the well-known activities of atropine and scopolamine in producing a temporary toxic, psychotomimetic state. Most of the reports in this survey belong to this later period, which started in about 1953. TAB, the last substance discussed in this survey, was intended to be a therapeutic agent in nerve-gas intoxication for self-administration by soldiers, as well as for use by medical personnel. It combined the peripheral and central anticholinergic actions of atropine and benactyzine with the principally peripheral cholinesterase-reactivating activity of trimedoxime (TMB-4). It was devised in approximately 1975 after Russian chemical protective kits captured by the Israelis were found to contain somewhat similar preparations.

Although the anticholinergic substances considered in this survey differ in chemical constitution, all seem to produce about the same effects. There is some variation in the balance between central and peripheral actions among these substances, the compounds containing quaternized amino functions having almost exclusively peripheral actions when moderate doses are administered. The anticholinergic compounds said to have been administered to volunteers, but not mentioned in the reports submitted for this survey, may reasonably be expected to have actions similar to those of the 24 that have been discussed, because of the similarity of actions among the 24.

Single-dose LD_{50}s for mice, rats, and rabbits have been found for 17 substances other than atropine that permit their lethal activities to be related to that of atropine. On the basis of atropine=100, the lethal activities of single doses of the 18 substances fall in the following order of increasing potency: methylscopolammonium, scopolamine, d-hyoscyamine, atropine, methylatropinium, WIN 2299, caramiphen, EA 3834, 302,668, benzetimide, mepiperphenidol, 3-quinuclidinyl benzilate, benactyzine, L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate, TAB, tropinyl benzilate, Ditran, and L-2-alpha-tropinyl benzilate. No information on the single-dose lethalities of EA 3443, EA 3580, 302,196, 302,212, 302,282, 302,537, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, or 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate were provided or could be found in the literature. Such information should exist; in its absence, no precise judgment on the safety of these compounds can be made. That is not to say that they are unsafe. The human studies carried out with these compounds indicated that none had persistent effects on the volunteers beyond several days after single doses.

Among the 13 former volunteers who had been given at least one of the compounds with which this report is concerned and who were re-examined by Klapper et al. (161) in 1970–1971, only one, of two who had received 3-quinuclidinyl benzilate, reported having experienced a flashback. The other abnormalities reported by or found in the nine former volunteers who had been given atropine, scopolamine, or 3-quinuclidinyl benzilate probably were unrelated to their serving as volunteers. The four men who had been given EA 3834, 302,668, L-2-alpha-tropinyl benzilate, or benzetimide apparently had no abnormalities.

Among the 16 compounds for which doses effective in producing some definite alteration of function were stated, data are available for 11 after intramuscular injection. The other five compounds were administered by intravenous injection. Because we have in no case an indication of the relative effectiveness of intramuscular and intravenous injections of these five agents, it is impossible to rank all 16 in order of relative effectiveness. Among the compounds administered to volunteers by intramuscular injection, the most active in inducing a degradation in normal function—most commonly assessed on the basis of the dose required to cause loss of at least 25% of the predose score in the Number Facility Test by 50% of a group of subjects—was EA 3443, followed fairly closely by EA 3580, EA 3834, and 3-quinuclidinyl benzilate. This ranking agrees with that by Lavallee (50), except for EA 3834, which was not included in that author's rating. Less active than 3-quinuclidinyl benzilate, in order of decreasing effectiveness, were mepiperphenidol, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, methylscopolammonium, and atropine.

Among the compounds administered by intravenous injection, the most active were 302,212 and 302,537. The intravenous doses of these two compounds required to induce a definite degradation in normal function in the volunteers were larger than the intramuscular dose of 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate required to satisfy the same criterion; thus, one surmises that these two compounds are probably weaker than the one or two agents that follow 2-methyl-3-quinuclidinyl-L-(phenyl-cyclopentyl)-glycolate in the list of compounds injected intramuscularly. The other compounds injected intravenously, in order of decreasing activity, are 302,668, 302,282, and benzetimide.

Regarding the 16 compounds for which incapacitating doses—usually calculated as the doses required to degrade the score in the Number Facility Test to not more than 10% of the predose score—were given, three routes of administration were used: intramuscular injection, intravenous injection, and ingestion. The most effective of the compounds injected intramuscularly was 3-quinuclidinyl benzilate, followed in order by EA 3443, EA 3580, 3-quinuclidinyl-L-(phenyl-cyclopentyl)-glycolate, L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, 302,196, atropine, and Ditran.

The most actively incapacitating of the intravenously injected compounds was 302,212; its incapacitating dose was larger than that of 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, so it probably fits into the series of compounds given by intramuscular injection two or more compounds below 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate.

Of the two compounds administered orally, EA 3834 was slightly more than twice as effective as WIN 2299. Its effective oral dose was about 44% greater than the ID_{50} for intramuscular L-2-alpha-tropinyl benzilate. EA 3834 apparently ranks fairly high in comparative ability to produce incapacitation, and WIN 2299 probably falls near the middle of the hierarchy of compounds with this type of activity.

Among the seven compounds injected intramuscularly for which times to onset of effect were stated, the most rapidly acting was 302,196, followed closely by scopolamine. Then followed in order L-2-alpha-tropinyl benzilate, 3-quinuclidinyl benzilate, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, EA 3580, and 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate. Among the intravenously injected compounds, the one with the fastest onset of action was 302,668, with a time to onset of 30 min, whereas intramuscularly injected scopolamine and L-2-alpha-tropinyl benzilate had times to onset of 15 and 60 min, respectively. WIN 2299 had a time to onset of 40 min after ingestion, whereas EA 3834, by the same route of administration, had one of 168 min. The two compounds other than 302,668 injected intravenously, 302,537 and 302,212, had times to onset of 90 and 240 min, respectively, so they probably fall in the middle and lower parts of the entire hierarchy.

Two of the 12 compounds for which durations of effects were stated are outstanding in this regard: 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate and 3-quinuclidinyl benzilate, with mean durations of incapacitation of 69 h. L-2-alpha-Tropinyl-L-(phenyl-cyclopentyl)-glycolate came in a weak third, with an estimated duration of incapacitation of 27 h. The other nine compounds for which this measure is available had durations of action between 90 min and 6 h. In order of increasing duration of effect they are EA 3580, 302,196, 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, EA 3834, WIN 2299, 302,668, and 302,212.

Because of the particularly long durations of their effects, 3-quinuclidinyl benzilate and 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate (EA 3167) might be more hazardous to both corporeal and mental health than the other compounds on which reasonably complete information is available. However, 3-quinuclidinyl benzilate has one of the most favorable ratios of LD_{50} for small rodents to ID_{50} for humans. The corresponding ratio for EA 3167 cannot be stated, because no data on its LD_{50} for experimental animals have been provided.

Of the five quinuclidinyl compounds on which information derived from experiments with human subjects was provided, we have measurements of lethality for experimental animals only for 3-quinuclidinyl benzilate. The only basis on which we can compare this group of compounds is effect on the volunteers. Of these five compounds, 3-quinuclidinyl benzilate is the most potent as an incapacitating agent, and it and EA 3167 (the next most potent of the five as an incapacitating agent) have particularly long durations of action, requiring 3–5 d for complete recovery. The other three compounds in the group have durations of incapacitation of about 2–6 h. As a group, these compounds are not particularly rapid inducers of whatever changes follow their administration; even after intravenous injection, 302,212 and 302,537 required 4 and 1.5 h, respectively, to induce incapacitation. After intramuscular injection, 3-quinuclidinyl benzilate, EA 3167, and 301,060 required 1.25, 2, and 5 h, respectively, to induce incapacitation. These long times contrast with 12 min for 302,196—N-methyl-4-piperidiylcyclopentyl-(1-propynyl)-glycolate. They probably reflect comparatively slow penetration into the brain and adsorption onto nerve cells there. The basis for the differences is not readily apparent.

In both animals and man, the earliest symptoms and signs that follow administration of any of the anticholinergic compounds are restlessness, a feeling of dryness of the mouth, dryness and flushing of the skin, and decreased motility of the gastrointestinal tract. The effects produced by these compounds can be categorized as effects on or through the central nervous system, effects on peripheral effectors, and effects on or through nucleotides.

The effects mediated through anticholinergic actions on the central nervous system include restlessness, shortened attentiveness and decreased ability to concentrate on a topic, sensation of dizziness or faintness, tiredness, lassitude, drowsiness, sensations of heaviness and of altered shape of the limbs, apprehension, flattening of the EEG and decrease in the predominant frequency, weakening or abolition of the visual alerting reaction in the EEG, increase in photic driving of the EEG, ataxia, nausea, mental slowing, underestimation of elapsed time, decreased sensitivity to pain, obstinate progression, vomiting, incoherent and extravagant ideation, xanthopsia and other chromatopsias, disturbed sleep, hyperreflexia with development of Babinski's sign, illusions, disorientation, hallucinations (predominantly visual), somnambulism, delirium (possibly violent), and coma with occasional convulsions.

Effects of anticholinergic compounds that may depend primarily on actions at neuroeffector junctions include dryness of mouth, difficulty in swallowing, hoarseness and dry cough, dryness of nasal mucosa and nasal stuffiness, dryness of conjunctivae, relaxation of smooth muscles of the intestinal tract with decreased peristalsis, vasodilatation in conjunctivae and sclerae, brightness and dryness of the eyes, mydriasis and cycloplegia, flushing of face and upper chest, increased body temperature during exercise or in a hot environment, temporary bradycardia (rarely, but possibly, with cardiac standstill), tachycardia, slightly decreased systolic blood pressure, increased diastolic blood pressure, headache or eyeache, difficult urination, dyspnea, atrial fibrillation, atrial ventricular dissociation, and ventricular fibrillation.

The only compound in this group whose fate in the body has been studied to a moderately satisfactory extent is atropine. Some information on the metabolic fates of 3-quinuclidinyl benzilate and of diethylaminoethyl benzilate is available (193, 219), but it does not account completely for all parts of the molecules. The binding of 3-quinuclidinyl benzilate to nerve cells (193,194) and to organelles from such cells (195) has been studied, but no detailed studies of its detoxification, pharmacokinetics, and molecular pharmacology have been found for use in this review. Little or no information on the biochemical aspects of the activities of the anticholinergic compounds surveyed here has been found. Whether such information exists in literature that has been withheld from consideration for this survey is unknown.

So far as the general impact of anticholinergic compounds on human health is concerned, the principal ill effect that has been attributed to long-term administration of these compounds is the induction or exacerbation of the dyskinesia that may occur after long-continued use of neuroleptic compounds. This condition is characterized by intermittent protrusion and rotation of the tongue and by chewing or biting movements of the jaws and mouth, in many cases with athetoid or choreiform movements of the limbs (292–296). The condition has been reproduced in animals, at least in part, by administration of neuroleptic drugs (297, 298). Scopolamine was found to increase the intensity of the experimental syndrome induced in mice by such drugs as methylphenidate and teflutixol (298,299). By analysis of electromyographic records from patients with tardive dyskinesia during administrations of various drugs and procedures, Mano and associates (300,301) concluded that the dyskinesia is induced by relative hyperfunction of dopaminergic neurons in the striatum established by excessive inactivation of cholinergic neurons by anticholinergic drugs.

There is abundant evidence that medication with anticholinergic drugs is contraindicated in human tardive dyskinesia (302–309). Burnett et al. (308) found that the improvement in tardive dyskinesia after discontinuance of the anticholinergic drug, when both an anticholinergic compound and a neuroleptic drug had been used to treat schizophrenia, was not due to any change in the patients' serum concentration of the neuroleptic compound, but must have been due to withdrawal of an effect by the parasympatholytic compound. Gerlach et al. (310) had advanced essentially the same idea in suggesting that centrally active anticholinergic drugs administered to patients with tardive dyskinesia increased the predominance of dopamine in the brain stem (established there by the induction by neuroleptic drugs of supersensitivity to that effector substance) by decreasing the effect of acetylcholine on striatal receptors and thereby disturbing the balance between cholinergic and dopaminergic effects.

Martys (311) investigated the drugs responsible for "certain" and "probable" reactions in 335 patients in a total of 817 patients treated during a period of years, finding that 51% of all the reactions were caused by drugs that had actions on the central nervous system. The most common of these reactions were drowsiness, nausea, dizziness, diarrhea, headache, and dry mouth. When the symptoms collected by investigators (311–314) who had used a variety of anticholinergic drugs for various purposes are arranged in order of decreasing frequency of specific complaints, the following list results: incoherence, disorientation, flushed facies, hallucination, mydriasis and cyclopegia, restlessness, hyperactivity, picking or plucking motions with the fingers, ataxia, motor incoordination, coma, tachycardia, confusion, dryness of the mouth, hyperreflexia, apprehension, fear, somnolence, fever (above 37.8°C), retrograde amnesia, toxic delirium, nausea, diarrhea, headache, nocturia, urinary retention, impotence, painful ejaculation, tremor, gastralgia, rash, itch, and vomiting.

Pfeiffer et al. (312) found that children generally were somewhat more sensitive to antimuscarinic drugs than adults; the root mean square of the difference in order numbers based on the frequencies of signs and symptoms of intoxication by 3-quinuclidinyl benzilate between adults and children was 2.66. Children had higher frequencies of occurrence than adults for 10 of the 15 effects counted.

Craig (315) analyzed the nature of death of 48 patients in a mental hospital whose deaths were attributed to asphyxia and found that 14 of the patients had choked to death. Because therapeutic regimens for psychotic patients commonly include combinations of neuroleptic, antimuscarinic, and tricyclic antidepressant drugs and because all these categories of drugs have the ability to induce sensitization of neuronal effectors to dopamine or to prevent access of acetylcholine to cholinergic neuronal effectors, or both, Craig and associates (316) undertook a further study of the association among alteration of the gag reflex, possible drug-induced neurologic syndromes, and the administration of drugs to chronically hospitalized psychiatric patients.

Of the 58 patients in the study (316), 34 were considered to have normal gag reflexes, nine to have variable responses to touching the posterior pharngeal wall, and 15 to have definitely impaired gag reflexes. The last two groups were combined for the analysis of drug use. All 58 patients had been receiving neuroleptics and antiparkinsonism drugs; 12

(35%) of the 34 patients with normal gag reflexes had received anticholinergic drugs, whereas 15 (62%) of the 24 patients with variable or impaired gag reflexes had received anticholinergic drugs. In the group with normal gag reflexes, only 14 (41%) had facial-oral dyskinesia, and only 19 (55%) had dyskinesia of some part of the body. In contrast, 19 (79%) of the patients with variable or impaired gag reflexes had facial-oral dyskinesia, and 20 (83%) had dyskinesia of some part of the body. Craig et al. concluded that patients with tardive dyskinesia, and particularly those receiving anticholinergic drugs, are at high risk of having impaired gag reflexes and, consequently, of having swallowing impairments that increase their risk of death by asphyxia associated with entrance of food or other foreign material into the larynx.

Injections of atropine and hyoscine into chicken eggs on the fourth day of incubation have resulted (291) in increased rates of death of the embryos (e.g., 33% living on the twelfth day of incubation vs. 91% alive in eggs into which saline was injected) and of malformation (e.g., 33% of those alive on the twelfth day of incubation vs. 0% occurrence in the control eggs). Gastroschisis was the most common malformation, accounting for about 71% of the abnormalities found in the embryos. Despite this finding in the closed environment of the egg, there have been few findings suggestive of a teratogenic effect of anticholinergic compounds in mammals. One report (317) described an acranial and anencephalic fetus with complete atelectasis of the lungs, hemorrhage into the lungs, and hypoplasia of the adrenals born to a woman who had been treated during pregnancy for a duodenal ulcer with 10 mg of oxyphencyclamine two or three times a day. (As the saying is, one swallow does not make a summer; or, to corrupt Virgil's imperative, ab uno non disce omnes.)

The survey by the General Practitioner Research Group (136) found only one among 57 instances of reproductive accidents in the group of 661 women who had taken drugs of some sort during the first trimester of pregnancy that could possibly be related to an anticholinergic drug. It should be noted, however, that two-thirds of the accidents of reproduction found in this survey were related to the use of antihistaminic drugs, many of which have some anticholinergic activity. Although anticholinergic drugs are useful in ameliorating the results of lack of inhibition of contractions of the detrusor muscles of the bladder, the danger of causing the development of hydronephrosis by using such drugs in cases with obstruction of urinary outflow from the bladder has been noted (318).

With respect specifically to 3-quinuclidinyl benzilate, several drugs containing the quinuclidine ring, including quinidine, are in more or less regular use. The closest relative to 3-quinuclidinyl benzilate is clidinium bromide, which is a component of Librax. This preparation consists of one-third clidinium bromide and two-thirds chlordiazepoxide and is administered usually in unit doses of 7.5 mg, one or two such doses being prescribed three or four times a day. Several papers (319–323) have reported side effects experienced by patients advised to take Librax, usually for ulcers of the gastrointestinal tract. The most common side effects of use of this mixture were dry mouth and constipation. Other fairly common effects were drowsiness, headache, gastritis, nausea, and vomiting. Also mentioned in these papers were blurred vision, ataxia, tachycardia, and difficult micturition. Females were reported (319) to develop higher heart rates and greater tendencies to constipation than males; males had a greater incidence of difficult micturition than females. There was one report (321) of petechiae and thrombocytopenia in a woman who developed these conditions while taking one unit dose of Librax three times a day. After discontinuance of Librax, her platelet count, which had fallen to 12,000/mm^3, became normal (200,000–500,000/mm^3) within 5 d.

Although N-benzylnortropinyl benzilate was found (324) to be more potently antimuscarimic than 3-quinuclidinyl benzilate, the latter compound produced greater disruption of the behavior of mice and rats than any of eight other antimuscarinic compounds tested, including N-benzylnortropinyl benzilate.

Although one of the two former subjects who had been given 3-quinuclidinyl benzilate re-examined by Klapper et al. (162) was the only former subject to have had an apparent flashback, a conclusion that 50% of these subjects experienced flashbacks probably would be highly erroneous. The available evidence from studies with both animals and man is that recovery from intoxication with this benzilate, although it occurs slowly, is complete. Mice and guinea pigs given five subcutaneous doses per week of 3-quinuclidinyl benzilate at 150 ug/kg for 3 wk (total maximal dose, 2.25 mg/kg) were reported (184) to have developed no lasting signs of toxicity. The experience in experiments with both laboratory animals and man has been that generally the mydriatic and cycloplegic effects on the intrinsic smooth muscles of the eye are the ones that persist longest after doses of 3-quinuclidinyl benzilate. The cytotoxic actions of this benzilate (206,207) do not seem to have been reflected in definite heritable mutagenic effects.

The general conclusion of this survey is that there is no firm evidence that any compound surveyed carries a direct hazard of persistent diminution of human health and normal function in the doses used by Edgewood Arsenal and its contractors or an indirect hazard of abnormality of structure or function in offspring of the former volunteers. However, the cryptic activities of these compounds need to be studied much more intensively than they have been.

TABLE I-1 Representative LD50s of Tropic Acid Esters in Laboratory Animals

	LD_{50}s, mg/kg											
	Mouse				Rat				Guinea Pig			Rabbit
Ester	P.O.	S.C.	I.P.	I.V.	P.O.	S.C.	I.P.	I.V.	P.O.	I.P.	I.V.	I.M.
Atropine	693	695	236	74	773	1,750	256	41	1,100	277	163	588
d-Hyoscyamine	—	—	—	81	—	—	—	—	—	—	—	—
Scopolamine	—	1,700	119	163	1,270	3,800	—	—	—	—	—	—
Methylatropinium	1,320	—	110	—	1,605	1,800	—	—	—	—	—	—
Methylscopolammonium	3,200	—	—	3,800	2,480	2,060	126	—	—	—	—	—

TABLE I-2 Representative LD50s of Benzilic Acid Esters in Laboratory Animals

		LD_{50}s, mg/kg			
Animal	Route of Administration	3-Quinuclidinyl Benzilate	Tropinyl Benzilate	L-2-alpha-Tropinyl Benzilate	Diethyaminoethyl Benzilate
Mouse	I.V.	22	14	29	14
	I.P.	110	—	—	115
	S.C.	215	—	—	350
	P.O.	490	—	55	160
Rat	I.P.	—	—	—	113
	P.O.	—	—	—	166
Guinea Pig	I.V.	14	—	—	—
	I.P.	—	—	—	115
Rabbit	I.V.	10	—	—	15
	I.P.	—	—	—	115
Cat	I.V.	12	—	—	—
Dog	I.V.	12	—	—	—
Pig	I.V.	5	—	—	—
Goat	I.V.	7	—	—	—

TABLE I-3 Single-Dose LD50s of Phenylisopropylglycolic Acid Esters

		LD_{50}s, mg/kg	
Animal	Route of Administration	EA 3834	302,668
Mouse	I.V.	63.0	81.1
	I.P.	—	120.0
	P.O.	—	383.0
Rat	I.V.	—	63.5
	I.P.		153.4
	I.M.	262	—
Rabbit	I.V.	25.1	30.0
Cat	I.V.	—	49.0
Dog	I.V.	49.5	65.0
Monkey	I.V.	29.4	100.0

TABLE I-4 Single-Dose LD50s of Nonester Anticholinergic Compounds

		LD_{50}s, mg/kg	
Animal	Route of Administration	Mepiperphenidol	Benzetimide
Mouse	I.V.	20.0	46.0
	S.C.	—	640
	P.O.	970.0	305
Rat	I.V.	—	37.6
	S.C.	—	160
	P.O.	—	` 0 4
Guinea Pig	S.C.	—	153
	P.O.	—	825

TABLE I-5 Single-Dose Minimal Effective and Lethal Intramuscular Doses of TAB

Animal	ED_{50}, mg/kg[a]	LD_{50}, mg/kg[a]
Male mice	—	61±4.2
Male rats	36 (27,48)	251 (210,301)
Female rats	36 (10,134)	154 (131,179)
Male rabbits	45	66
Dogs	21	80
Monkeys	21	50

[a]Figures in parentheses are 95% confidence limits. The standard deviation of the mean is given for mice. Single values were derived from experiments whose results were not suitable for calculation of appropriate statistics.

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APPENDIX J

ANTICHOLINERGIC DRUGS AND THE EEG

by

Max Fink, M.D.

This review examines the effects of anticholinergic compounds on the human central nervous system, in relation to their military testing. Such compounds were tested in volunteers to define their role as incapacitating agents for military use. The question presented is whether, as tested, the chemicals are likely to produce adverse health effects or delayed sequelae in the test subjects, or whether it is possible even to predict that likelihood This review focuses on the data derived from electroencephalography.

The data include protocols of military experiments declassified for this review, reports published in the scientific literature on animals and man, and my own studies. Between 1956 and 1966, experiments were undertaken in my laboratories in New York and St. Louis to define the effects of anticholinergic drugs on brain function and behavior. We sought to determine whether any of the substances had persistent behavioral effects that could be useful in treating the severely mentally ill (1–7).

Atropine and scopolamine (hyoscine) are prototypic anticholinergic compounds. Their effects are principally antimuscarinic. Compounds usually classified with these prototypes are those used in the treatment of parkinsonism—such as procyclidine (Kemadrin), benztropine mesylate (Cogentin), and trihexyphenidyl (Artane)—and such medicinals as glycopyrrolate (Robinol) and methantheline (Banthine). Such experimental anticholinergic compounds as Ditran, JB-329, JB-336, and WIN 2299 are included in the same class. In some studies, particularly EEG studies in patients, the tricyclic antidepressants imipramine and amitriptyline have been shown to have atropine-like properties.

Electroencephalography developed from studies published in 1929. In the early studies, changes were assessed by visual inspection of ink-written records. These have since been replaced by electronic and, lately, digital computer methods of quantitative analysis. These methods provide excellent assessments of minimal changes in brain function in man. Unfortunately, the military reports are limited to inspection.

LITERATURE REVIEW

The effects of anticholinergic drugs on brain function have been studied extensively. CNS effects in man are defined principally on the basis of self-reports, observer evaluations,

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neuropsychologic tests, and physiologic measures. Of these, the EEG, especially in its present quantitative form, is a sensitive index of changes in CNS activity, with particular relevance to human performance and states of vigilance (8,9).

Anticholinergic drugs have well defined effects on the EEG, accompanied by measurable behavioral effects. In most drug studies, EEG patterns correlate well with behavioral changes. Studies of atropine in animals, however, elicited reports that their EEG showed increased amplitudes and lowering of frequencies at times when the animals were apparently restless. Animals examined in halters often exhibited running movements, but their EEG patterns were similar to those seen in deep sleep (10). The apparent purposeful movements associated with "sleep-like" EEG records led some observers to define an "EEG-behavioral dissociation" with anticholinergic drugs (1,11–15). In studies of psychoactive substances in man, we and others found a close relationship between the changes in EEG patterns and the behavioral effects of drug administration (8). The difference in observations between animals and man led to a symposium in 1966 that summarized the data available to that time (1). The symposium participants concluded that the apparent "dissociation" in EEG and behavior with anticholinergic drugs was limited to observations in animals and was an artefact of the gross nature of the measures used in animal trials, rarely, the inability to assess changes in cognition, vigilance, mood, and affect (which are principal targets of anticholinergic drugs).

Anticholinergic drugs have a characteristic dose-related effect on brain function, and particularly on the resting, alert scalp-recorded EEG (3,4,6,7,16–27).

Low doses, such as 1–2 mg of atropine, are sufficient to induce mild tension, irritability, and anxiety. The subjects are aware of changes in their perception and mood, and they make errors on cognitive tests. At these times, the EEG patterns exhibit an increase in high frequencies and decreases in the mean alpha frequency and in the amplitude of the dominant (alpha) frequencies. These effects may be accompanied by a decrease in heart rate and some minimal effects on salivation and skin conductance (28,29). With higher doses, such as 10–30 mg, or repeated administrations, the subjects become delirious, showing restlessness, impairment of motor and sensory functions, cognitive defects, illusory sensations, and thought disorder, including hallucinations and delusions. Heart rate is increased, and the peripheral effects of dry mouth and skin, decreased urination, and difficulty in near vision are prominent. The EEG shows an increase in slow waves, a decrease in mean frequency, a decrease in the percent time and amplitudes of alpha activity, and an increase in the high frequencies, which can be seen to be "riding" on the slow waves. There is a direct association between the amount of EEG fast waves with behavioral restlessness and the amount of EEG slow waves with stupor and cognitive defects. At "toxic" doses, patients are in stupor or coma, with rapid heart rate and lowered blood pressure. The EEG demonstrates persistent high-voltage slow waves, with a minimum of alpha and high frequencies.

In epileptic patients, single intramuscular administrations of atropine at up to 2 mg/kg (30) and 1.2 mg of intravenous hyoscine (24) elicited epileptic spike EEG activity. The changes were transient, and the records and behavior of the patients returned to normal within 24 h.

EEG and behavioral effects may be modified by the concurrent administration of other drugs. Thus, atropine (or Ditran) and chlorpromazine resulted in behavioral stupor that was very deep (allowing surgery without pain responses), accompanied by persistent EEG high-voltage slow waves and decreases in fast waves. When Ditran and yohimbine or Ditran and imipramine were given together, restlessness increased, as did the ratio of fast waves to slow waves in the EEG. When patients who exhibited a toxic delirium to Ditran or atropine were given tetrahydroaminacrin (THA), the stupor was relieved and the EEG showed decreases in both the low and high frequencies (1,7,31).

After acute administration of various compounds, the time for recovery varies with dose—at low doses, the peak effects on parenteral administration are seen in 0.5 h and last for up to 6 h; at high doses, the effects persist for up to 24 h; at toxic doses, there is a return to baseline values the second day after administration. Followup EEG data are limited, the principal data being reports of atropine toxicity. The few statements about EEGs sugggest that the effects are gone within a few days of the last exposure (32–37).

In studies of imipramine, some patients developed an acute psychotic reaction. These patients were identified as being young and as exhibiting a "schizophrenic" syndrome—an observation that led to administration of imipramine as a test of "schizophrenia" (2,38).

Although no long-term EEG study of anticholinergic drugs is available, one study of cholinesterase inhibitors may be cited. Duffy et al. (39) reported persistent quantitative differences in EEG patterns between workers exposed to the organophosphate compound sarin and a control group of workers in the same plant not so exposed.

MILITARY DATA

The principal military data on EEG studies are in the summaries provided in Case Report Summaries—Anticholinergics, and an addendum provided in a letter by Dr. Frank Marzulli of October 8, 1981. This latter summary included all the useful EEG records cited in the formal protocols of the military studies. No EEG records were found of subjects receiving atropine, scopolamine, EA 3443, or EA 3167. Of the available records, five were related to BZ, two to EA 3580, and one to EA 3834.

The reports stated that the pretreatment records were within normal limits and the postexposure records, taken at various times, were also within normal limits. The records were assessed visually. The reports did not state the conditions of testing, nor is it clear how long after exposure the testing was done.

These records were customary for the time and reflected nonspecific effects that are similar to those reported for many CNS active compounds. The reports did provide neuropsychologic-test data, however, which indicate that the effects of exposures to the anticholinergic substances on the military volunteers were transient, reverting to baseline values within a few hours or, in a few tests repeated at longer intervals, within a few weeks (40–49. In other studies of the relationship between the changes induced in EEG and in neuropsychologic tests by CNS-active drugs, there was a parallel in the onset and duration of the effects in the different measures. Arguing by analogy, we would anticipate similar reversions to normal for the EEG changes in these volunteers.

Conclusions

In the experiments in which patients and normal volunteers were exposed to single or multiple doses of anticholinergic drugs, a consistent pattern of EEG and behavioral change has been described. In most volunteer studies, doses have been low, exposures usually single, and effects transient. There is no evidence of persistence of behavioral or EEG effects in these experimental trials (50).

In patients who have been given heroic doses of atropine and scopolamine (up to 250 mg) and in whom the doses have often been repeated three times a week for up to 4 mo, there have been few signs of persistent toxicity. The patients have been subjected to periods of coma lasting up to a day. Death has been reported in only one instance. In many patients, the persistent effects have been considered salutary—i.e., the patients have been considered improved in mental state and discharged to the community! In the remainder, they have been reported to be no worse than before treatment. Considering the many hundreds of patients so treated and the continuation of this form of therapy in patients in eastern Europe today, it is unlikely that there is a behavioral syndrome of toxicity.

Focusing exclusively on the anticholinergic properties of the drugs examined in military volunteers, considering the low doses used and the minimal exposures, and aware that heroic doses of anticholinergic drugs (inexplicably) fail to stimulate a defined toxic syndrome, we deduce that the single exposures of doses of anticholinergic drugs used in the volunteers were insufficient to stimulate a persistent toxic syndrome. The data available are sufficient to conclude that, as tested, the chemicals are not likely to produce adverse health effects.

For a more definitive conclusion, a prospective study or a study similar to that reported by Duffy et al. (39) in parkinsonism patients or industrial workers exposed to anticholinergics is required.

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APPENDIX K

BIOCHEMICAL ASPECTS OF ANTICHOLINERGIC CHEMICALS

by

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There have been few published studies—an example is that by Jovic and Zupanc (1)—on the biochemical mechanisms associated with quinuclidinyl benzilate (BZ). A major attribute of atropine, scopolamine, and other anticholinergic compounds is their ability to interact with postsynaptic muscarinic binding sites. It is through such an action on smooth muscle that our ideas about BZ and other potent anticholinergics have developed. A property common to all is a high affinity for muscarinic receptor sites (K_D=0.05−0.1× 10^{-9} M). A regional study with [^{3}H] BZ of binding capacity in hippocampus, frontal cortex, and caudate nucleus of human brain showed (2), small decreases in these areas in Huntington's chorea patients as compared with normal human brain. Atropine and BZ were alike in binding capacity to these sites. Because of the high affinity of BZ for muscarinic receptors, Yamamura and Snyder (3) were first to show its binding to postsynaptic sites, but failed to find evidence of presynaptic sites in the CNS. The suggestion by Polak and Meews (4) that the increased release of acetylcholine in vivo could be accounted for by blockade of "presynaptic" muscarinic receptors therefore requires another explanation.

An early finding by Sacktor (5) is that acetylcholine (8.6× 10^{-5} M) increases phosphatidyl-L-serine, as well as phosphoinositide and phosphatidic acid. In addition, atropine (1.6 ×10^{-6} M) and BZ (7×10^{-5} M) blocked the ACh-stimulated incorporation of $^{32}PO_4$ into phosphoinositides, but stimulated incorporation of labeled phosphate into phosphatidyl serine. This suggests that, although events related to muscarinic receptor action are blocked by antimuscarinic drugs, other actions may be stimulated. It is known that calcium ions activate phospholipase-C and stimulate the turnover of phospholipids, such as triphosphoinositides, in excitable membranes. Calcium ions have diverse functions in the regulation of cell function. They are required for the release of acetylcholine (6) in neuromuscular transmission and they influence threshold and other attributes of CNS action potentials. It is estimated that intracellular "ionized" calcium is low in neurons (about 10^{-7} M). A small rise in intracellular calcium will produce a sharp rise in K^+ conductance, hyperpolarization, and depression of excitability. The slowed excitation of central neurons by the muscarinic actions of acetylcholine may be countered by anticholinergic drugs and thus account for many of the central effects of BZ in volunteers reported

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by Ketchum (7) and by Sidell (8). Most prominent among these are amnesia, confusion, and disorientation lasting 2 to 6 d. Although the effects of BZ are qualitatively similar to the pharmacologic actions of atropine and scopolamine, the potency and especially the duration of action of BZ are considerably greater. That situation is very likely related to properties other than the binding affinity, which is about the same for all these compounds for the muscarinic receptor (K_D=10^{-9} M).

The π -bond properties of quinuclidinyl compounds probably account for their "sticking" to tissue constituents for long periods. Experimentally, a 5-mg/kg dose of radiolabeled BZ prevented auditory stimuli for 7–8 d, but labeled drug was hardly detectable in brain after 3 d. Unfortunately, the only thorough study was that by Gosselin et al., (9), on (^{14}C]atropine, so direct comparisons are difficult. Although atropine abuse with 2,5-dimethoxy-4-ethyl amphetamine (STP) has been reported (10), the number of "bad trips" made its popularity short-lived. The absence of further reports on abuse of atropine-like drugs suggests that their abuse by the volunteers in question does not constitute a long-term problem.

Administration of atropine or scopolamine to animals increases acetylcholine output from the exposed cerebral cortex (11). This has led to the hypothesis of a direct effect on presynaptic muscarinic receptors that regulate ACh release. The finding of release when Ca^{2+}-free Ringer's solution is present (12) led to the suggestion that interneurons are more sensitive to a local decrease in Ca^{2+} ions than the cholinergic nerve endings whose release they modulate. Alternatively, the ability of BZ, Ditran, and other anticholinergic drugs to cause the release of calcium from intraneuronal binding sites (13) could also explain the in vivo observations. The role of ionized calcium in the release of ACh in neuromuscular and electroplax preparations is well characterized. It is persuasively argued that, when ACh emerges from a terminal, it has to pass through some sort of "gate" that is a Ca^{2+}-dependent channel; the "gate" is more likely to be open when the terminal is depolarized. The suggestion that control of ACh release is at the level of calcium displacement by BZ is important, because it is the Ca^{2+}-dependent property of "anticholinergic" drugs, and not their antimuscarinic actions, that explains why BZ and Ditran are much more active than atropine centrally. They appear to be equally effective peripherally, when comparisons are based on affinity for muscarinic binding sites.

Calcium ion contents can be regulated by any of the membrane systems that are in contact with the cytosol, e.g., plasma membrane, endoplasmic reticulum, and mitochondrial inner membrane. In brain, mitochondria are in the highest concentration at nerve terminals and represent the major supplier of cellular energy (ATP). Mitochondria can take up Ca^{2+} ions by an energy-requiring process and release free Ca^{2+} in exchange for Na^+ ions. The high capacity of isolated brain mitochondria to transport Ca^{2+} ions has led to the suggestion of a significant role for this organelle in ionic homeostasis. The early observations on energy metabolism demonstrated that Ditran and BZ selectively interfered with the increased energy needs in vitro of depolarized cerebral tissue. In contrast, atropine and scopolamine were either without effect or active only at much higher concentration. In the presence of isolated brain mitochondria, Ditran (JB339) was shown to interfere with ATP production (13). Calcium retention by brain mitochondria is lost when energy generation (ATP) is interfered with (14). The significance of these and other studies is that BZ and Ditran are quantitatively different from the less-potent atropine and scopolamine. A selective action on Ca^{2+} channels by BZ and Ditran on particularly vulnerable neurons would explain the unevenness in the correlation between the receptor-binding properties and behavioral alteration by antimuscarinic compounds in animals and man.

CONCLUSION

Reports of biochemical effects of anticholinergic compounds contain data on animals and may or may not permit extrapolation to man. There is evidence that the actions of quinuclidine derivatives are longer-lasting and the limited metabolic data available suggest that they may be retained in tissues for a longer period. Peripherally, the benzilates are acetylcholine antagonists with high affinity (low KDs). They bind reversibly to muscarinic receptors. Centrally, however, their actions are more complex, and pharmacodynamic and pharmacokinetic properties play an essential role. The available evidence is not entirely convincing that their basis of central action is through postsynaptic muscarinic receptor binding, and their presynaptic role in calcium metabolism must be seriously considered. Without morbidity data, our present biochemical information cannot help to predict long-term effects of exposure to anticholinergic agents.

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APPENDIX L

STRUCTURE-ACTIVITY RELATIONS OF THE CENTRALLY ACTIVE ANTICHOLINERGIC AGENTS

by

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The structure-activity relationships of a large number and variety of anticholinergics have been described in several reviews (1–3), and only the general features will be described here. The main difficulty in comparing chemical structure with psychopharmacologic potency is that unequivocal behavioral measurements of potency are deficient. Although some anticholinergics have been evaluated for their psychotomimetic potency in man (2,4), the comparisons are based largely on a battery of psychopharmacologic measures in animals, including hyperactivity (5), swim maze (6), characteristic exploratory head movements (2), and operant conditioning (7–9). The limitations and reliability of these comparisons have been discussed elsewhere (3), and they do permit generalizations concerning the relative ability of the anticholinergics to produce behavioral disturbances in animals that may reflect psychotogenic potency in man. Structural variations in the heterocyclic amino alcohol result in marked changes in potency; the most potent anticholinergic is 3-quinuclidinyl benzilate, with its rigid conformation (Figure L-1).

With respect to the acid moiety of the anticholinergics, the following structure-activity relationships are found:

As R_1 is increased from methyl to higher alkyls or becomes hydrogen, alkenyl, amino, or aminoalkyl, psychotropic potency diminishes without much effect on peripheral anticholinergic action.

R_2 should be an unsubstituted phenyl group, wherase R_3 must be either a cycloalkyl, alkynyl, thienyl, or unsubstituted phenyl. Alkyl, aryl, halide, or hydroxyl substituents on the phenyl rings abolish central action and diminish anticholinergic potency. R_2 and R_3 can also be replaced by hexahydrofluorenyl (10).

- As “n” is increased beyond 2 or “Y” beyond zero, psychotropic, but not anticholinergic, potency decreases. The position of the ester side chain affects central, but not peripheral action, with the 4-piperidyl ester being most potent, the 3-ester second most and the 2-ester least.
- R_4 must be a hydroxyl group, whereas compounds with hydrogen or an isosteric methyl group are devoid of central action and have diminished anticholinergic action. If R_4 is hydrogen and the hydroxyl group is present on phenyl, central potency is retained.

The duration of the psychotropic action of the various anticholinergics depends both on the type of heterocyclic amino

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group and on R_2 and R_3. The quinuclidinyl and pyrrolidyl amino derivatives tend to be longer-acting than piperidyl, tropanyl, or granatonyl; a cycloalkyl group in R_2 or R_3 prolongs duration. An alkynyl group in R_2 or R_3 decreases duration, whereas increasing chain length or branching of the alkynyl group correspondingly increases duration.

STEREOSPECIFICITY

The anticholinergic glycolate esters of heterocyclic amino alcohols, including scopolamine and related natural alkaloids, exist as optical isomers, resulting from the asymmetric C of both the amino alcohol and acid moiety. Of the two enantiomers of 3-diphenylacetyl quinuclidine, the (−) isomer had 25 times the antispasmodic potency of the (+) isomer; however, the isomers were of equal toxicity, whereas no difference in antispasmodic potency was noted between the two isomers of the quaternary derivative of 3-quinuclidinyl benzilate (11). With respect to their central action, the (+) and (−) enantiomers, which were prepared from the respective quinuclidinols resolved with (+)-camphor-10-sulfonic acid (11), differ markedly in potency (12,13). The (−) isomer was reported to have about 20 times the potency of the (+) isomer in producing ataxia in dogs (12); however, with the use of more elaborate behavioral measurements in cats, the potency difference was in excess of 100-fold (13). It is conceivable that the (+) isomer of 3-quinuclidinyl benzilate is devoid of activity on the central nervous system, and the slight activity observed may be attributed to a 1% contamination by the (−) isomer.

COMPARISON OF ANIMAL AND HUMAN DATA WITH VARIOUS ANTICHOLINERGIC DRUGS

In general, there is good agreement between the relative pharmacologic potency of various anticholinergic psychotomimetic agents in animals and that in humans. The comparison is applicable to both central and peripheral effects of the anticholinergics and to their duration of action. Table L-1 summarizes the data obtained on the Edgewood volunteers who were given a single dose of an anticholinergic agent. Central nervous system (CNS) potency and peripheral antimuscarinic potency are expressed on an arbitrary scale of 0–10, with 10 being the most potent. Most of the agents used were of comparable peripheral antimuscarinic potency, whereas the CNS potency extended over the whole range.

The CNS data in Table L-1 can be summarized as follows. BZ and other quinuclidinyl glycolates containing at least one phenyl and a cycloalkyl group in the acid moiety were the most potent and had the greatest duration of action. The corresponding piperidyl glycolates were one-fifth to one-half as active as the quinuclidinyl esters. It can be concluded from such structure-activity studies, which are based on extensive psychopharmacologic stests in both animals and human volunteers, that BZ is the most representative of the most potent anticholinergics. However, atropine and methylatropine are the most representative of the relatively inactive anticholinergics and could serve as control drugs, particularly because atropine is equipotent, although of shorter duration, to BZ in peripheral anticholinergic effects.

SITES OF ANTICHOLINERGIC ACTION IN BRAIN

Studies have been performed on the regional and cytoplasmic distribution of high-affinity radiolabeled anticholinergics in mammalian brain (14,15). In monkey brain, the regional distribution of [^{3}H]3-quinuclidinyl benzilate was found to correlate well with other cholinergic measures, such as [^{3}H]choline uptake, choline acetyltransferase, and acetylcholinesterase (16). By far the highest level of all four parameters was in the putamen and caudate nucleus. The cerebral hemispheres, amygdala, and hippocampus contained about half the concentration of the benzilate, but the other concentrations were small fractions of those in the caudate nucleus. Apart from the caudate nucleus, the correlation between the distribution of the anticholinergics and the measures of cholinergic function was not impressive. By determining the extent to which [^{3}H]3-quinuclidinyl benzilate binding was diminished by pretreatment of rats with atropine, the degree of specific binding of the anticholinergic can be determined in various brain areas (16). It appears that, although a correlation was found in some brain areas (e.g., the caudate nucleus,) between the distribution of the anticholinergics and other measures of cholinergic action, the binding studies do not permit the conclusion that the drugs' distribution is an accurate reflection of the pattern of muscarinic cholinergic receptors in brain.

The binding of [^{3}H]-anticholinergics to tissue preparations after lesions are produced in specific brain areas is another means of measuring specific receptors for the anticholinergics. If nerve terminals of the cholinergic afferents to the hippocampus contain muscarinic cholinergic receptors, as suggested by pharmacologic studies (17,18), then lesions in the septal-hippocampal cholinergic tract should reduce the drugs' binding—a conclusion that was experimentally verified (19).

COMPETITION BETWEEN BEHAVIORAL POTENCY AND RECEPTOR AFFINITY

To determine whether the centrally active anticholinergics bind to a physiologic receptor, an attempt was made to correlate inhibition binding constants of the various anticholinergic agents with their psychopharmacologic potency (20). Such a correlation between behavioral and binding data has been attempted with a series of 14 glycolate esters (Figure 2). A linear relation was observed between the behavioral data and the logarithm of the inhibition constants for binding. The behavioral data were taken from previously published accounts and are expressed quantitatively as BDI (behavioral disturbance index, or BDI (21). From the evidence presented in this study, it can be concluded that [^{3}H]quinuclidinyl benzilate binds to a muscarinic site in brain that is involved in producing the behavioral disturbances elicited by the glycolate esters. This conclusion is based on the low K_d, the saturability and stereospecificity of binding competition studies, and especially the reasonable correlation observed between behavioral and binding data (Figure 2). Although the correlation can be useful in predicting the behavioral potency of new glycolate esters, on the basis of their inhibition constants, there are notable exceptions, such as atropine, scopolamine, and compound IV. All three drugs had very high affinities for the [^{3}H]quinuclidinyl benzilate binding site, but their behavioral potencies were relativey low. They all contain heterocyclic ring systems other than piperidine or quinuclidine. In a previous study, in an attempt to correlate the behavioral potencies of a series of glycolates with some physical constants, the correlation tended to be excellent for the quinuclidinyl and piperidyl esters, but not for those having other heterocyclic amino rings, such as tropanol and granatonol. However, an excellent correlation was observed between affinity constants and the ability of the anticholinergics to block the acetylcholine-induced contraction of ileum, the correlation being independent of the type of heterocyclic amino ring (20).

A comparison of the receptor binding affinities of the two optical isomers of 3-quinuclidinyl benzilate reveals that the (−) isomer has about 50 times the affinity of the (+) isomer for a synaptic-membrane preparation from rat caudate nucleus (unpublished). It had been reported that the (−) isomer had only 20 times the affinity of the (+) isomer for a neural-membrane preparation from whole rat brain (20). The reasons for the difference may be that caudate nucleus contains a greater concentration of cholinergic receptors and that a purified synaptic membrane preparation was used in the later study. The relative binding affinities of the two isomers however, still had less than the 200:1 potency ratio found in the cat behavioral test (22). A plausible explanation is that the specific neurons associated with the behavioral disturbances of the drugs may have a greater degree of binding stereospecificity than that exhibited by membrane preparations from either whole brain or caudate nucleus.

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TABLE 1 Structure-Activity Data on Anticholinergics in Human Volunteers

Code Name	Dose µg/kg	Duration of Effect, h	Potency[a] Peripheral	CNS	No. Subjects
BZ	5–8	48–96	10	10	354
22,608	1–2	48–72	10	10	21
EA 3167	3–4	48–120	10	10	24
301,060	3–5	48–120	10	9	29
CS 27349	7–8	6–24	10	5	50
302,282	7–14	6–12	10	4	8
EA 3580	4–37	12–48	8	3	136
EA 3443	4–60	24–48	8	3	101
302,196	22–54	4–10	8	4	56
Scopolamine	4–16	6–24	10	5	637
302,537	4–6	6–24	10	4	18
Ditran	100	12–24	10	3	12
EA 3834	3–24	12–24	9	4	171
302,668	13–18	12–48	8	8	39
Atropine	1–70	12–24	10	1	602
Benactyzine	100	6–24	4	1	17
Methylscopolamine	1–30	6–30	5	0	66
Methyl atropine	2–20	6–48	8	0	15
302,368	3–4	6–24	10	1	10
Atropine+benactyzine	(100+20)	4–10	10	1	5

[a]Potency is expressed in terms of peripheral (mydriasis, dryness of mouth, etc.) and central nervous system (CNS) effects. The latter involve confusion, hallucinations, memory loss, and delirium. Drugs are evaluated relative to BZ.

Figure F-1 Chemical structure of various heterocyclic amino esters of benzilic acid, I=3-quinuclidinyl, II=N-methyl-4-piperidyl, III= N-methyl-3-piperidyl, IV=N-methyl-3-pyrrolidyl, V= N-methyl-N-methyl-2,3-piperidienyl, VI=1,2,2,6-tetramethylpiperidyl, VII=N-methyl-3-tropanyl, VIII=N-methyl-3-granatonyl, IX= 1-pyrrolizidinyl, X=1,2,2,6,6-pentamethyl-3-piperldyl. R=benzilate. Psychotomimetic potency decreases from I to X.

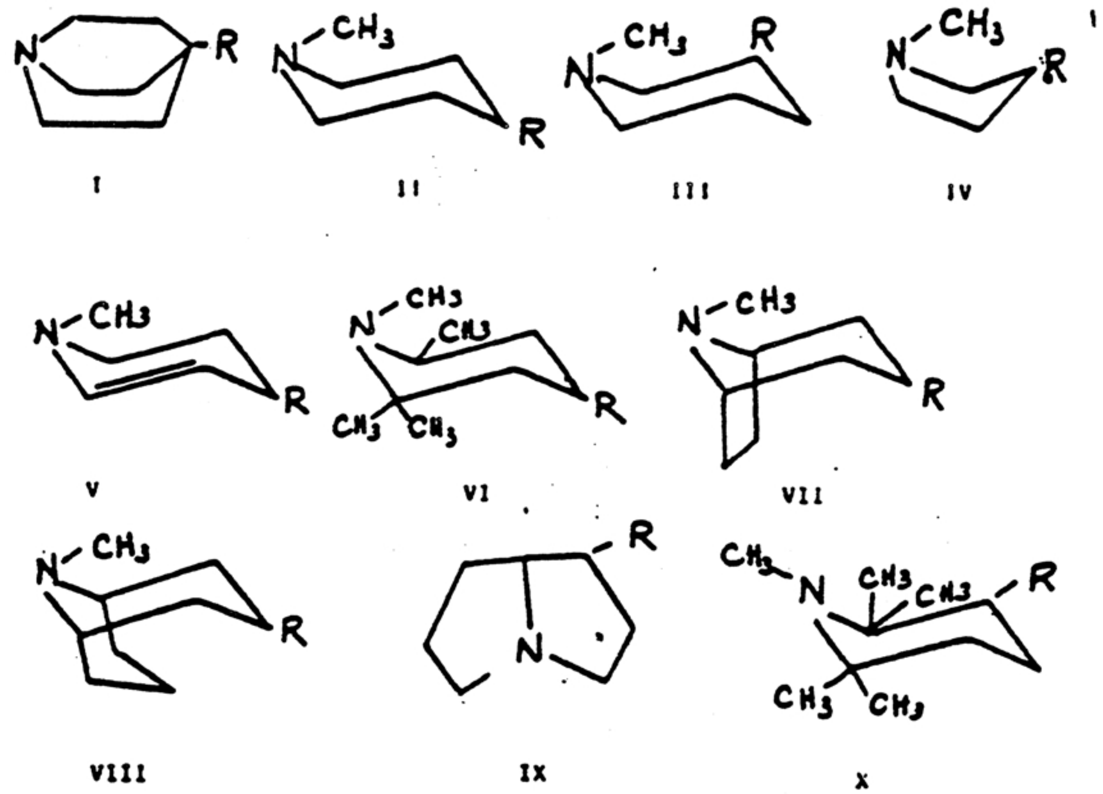

Figure F-2 Correlation of binding affinity of various centrally active anticholinergics to their behavioral disturbance index (BDI). Binding affinity is measured by K_i, the drug concentration producing 50% inhibition of [^{3}H] quinuclidinyl benzilate binding to brain-membrane preparations. BDI=composite index of behavioral measurements in various animals as a measure of psychotropic potency. Data adapted from Baumgold et al.(20).