Spontaneously Hypertensive (SHR) Rats: Guidelines for Breeding, Care, and Use (1976)

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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Preface

This report has been written for a broad audience, including scientists and laboratory assistants with different backgrounds and laboratory animal care personnel. For this reason some of the sections begin with rather detailed introductions, e.g., blood pressure, genetics. A more exhaustive discussion of some of the subjects covered in this report can be found in the proceedings of the first symposium on the spontaneously hypertensive (SHR) rat.[1]

The report is intended to provide guidelines for breeding, colony management, and measurement of blood pressure. It is also intended to aid investigators, producers, and journal editors to recognize the special problems involved in using this animal model. Standardization of requirements and conditions for breeding will make it possible for investigators to obtain the same animal model from any producer and thereby be able to compare their findings with those of other investigators around the world. Removing the variable of local breeding of this animal model will encourage many investigators to apply their own expertise to the problem of hypertension. Where established experimental data are lacking, recommendations are made on the basis of the cumulative experience of the committee members. No details have been given of the sources for the specialized equipment used for measuring blood pressure in rats, as this is common knowledge.

The term SHR, used throughout this report, refers specifically to the spontaneously hypertensive rat developed by Okamoto and Aoki. Although the "R" in the strain designation SHR stands for "rat," the term SHR has been used in adjective form, i.e., SHR rat(s). It should be noted that the term SH rat is currently used to designate the Sherman strain of rat. With respect to control animals, WKY signifies Wistar-Kyoto. In Japan, this is abbreviated as WK.

The advice provided by Dr. Ronald Geller of the National Heart and Lung Institute, National Institutes of Health, by Drs. Akira Ooshima, Conrad Richter, and Sydney Spector of The Roche Research Center, and Dr. George J. Pucak, Charles River Breeding Laboratories, Inc., in developing the present guidelines is acknowledged. Since the SHR is a recently developed model, the recommendations and guidelines suggested here should be considered as tentative and subject to modification as new data become available.

S. Udenfriend, *Chairman*

1 Summary of Recommendations

The most important issues raised in this report are summarized below and presented to help guide those involved with the spontaneously hypertensive (SHR) rat as producers, investigators, or reviewers. The last category includes peer review groups and journal editors who are asked to review manuscripts or proposals relating to the SHR rat.

Producers should emphasize the following:

- Quality control, including maintenance of records, by continual monitoring of blood pressures of the foundation stock. Commercial producers should include blood pressure information in their catalogs, and provide animals with blood pressures *no lower* than those of the F_{30-32} generation (see p. 6).
- Periodic introduction of original stock from the SHR colony at the Veterinary Resources Branch, National Institutes of Health, in order to minimize genetic variables between laboratories.
- Measures to prevent and control disease.
- Diets that contain no more than 1 percent sodium chloride.

Investigators should emphasize the following:

- Adequate equilibration time after receiving animals from outside sources or after intramural transfer to permit blood pressure to adjust.
- Use of an individual room to house SHR rats as well as to monitor blood pressure.
- Blood pressure taken by the indirect method without anesthesia, using appropriate instrumentation and procedures.

Reviewers should insist that manuscripts or proposals relating to the SHR rat clearly state:

- Specific criteria used in determining the hypertensive condition of the animal (i.e., blood pressure and method used to measure it) and source of the animals.
- Control strain used and its source.
- Details of the methods and equipment used for monitoring blood pressure.

2 Introduction

The spontaneously hypertensive (SHR) rat exemplifies the use of the resources of the National Institutes of Health (NIH) to introduce to the scientific community important findings of an individual laboratory. By 1966, Professor Kozo Okamoto and his colleagues in Kyoto, Japan, had carried their SHR rats to the thirteenth generation as an animal model of essential hypertension. At that time they were receiving periodic requests for animals from around the world. In most cases an animal model is studied mainly in the laboratory of origin and only on occasion by other investigators. However, Professor Okamoto approached the NIH through Dr. Sidney Udenfriend, who, together with Dr. Albert Sjoerdsma, then head of the Experimental Therapeutics Branch of the National Heart Institute (now National Heart and Lung Institute), convinced the NIH authorities to import SHR rats and to carry on enough studies to substantiate the value of breeding. It should be noted that Professor Okamoto developed the SHR strain intentionally and that he is noted for the development of animal models of other human diseases, particularly diabetes.

Early in 1967, Dr. Okamoto sent one of his colleagues, Dr. R. Tabei, with a colony of SHR rats to Dr. Sjoerdsma's laboratory, where, with Dr. Sydney Spector, they started their studies with these animals. By 1968, their findings corroborated those of the Kyoto group. The Veterinary Resources Branch (VRB) of the NIH, which then began producing SHR rats, has continued to breed and distribute them after being designated as a World Health Organization (WHO) Genetic Repository for laboratory rodents.

The SHR rat is not the only animal model for hypertension. However, because it is now so widely used, far more information has been obtained with it than with any other model of spontaneous hypertension, and laboratory findings may be easily compared.

3 History of the Spontaneously Hypertensive Rat

DEVELOPMENT

In an effort to develop a genetic animal model of human essential hypertension, Okamoto and Aoki examined the blood pressures of several hundred rats from the Wistar colony of the animal center at Kyoto University. The blood pressures in these Wistar rats averaged 120–140 mm Hg as measured by tail-plethysmograph techniques without anesthesia. One of the male rats examined exhibited blood pressures of 145–175 mm Hg. A female rat with blood pressures of 130–140 mm Hg was chosen for mating with this male rat. In 1959–1960, the male rat maintained a sustained elevated blood pressure (more than 150 mm Hg). Mating between the pair was repeated four times.

The offspring of these matings consisted of 16 males and 20 females. Of these, 12 males and 6 females on normal laboratory diets at the age of 20 weeks and 14 males and 11 females at the age of 30 weeks were found to have sustained blood pressures above 150 mm Hg. The offspring that exhibited hypertension for over 1 month were used for further brother-sister matings. Successive generations of hypertensive animals were obtained by brother-sister matings of animals selected for sustained high blood pressure. As seen in Figure 1, the mean blood pressures of the succeeding generations increased quickly, with a plateau being approached by about the sixth generation (F_6). This process was continued until a colony of rats was produced in which the blood pressures were uniformly more than 180 mm Hg by 20 weeks of age.

The typical hypertensive lesions often associated with human essential hypertension were frequently observed in these rats, suggesting that they might be good models for the human disease (see Chapter 4). The animals were called Spontaneously Hypertensive Rats.[2,3,4] Successive brother-sister matings were continued in order to produce the inbred strain of SHR rats, which was obtained in October 1969.[5] This inbred strain was in the thirty-sixth generation in 1974. The colony of rats currently maintained at Kyoto University numbers some 2,000 animals. Animals derived from this colony have by now been introduced into about 200 institutions.

Experience in many laboratories indicates that, for comparative purposes, only the parent WKY strain serves as an adequate control (see Chapter 5).

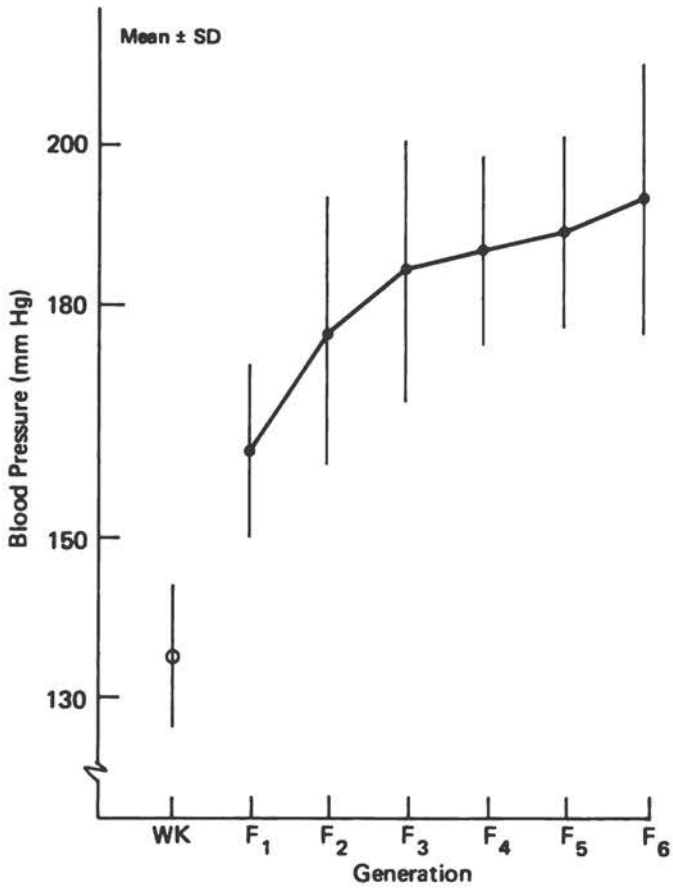


FIGURE 1 Development of hypertension in the first six generations of SHR rats. Data indicate the average (mean ± SD) of blood pressure measured by a tail-plethysmographic method without anesthesia in male SHR rats at the age of 20 weeks. Basic data cited from Okamoto.[4]

DESCRIPTION

Blood Pressure

The SHR rats develop hypertension in 100 percent of their population. In the most recent generations of SHR rats, hypertension (over 150 mm Hg) develops by the fifth week, earlier than in their antecedents, and is maintained in adult animals thereafter at a higher level (frequently over 200 mm Hg) than in the previous generations (Figure 2).[6,7] The average blood pressure (mean ± SD) in males and females of F_{30-32} generations is 184 ± 17 and 178 ± 14, respectively, at the age of 10 weeks. Producers of SHR rats and experimental groups should aim for blood pressures *no lower* than these.

Hypertensive Complications

With continued hypertension these rats exhibit complications similar to those in man, such as cerebral lesions (infarction, hemorrhage), myocardial lesions (infarction, fibrosis), and nephrosclerosis (benign, malignant). The incidence of these lesions in adult SHR rats is shown in Figure 3. These lesions are not generally observed before the age of 3 months. Because of these complications, the average life span of the

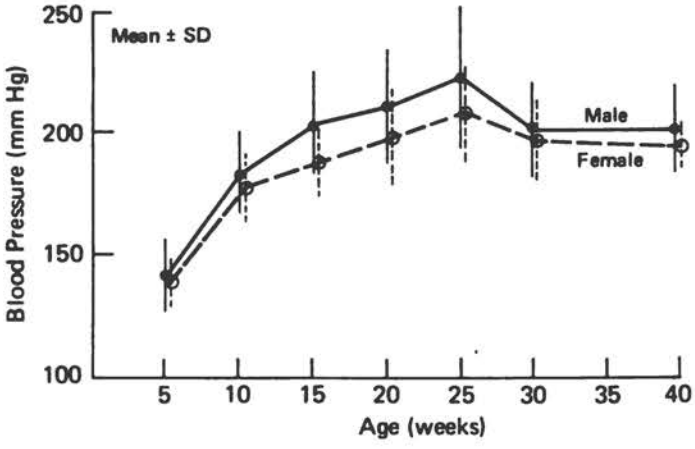


FIGURE 2 Blood pressures in male and female SHR rats at various ages. Data indicate blood pressures (mean ± SD) measured by a plethysmographic or pulse-pickup method on groups of 50–350 unanesthetized SHR rats. These were F_{30-32} offspring of the original colony maintained at the Department of Pathology, Faculty of Medicine, Kyoto University, Japan.

male SHR rat under conventional conditions is shortened to an average of about 18 months; that of the WKY is at least 24 months.

Litter Size

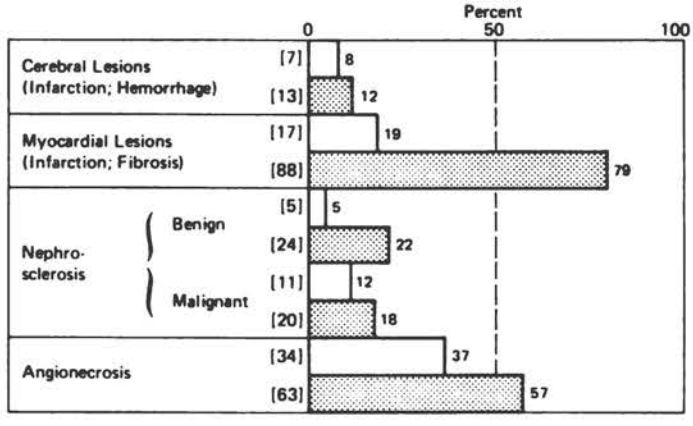
Litter size has been about 8–10, with no sex difference noted in the numbers.[4]

Genetic Markers

The SHR rat has strain-specific isozyme patterns of arylesterase in the kidney, liver, and gastrointestinal tract, as well as other enzymes.[8,9] Characteristic serum esterase patterns have also been reported.[10,11]

Substrains and Related Models

In recent years several substrains of the SHR rat have been isolated.[10] One of the most important of these is the stroke-prone SHR rat. This substrain exhibits severe hypertension, with adult males having blood pressures of over 230 mm Hg. These animals have a very high incidence of stroke; some 80 percent of the animals have strokes by 2–3 months of age.[6] Another related animal model is the obese hypertensive rat developed by Koletsky from the offspring of crosses between SHR and Sprague-Dawley rats.[12]



SHR rats with blood pressure below 200 mm Hg (□ 91 cases: 51 males and 40 females) and over 200 mm Hg (▨ 111 cases: 57 males and 54 females).
[ ]: Number of SHR rats examined.

FIGURE 3 Pathologic findings in SHR rats (F_{9-17}) after natural death. Data indicate blood pressures (mean ± SD) measured by a plethysmographic or pulse-pickup method on groups of 50–350 unanesthetized SHR rats. These were F_{30-32} offspring of the original colony maintained at the Department of Pathology, Faculty of Medicine, Kyoto University, Japan.

4 Evaluation of the Spontaneously Hypertensive Rat as a Model of Human Hypertension

Chronic hypertension in man may be divided into primary and secondary forms. The latter includes hypertension of known causes, such as coarctation of the aorta, pheochromocytoma, primary aldosteronism, chronic glomerulonephritis, and renovascular hypertension. However, the majority of patients with elevated blood pressure (approximately 90 percent) have primary or essential hypertension.

No cause has been demonstrated for essential hypertension. Among the hypotheses that have been proposed are the following: (1) essential hypertension encompasses a variety of specific but still undefined pathophysiologic disturbances, (2) it is a disorder of regulation in which a mosaic of blood pressure control mechanisms (endocrine, neurogenic, renal, and others) interact to result in an elevated blood pressure,[13] and (3) essential hypertension is not a distinct entity, as there is no true dividing line between normal and raised blood pressure.

Distribution curves of blood pressure in the general population are smooth and provide no evidence that there are two components–one normotensive and the other hypertensive.[14]

The spontaneously hypertensive rat serves as an experimental model that has many characteristics resembling those found in essential hypertension of man. One of the great advantages of this model is that the entire life history of the disease is compressed within a period of less than 2 years.

The similarities between essential hypertension in man and spontaneous hypertension in rats include the following:

- There is an important genetic component in both conditions. It has long been recognized that essential hypertension tends to occur in families. This tendency is polygenic rather than related to a single gene. While available evidence is difficult to quantify, it indicates that the contribution of inheritance is probably between one-third and two-thirds of the whole.[15] The inheritance of hypertension in the SHR rat also is polygenic, even though the number of major genes involved may be relatively small.[16] (See Chapter 8 for a detailed discussion of genetic considerations.)
- No specific pathogenic mechanism has yet been found for essential hypertension in man or in the SHR rat. Experimental investigations of the factors raising the blood pressure in the rat may provide insights into the nature of human essential hypertension.
- The course of the hypertension is similar. While there is wide individual variation, the blood pressure in man tends to rise with age. Individuals showing the steepest rise with age, and those with high normal blood pressures in youth, generally become hypertensive in middle age. In the SHR rat blood pressure also rises with age. The blood pressure is above that of normal control Wistar rats from 5 weeks of age (the youngest age in which the blood pressure has been measured) and rises progressively with the passage of time to a plateau at about 6 months of age (see Chapter 8).
- The major cardiovascular complications in essential hypertension include left ventricular hypertrophy and dilation often leading to congestive heart failure, hemorrhagic stroke, nephrosclerosis with impaired renal function, and a malignant phase of hypertension with fibrinoid necrosis of arterioles and small arteries. These various pathological changes are also found in the SHR rat and represent the leading causes of death in that animal.[4] (Also, see Chapter 3.) Patients with essential hypertension are also prone to develop atherosclerotic complications of the coronary and cerebral arteries leading to myocardial infarction and atherothrombotic strokes. Although the rat is resistant to atherosclerosis, it has been reported that lipid-containing arterial lesions resembling atherosclerosis can be induced in the SHR rat with special diets high in fat and cholesterol.[17]
- Hemodynamic changes in the established phase of essential hypertension are characterized by a normal cardiac output and an increase in total peripheral resistance.[18] This hemodynamic pattern also is found in the SHR rat.[19] In early or borderline hypertension in man, an elevation of cardiac output may be associated with a normal total peripheral resistance.[18] Similar hemodynamic changes have been reported in the young SHR rat prior to the development of a high resistance form of hypertension.[19]
- Salt has long been considered to be an important factor in the development of essential hypertension.[20] Salt-restricted diets and saluretic agents reduce the elevated blood pressure. The blood pressure of the SHR rat also is influenced by the amount of salt ingested. The development of this hypertension is accelerated by an excess of salt and is retarded by a low-salt diet.[21]
- Antihypertensive drug therapy reduces the incidence of major cardiovascular complications in patients with essential hypertension.[22] Controlled therapeutic trials have demonstrated that reduction of blood pressure with antihypertensive drugs results in a significant decrease in the incidence of stroke, congestive heart failure, renal failure, malignant hypertension, and dissecting aneurysm. The SHR rat also can be protected against the development of such complications by treatment with antihypertensive agents.[23] The same drugs or combinations of drugs that are used to control the blood pressures of patients are effective in lowering the blood pressures of SHR rats.[23,24]

Of greatest importance in considering the similarity of the hypertension seen in the SHR rat to that seen in human essential hypertension is that there are initially no evident pathological disturbances. In the early stages of the hypertension, both man and the SHR rat appear to be normal in all respects other than the elevation of total peripheral resistance. Important similarities (other than the initially higher blood pressure) include the strong genetic component (the tendency for blood pressure to rise with age), the late appearance of cardiovascular complications, the types of complications, the aggravating effects of salt, and the response to antihypertensive drugs.

5 Selection of Suitable Controls

One of the most important decisions made in planning an experiment is the choice of the control. The basic experimental technique in the biomedical sciences is comparative. That is, our understanding of basic biological phenomena is determined by comparing the effect of a certain experimental procedure on groups of animals that presumably are identical to others not subjected to these procedures. Obviously, there are many adaptations to this basic concept, and the SHR rat represents an example. One of the important purposes of using the SHR rat model is to determine the pathogenesis of the elevated blood pressure; in other words, to locate cause and effect relationships. Since the SHR rat represents a unique genetic entity, the ideal experimental system would consist of two populations, each genetically identical (congenic strains) except that one would carry the SHR genes for elevated blood pressure. Since we do not operate in an ideal world, the next best approach probably is to use as a control the WKY rat strain, the base normotensive stock from which the SHR rat was derived. It should be emphasized, however, that the SHR rat strain has been subjected to selection, which might have the effect of fixing additional genes that are still segregating in the WKY rat strain.

Investigators have sought to explain hypertension at a biochemical level. Comparative biochemical studies between the SHR rat and its normotensive control illustrate the need to select the proper control animal. Biochemical differences have been detected between colonies of different strains of normotensive rats that have been maintained in different laboratories. Lovenberg *et al.*[25] have compared the enzymatic activities of tyrosine hydroxylase, aromatic L-amino acid decarboxylase, and dopamine beta hydroxylase in the brain stem and in the adrenal glands of a number of inbred rat strains. They found a significant variation in the activities of the catecholamine biosynthetic enzymes in these inbred strains, which were all selected for normal blood pressures. These findings indicate that in these various rat strains there are significant genetic variations in the catecholamine biosynthetic enzymes.

Yamabe *et al.*[26] have shown that a WKY strain maintained for several generations at the NIH differs from the Wistar strain in its norepinephrine content, activities of the catecholamine synthesizing enzymes, and catecholamine turnover rate. DeJong *et al.*[27] showed that the SHR rat had increased plasma renin activity as compared to a normotensive Wistar strain from the NIH. However, when plasma renin activity was measured in a Wistar-Kyoto strain, there was no significant difference from that of the SHR rat. The need for proper controls is illustrated further in the studies of Spector *et al.*[28] and Clineschmidt *et al.*[29] on the reactivity of aortic strips to norepinephrine.

This committee recommends that the WKY be used as a control for the SHR rat. However, it is also suggested that other established rat strains be considered as parallel controls with the WKY. Such comparisons may yield new insights into the factors responsible for the elevated blood pressure of the SHR rat, which would not be apparent if only the WKY rat were used as a control.

6 Current Usage

SOURCES AND VOLUME

Study of the SHR model has been pursued with extreme vigor in Japan since it was first reported by Okamoto.[30] The use of this rat as a model for hypertension is now widespread throughout the world. In 1975, grants from the National Heart and Lung Institute supported research involving studies on the etiology of this spontaneously occurring disease in SHR rats in 30 laboratories. Numerous pharmaceutical companies have found these rats to be valuable models for developing and testing antihypertensive drugs.

Research during the past 12 years has utilized SHR rats from many sources. In Japan, the animals have been distributed from the laboratory of the originator and are presently bred in various laboratories. All the breeding stock in Europe and the United States came from Japan. From the breeding stock supplied to the WHO Genetic Repository, Veterinary Resources Branch of the NIH, Dr. Carl Hansen supplied breeding pairs to various investigators, who in turn supplied commercial companies. Over the past 3 years, the NIH facility has distributed to investigators approximately 240 rats per year. It has been estimated that the commercial sales volume in the United States exceeded 30,000 rats in 1974, with one company reporting a sex ratio of 8 males to 1 female in its sales. The SHR rat is one of the more widely used inbred rat strains in the United States. A listing of currently available sources of SHR rats may be obtained from the Institute of

Laboratory Animal Resources, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.[31]

EXPERIMENTAL USES OF THE SPONTANEOUSLY HYPERTENSIVE RAT

Symptomatically, the disease entity of this animal model closely resembles human hypertension, even though the etiology of the disease may be different from that of most human essential hypertension. The current use is largely limited to studies relating to hypertensive disorders, although several other abnormalities have been discovered or may become apparent in future studies.

MODEL FOR HYPERTENSION

Studies on the etiology of hypertension in this model were reviewed in a symposium held in Kyoto, Japan, in 1971. The proceedings of this meeting were published in 1972.[1] Research on inborn errors relating to metabolism and biosynthesis of catecholamines has been pursued by many investigators. Even though some investigators have demonstrated certain relationships between organ content, metabolism, or biosynthesis of catecholamines and hypertension,[1] others have produced evidence that clearly questions that the etiology of this form of genetic hypertension relates to abnormalities in catecholamine metabolism.[1,26,32,33,34,35,36,37,38] Disruptions in the nervous system and behavioral patterns may be involved.

In the etiology of hypertension in the SHR rat, various parameters are being measured. The possible roles of the prostaglandins, kallikrein, bradykinin, and adrenal steroids are being studied. To determine the possible involvement of the kidney, more detailed renal studies, such as single nephron pressures, flow, and resistances are being conducted. Smooth muscle reactivity and transmembrane fluxes on isolated muscle preparations may uncover some differences between this strain and its precursors.

The cardiovascular hemodynamics do not differentiate this condition from other animal forms of hypertension. The SHR rat has been an excellent model for hypertrophy of both the blood vessel wall and heart.[39,40] Renal involvement in the disease appears to be more secondary than primary. Sen *et al.*[41] and DeJong *et al.*[27] have reported that renin increases in the early phases of hypertension in these animals. Czyzewski and Pettinger[42] have suggested that this enzyme is not increased in plasma, but that its secretion is more easily stimulated by anesthesia. However, Sinaiko and Mirkin[43] found that kidney renin is elevated in the neonatal SHR rat. DeJong *et al.*[44] reported high levels of renin in the submaxillary gland of the SHR rat and the normotensive strain from which they were derived. The enzyme level in these glands increased with age.

MODEL FOR ATHEROSCLEROSIS AND STROKE

The cardiovascular pathology of the SHR rat is similar to that of other hypertensive models. However, since the onset of hypertension requires no special diets or surgery, the SHR rat has proven to be valuable for studying the interrelationship between hypertension and atherosclerosis.[17,45,46] Two recently established substrains may become valuable models for studying the etiology of atherosclerosis and stroke. These are the obese SHR rat developed by Koletsky[12] and the stroke-prone SHR rat.[6]

MODEL FOR DRUG EVALUATION

Early investigations showed that antihypertensive drugs taken orally were effective in reducing blood pressure in SHR rats.[4,24] Freis[47] used a combination of reserpine, hydralazine, and chlorothiazide to maintain a normotensive state in these animals for long periods of time without apparent toxicity. These, as well as many more recent experiments, validate the usefulness of this strain as a model for drug evaluation. Most pharmaceutical companies interested in hypertension now maintain colonies of these animals for this purpose. Because of the large percentage of human hypertension that falls into the broad classification of essential hypertension, the use of the model for screening drugs is obvious.

Cardiac hypertrophy, which usually accompanies hypertension in man, also occurs in the SHR rat and begins at a very early age (9–10 weeks). Research on the evaluation of drugs to reverse this hypertrophy, as well as research on the etiology of this phenomenon, has been reported by Sen *et al.*[40]

MODEL FOR ERYTHROCYTOSIS

Increased red cell production and elevated erythropoietin levels in SHR rats were reported by Sen *et al.*[48] This abnormality paralleled the increase in kidney renin. No causal relationships have been established, but the animal provides one of the simpler models for additional mechanistic studies. This increase in erythropoietin does not occur in the experimental renal and DOCA salt hypertension models.

7 Techniques for the Measurement of Blood Pressure

A simple, reliable means of measuring blood pressure must be available for investigators to conduct meaningful research using SHR rats and for producers to provide high-quality animals. Suggested types of measurement, instrumentation, comparison of methods, and precautions are presented in this section.

TYPES OF MEASUREMENT

Arterial blood pressure is the hydrostatic pressure generated within the arterial system by the heart pumping blood against the peripheral resistance of the vessels. The parameters of interest are the maximum or systolic pressure generated during contraction of the heart, the minimum or diastolic pressure occurring just prior to the next contraction, and the pulse pressure or difference between systolic and diastolic pressures.

Direct Techniques

Direct measurement of blood pressure requires surgical cannulation of a major artery. The cannula is connected to a pressure transducer that has been calibrated with a recording device. While this approach probably provides the most accurate measure of both systolic and diastolic pressure, it has several drawbacks in physiological experiments and is not particularly useful for breeders who wish to monitor the blood pressure of the animals they are producing. In acute experiments the trauma of the operation and the anesthesia can profoundly alter blood pressure. For chronic experiments maintenance of infection-free postoperative animals and unclogged cannulas present significant problems. The details of a successful system for chronic direct arterial pressure recording have been reviewed by Stanton.[49] This system is only practical in laboratories primarily devoted to this type of research.

Indirect Techniques

The indirect approach permits repeated measurements on large numbers of animals. Although the accuracy of the measurement is somewhat reduced, reliable comparative measurements can be made if the conditions during measurement are strictly controlled. All indirect systems require the use of a cuff for occluding the blood flow in an appendage. The tail of the rat is the most convenient appendage for occluding the arterial blood supply. Most indirect methods of monitoring blood pressure utilize a tail cuff with a variety of techniques to ascertain the occluding pressure. Much of the work on rat blood pressure has been done using plethysmographic systems, based on techniques originally described by Byrom and Wilson[50] and Williams *et al.*[51]

In general, an inflatable cuff is applied to the proximal portion of the tail, while the distal portion of the tail is enclosed in the plethysmographic chamber. The pressure in the pneumatic cuff, which is connected to a mercury manometer, is raised above the systolic pressure and then released slowly. The increase in volume of the tail due to the inflow of blood when the pressure of the occluding cuff reaches the systolic blood pressure can be observed in the plethysmographic manometer. Alternatively, various types of pulse sensors can replace the plethysmograph as a means of detecting the systolic pressure. Indirect methods are largely used to detect systolic blood pressure; measurement of diastolic pressure by these techniques appears to be somewhat less reliable. The width of the tail cuff in relation to the diameter of the tail is extremely important and has been discussed by a number of workers.[52,53,54,55] Cuff widths of 15–20 mm appear to be appropriate for rats 10 weeks and older. It should be noted that a system for warming rats (*vide infra*) prior to using a tail cuff is absolutely essential.

Another indirect approach is to use a leg cuff and measure blood flow in the paw by a photoelectric scanner.[56,57] Although this method eliminates the need for warming the rats, it is more cumbersome than the tail cuff procedure and has not been widely used.

INSTRUMENTATION

A number of commercial suppliers of laboratory equipment carry the individual components necessary for recording blood pressure of the rat.

For acute, direct blood pressure recording, a Statham P23Db pressure transducer (or equivalent), which is connected to an arterial catheter and attached to a calibrated physiograph recorder, is commonly used. An investigator wishing to monitor blood pressure chronically should consult the studies of Weeks and Jones[58] and Stanton.[49]

The instrumentation required for indirect pressure measurement is as varied as the techniques. The four basic items needed are: a thermostated box for warming the rats, a tail cuff, a system to detect and measure the occluding pressure, and a rat restraining device.

Warming Box

The arterial pulsations during deflation of the cuff are most easily detected when the vessels of the tail are fully dilated. This vasodilation can be produced by warming the animal. Warming boxes of various designs can be constructed for rats in most laboratory shops or they can be purchased commercially. Accurate and reproducible conditions for warming the

animals prior to measuring blood pressure are essential for obtaining reliable results. (See Recommended Procedures and Precautions below.) Results will be variable and low if warming is not adequate. The boxes should be able to maintain rats at a constant environmental temperature of 38°-39° C. Other techniques for warming rats, including infrared lights and warm air blowers, have been used, but the most reliable method appears to be a constant temperature container.

Tail Cuffs and Pressure-Measuring Devices

Tail cuffs for various size rats can be purchased. The simplest and least-expensive approach to measure the occluding pressure is to construct a plethysmograph, as originally described by Williams *et al.*,[51] and connect the tail cuff to a standard mercury manometer. Complete recording units including a rubber pneumatic sensor with a sensitive pressure transducer to detect the tail pulse are available commercially. Such instruments can be used with an ordinary pressure cuff or coupled with an electrosphygmomanometer to inflate and deflate the pressure cuff automatically and at a constant rate.

Another widely used system for detection of the pulse following arterial occlusion is the microphonic method first described by Friedman and Freed.[59] In this system a small carbon microphone is taped to the ventral portion of the tail, and the pulse sounds are amplified and detected with earphones or displayed on an oscilloscope. Equipment for this system can be obtained from commercial sources. A Doppler ultrasonic flowmeter can also be used to detect the occluding pressure.

Restraining Devices

Devices to restrain the rats during blood pressure measurement can either be made or purchased commercially. They should be adjustable or available in various sizes for different-sized animals. The restrainers are basically tubelike devices that fit the rat snugly and allow for ventilation at the head and for protrusion of the tail.

Although there are a variety of instrumentation components that can be combined to provide an adequate system for measuring rat blood pressure, considerable care in calibration and adaptation of the equipment must be provided by the investigator.

ACCURACY AND REPRODUCIBILITY

Many workers have attempted to correlate the various indirect techniques with direct intra-arterial recording. While all the indirect techniques theoretically measure systolic blood pressure, it is generally observed that indirect measurements yield values that are slightly lower than those found by direct arterial recording. There appears to be a linear relationship between the two approaches. Pfeffer *et al.*[60] and Bunag[61] found a close correlation (correlation coefficient = 0.975) between values obtained by indirect methods and by direct recording in unanesthetized animals. A detailed comparison of the various methods for detecting the pulse following occlusion of the arterial flow has not been made. Recently, Yamori (unpublished experiments) compared the plethysmographic method of Williams *et al.*[51] with the DIE 20 crystal pulse pickup method and found a very close correlation. Blood pressures recorded by the indirect method during recovery from light ether anesthesia are significantly lower than in the unanesthetized animal.[62]

RECOMMENDED PROCEDURES AND PRECAUTIONS

The indirect tail cuff method is recommended for most monitoring of blood pressure in rats. Any of the several systems described above can be used to detect the pulse during or following occlusion of the caudal arteries. The rats should be maintained in a warming box at 38°-39° C for 10 minutes prior to blood pressure measurement. They then should be put in a restrainer and the cuff quickly placed around the proximal portion of the tail with the sensor in the cuff attached to the appropriate detecting device. If possible, the restrainer or the ambient temperature surrounding the rat should be maintained at 38°-39° C. If this is not feasible, the blood pressure should be recorded during the first minute after removing the animal from the warming box.

A great many factors can lead to variable and nonreproducible results. Generally the investigator needs considerable experience before he can have confidence in his results. The following procedures are recommended in order to reduce the variability and increase the reliability of measurements. The investigator should:

- Equilibrate newly delivered rats to their quarters before using them (see Chapter 9).
- Habituate rats to the procedure during several training sessions.
- Warm rats in a uniform and adequate manner.
- Place the tail cuff consistently about 2 cm from the base of the tail. Fregly[55] has shown that the measured blood pressure varies with the location of the cuff on the tail.
- Take measurements in a quiet laboratory free of extraneous activities.
- Compare blood pressure measurements of the same type only (indirect with indirect and direct with direct), since direct measurements are made on anesthetized animals.
- Record blood pressures 3–4 times and discard the lowest and highest values.

The results obtained should be compared to the values described in Chapter 3.

8 Management of a Breeding Colony

In a relatively short time, the SHR rat has become one of the most widely distributed rat strains reflecting its value for the study of essential hypertension in man. This widespread distribution is not without problems that ultimately could seriously compromise the strain's unique value. The primary problem is minimizing intercolony variation of blood pressures. The danger of intercolony variation becomes substantial unless the methods of colony management are standardized. There is still much to be learned about the pathogenesis of the elevated blood pressures, especially in the area of genetics. This is particularly important for managers of large-scale commercial breeding operations, but should also be kept in mind for smaller individual laboratory colonies. One of the purposes of this chapter is to consider some of the problems in the management of this animal model and provide some guidelines for the colony manager.

GENETICS

The essential problem in the long-term management of any animal model is maintaining the stability of the characteristic in question. It has been an article of faith that, once certain levels of inbreeding have been reached, the colony manager need only be concerned with maximizing reproductive yield, since all of the genetic variation has been fixed. Experience with a wide variety of inbred strains has indicated otherwise. Likewise, the genetic characteristics of subpopulations of so-called outbred stocks of animals will also change with time.

The primary reason for the differentiation of separated colonies of the same strain or stock is that only the genes and not the genotypes are transmitted from generation to generation. In a population of animals such as an inbred strain in which all genotypes, i.e., *AA, BB, CC, DD,* etc., are identical, there is no possibility of genetic segregation, and the progeny will be genetically identical to the parents. This is a technical definition of an inbred strain. Furthermore, if the phenotype accurately reflects gene action, the genes are said to act additively. If not, gene action is considered to be nonadditive. An example of nonadditive gene action, when only one locus is involved, is known as dominance. In the case of dominance, the *Aa* genotype cannot be distinguished phenotypically from *AA*. In this situation, genetic segregation is possible, with the result that progeny will not be identical to the parents. Nonadditive interactions between more than one locus are also possible and will result in continued genetic segregation.

A combination of inbreeding and artificial selection, which was used to form the SHR rat strain,[3] technically should be an effective method in developing a unique population of animals provided the genes responsible for elevated blood pressure act in an additive manner. This would have the consequence of fixing, i.e., making homozygous, those genes that contribute to the elevated blood pressures. When this point has been reached, no further progress to selection should be possible; but, on the other hand, blood pressures should not change once the selection pressure is removed. Also, there should be no reason to continue further inbreeding, as all of the genetic variation has been fixed.

The relatively rapid response to selection for elevated blood pressure during the developmental phases of this rat strain observed by Okamoto and Aoki[3] would suggest that gene action is additive. This is further confirmed by the observations reported by Schlager,[63] Hansen,[64] and Yen *et al.*[65] in a variety of species of laboratory animals.

Data reported by Okamoto *et al.*[6] suggest that the genetics of the SHR rat may be more complex than indicated by the above studies. Okamoto and his colleagues were able to separate the original SHR strain into a number of sublines, some of which differ significantly from each other as well as from the original strain. The occurrence of heritable differences in highly inbred strains of laboratory animals has long been a concern of geneticists and others who work with these animals. The expectation is that after 20+ generations of intense inbreeding all of the genetic variability is fixed, and such variability as occurs is due to environmental causes. However, the observation of substrain differences is relatively common. A number of factors may contribute to these differences, but at the present time there is no real agreement as to the source of this variation. Mutation is a real possibility, although Grüneberg,[66] studying skeletal variability between substrains of the same mouse strain, and Bailey and Kohn,[67] studying various histocompatability loci, also in mice, concluded that the observed variability was far greater than could be accounted for by known mutation rates.

Natural selection for reproductive fitness may also be a source of genetic variability with long inbred strains. The argument for this source of variability stems from the almost universal decline in reproductive fitness observed with the increase in the levels of inbreeding and its recovery when two inbred strains are crossed. That is, reproductive fitness is dependent upon heterozygosity. Continuation of the strain then depends upon the more heterozygous individuals, since the more homozygous ones fail to reproduce or to contribute progeny to the succeeding generations. This situation will lead to a point at which the progress to fixation or homozygosity is counteracted by the heterozygosity necessary to maintain sufficient reproduction to continue the strain. Selection can also have a similar consequence. Selection and inbreeding in certain respects have a similar effect. That is, both will lead to homozygosity, although in the case of selection only those genes that are under selection pressure will be affected, whereas for inbreeding the entire genome is affected. Natural selec-

tion can also counteract the progress resulting from artificial selection. That is, the more extreme phenotypes resulting from artificial selection may have a lower level of reproductive fitness than the "normal" phenotypes. This can also lead to a situation where the progress to fixation resulting from artificial selection is effectively halted despite the presence of genetic variability. Finally, the combined effects of inbreeding and artificial selection may serve to depress reproductive fitness to the point where only the more heterozygous individuals will continue to reproduce. Experimental evidence on these various questions is limited, and the concepts are somewhat controversial; nonetheless, they remain a possible source of variability in theoretically homozygous populations and cannot be ignored.

COLONY MANAGEMENT

The evidence presented by Okamoto *et al.*[6] emphasizes the importance of maintaining an adequate quality-control program in the maintenance and production of the SHR rat strain. A quality-control program in this context means the continual monitoring of blood pressure in order to ensure that it remains high and stable (see p. 6 for criteria). This introduces additional problems for the commercial breeder, since it will involve additional resources that in turn will increase the cost of production. Nonetheless, for the breeder's own protection, as well as to ensure the scientific community of a high-quality product, continual monitoring of blood pressure of this strain is necessary. The facilities required to monitor blood pressure are described in Chapter 7.

The amount of resources required to support a blood pressure monitoring program for a commercial producer can be substantially reduced by dividing the colony into two components–a small self-perpetuating foundation colony that provides replacement breeding stock to a second or larger production colony. The following example describes how a monitoring program may be carried out. Assume that a production colony of 1,000 breeding females is necessary to meet requirements. Further assume that 10 percent of the colony must be replaced monthly. Thus 100 females and sufficient males are required from the foundation colony each month for replacements. The size of the foundation colony then needs to be 50 females, assuming an average yield of 4 offspring per female per month, again replacing about 10 percent of this colony each month, or 5 females and sufficient males. On this basis, only those males and females used as replacements in the foundation colony need to be monitored.

The efficiency can be further improved if pedigreed controlled brother-sister matings are followed in the foundation colony. Choosing replacements in the foundation colony on the basis of their relationship to a common ancestor results in the formation of primary breeders consisting of one or two breeding pairs or groups. The remainder of the animals in the foundation colony that are close relatives to the primary breeders are for the purpose of providing replacements to the production colony. Under this scheme, only members of the primary breeding stock that are being considered for perpetuating this group need to be monitored each generation.

An alternative to this procedure is establishment of an additional multiplying colony between the foundation and production colonies. If this procedure is followed, only a very small foundation colony is necessary. Using the above example, the size of the foundation colony could be reduced to less than 10 females, since its only function would be to support the intermediate multiplying colony. The intermediate multiplying colony then functions as a source of replacement breeders for the larger production colony. This procedure has the advantage of minimizing the amount of record keeping necessary for the foundation colony.

The ideal management of a large production colony of this model would follow a pyramid system, beginning with a very small foundation colony at the apex and a larger intermediate multiplying colony that in turn supports a much larger production colony. This procedure has a number of advantages and can be used under a wide variety of conditions, depending upon the circumstances. One is that it markedly reduces the number of animals that need to be monitored. Secondly, it is very flexible in that it can be rapidly changed as demands on the colony change. Thirdly, the various colonies can be maintained separately, reducing the risk of loss due to disease or other causes.

REPRODUCTION

One of the key elements in determining the cost of a research animal is reproductive efficiency. Reproductive efficiency is probably one of the most sensitive indicators of the effectiveness of a colony management system, since successful reproduction requires completion of a series of complex but interrelated steps that are dependent upon a variety of internal and external stimuli. The roles of some of these stimuli are understood; others are not. Nonetheless, the colony manager must account for all of them when planning management strategies designed to maximize the reproductive yield from a given genetic group of animals.

The SHR rat strain is no exception. At the present time, however, there has not been sufficient experience with this model to determine what systems of management are the most effective. Initial experience with this strain by many breeders has been mixed. Some have reported no problems; others have experienced many problems. The primary problem appears to be an excessive rate of cannibalization of newborn young, especially in newly established colonies. This suggests that the stress of shipping may alter normal behavior, which is reflected in abnormal maternal responses. This pattern occurred in the original stock established at the NIH in 1966 and has occurred repeatedly in breeding stock shipped to other institutions from the NIH colony. It has been argued that the aberrant maternal behavior in the SHR rat strain is the consequence of its high blood pressure. However, experience at the NIH in

issuing a relatively large number of animals of other inbred rat strains that also exhibit cannibalism indicates that this problem is not unique to the SHR strain.

There appears to be no real solution to this problem other than eliminating the females that are prone to this behavior and attempting to minimize loud noises and rough handling in a colony. In subsequent generations, this problem tends to disappear. The tendency towards cannibalism points out that maternal behavior, of which relatively little is known, is an important component in determining the success or failure of an animal-breeding operation.

The SHR rat at the NIH has proved to be relatively trouble-free since the initial problem of cannibalism was resolved. This colony has produced an average of 3.77 weaned offspring per female parent per month over the past 5 years in a specific pathogen-free, open-room, barrier facility. The WKY strain weaned an average of 3.31 offspring per female parent per month with an average litter size at weaning of 9.08 (Hansen, personal communication, 1975).

A question frequently asked concerns the advantages and disadvantages of monogamous and harem mating systems. Both mating systems have been followed in the NIH colony without major problems. Harems were formed by mating two females to a single male and then isolating pregnant females in maternity cages until their litters were weaned. Under this system, very few litters were cannibalized. The disadvantages of this system are the necessity of extra handling of the animals and additional cages to house the pregnant and lactating females. The monogamous system is theoretically more efficient, as conception during the postpartum estrus is possible. Experience at the NIH, however, indicates a higher frequency of cannibalized litters with this system, although more offspring per female are weaned by this method.

Recommendation as to the "best" method in managing either the SHR or the WKY rat strains must be tempered with knowledge of the unique circumstances in a particular animal facility. There is no best method other than one that maximizes the output with a minimum amount of resource input. Each colony manager must make his own decisions.

9 Shipment and Transfer

Evidence gathered from shippers and receivers indicates that the standard procedures used for shipment of other rodent stocks are currently being employed successfully for the shipment and transfer of the SHR rat. There appear to be no special precautions or measures that one could recommend as an improvement over the basic standards established by the Institute of Laboratory Animal Resources.[68]*

An estimate of the time required for equilibration of the SHR rat following exposure to stress incurred in shipment can be determined from information collected by Dymsza *et al.*[69] In comparing weight loss and time in transit versus weight gain after arrival at the laboratory, the authors determined the animals required a minimum of 2 days to equilibrate to their new environment. It would follow that equilibration of other biologic values would follow this pattern; however, the equilibration period for the SHR rat can best be determined by monitoring the blood pressure until it plateaus and is reproducible.

*Document currently under revision; projected availability late 1977.

10 Diseases and Care of the SHR Rat

GENERAL

In general, the principles of care of the SHR rat do not differ from the basic guidelines outlined in *Rodents: Standards and Guidelines for the Breeding, Care, and Management of Laboratory Animals.*[70]* The chapters in that booklet, especially those relating to environment, equipment, and sanitation, should be consulted for supplemental information on these subjects.

This discussion is limited to salient considerations bearing upon problems or concepts that require emphasis or may be unique to the SHR rat.

DISEASES IMPORTANT TO THE SHR RAT

Chronic Bronchiectatic Pneumonia (CRD)

The most important disease entity of rats remains chronic bronchiectatic pneumonia (CRD), and the SHR rat has a high degree of susceptibility to this disease. It is essential that experimental stock be obtained and maintained free of this problem. Therapeutic measures are of little value against established CRD and may potentially interfere with experimental parameters.

The etiological factors and disease dynamics of CRD remain uncertain; however, several respiratory infections are known to occur in the rat and may play an important role in CRD.

Pneumonia Virus of Mice (PVM)

Serotiters to this virus are commonly present in most colonies of rats, including those of the SHR strain. Little is known of the pathology of this disease in the rat, and assumptions that it attacks lung parenchyma as in the mouse may be unwarranted (C. Richter, personal observation).

Sendai Virus

This virus is also commonly found in SHR and other rat colonies and more often produces a higher percentage of seroresponders than PVM. Unlike PVM, it is known to cause acute bronchopneumonia in the rat, but is rarely fatal itself. Because of its higher incidence in individual and colony infection, its potential role in CRD is probably greater than that of PVM. Serum samples from colonies should be periodically checked for positive reaction against PVM and Sendai, since these viruses are excellent measures of colony management practices.

*Document currently under revision; projected availability late 1977.

Mycoplasma pulmonis

The SHR rat is highly susceptible to this infection. *M. pulmonis* is the agent most consistently associated with CRD, although there is reason to doubt that it alone is entirely responsible for the disease.[71] Established infections typically cause otitis media and chronic progressive tracheobronchitis manifested by cavitating abscesses of the bronchial tree and lungs. Since otitis media is one of the most common pathological findings in CRD, culture of the middle ear (tympanic bulla) affords an excellent colony screening procedure for this infection. It is extremely undesirable to obtain experimental stock infected with *M. pulmonis.*

Sialodachryoadenitis Virus (RCV)

This is a Corona virus infection,[72] apparently limited to rats, and the SHR strain is quite susceptible. There is presently no indication that this virus plays a role in CRD; however, it is a common and important infection. The virus attacks the salivary, lacrimal, and harderian glands, as well as the ocular conjunctiva, cornea, and anterior uveal tract. Infection occurs as acute epizootics characterized by inappetence, photophobia, cloudy cornea, and swelling around the head. Although infected rats appear quite sick and should not be used for experiments during illness, individuals usually recover within 2 weeks and develop a durable immunity. The virus is highly contagious.

Other Infections

Sick rats typically fail to groom themselves properly. When rats do not groom, secretions of the harderian glands that are located behind and around the orbit of the eye accumulate on the eyelids, spread through the lacrimal ducts, and accumulate around the external nares. Since the secretion of the harderian glands is red, its accumulation around the external nares has been misidentified as "bloody nose." Sickness from almost any cause produces this phenomenon, which the rat quickly corrects upon recovery. The redness is most quickly detected on white rats such as the SHR.

Less is known about the role of other murine viruses in natural disease processes in the SHR rat; however, serotiters against them are an indication that these potential pathogens have gained access to the rat colony. These viruses include: Theiler's polioencephalomyelitis, Reo 3, K, minute virus of mice, mouse adenovirus, mouse hepatitis virus, LCM, Toolan's H-1, and Kilham rat virus.[73]

Experimental stock free of exo- and endoparasites should be obtained.

DISEASE SURVEILLANCE

It is highly desirable that necropsies by knowledgeable persons be employed to determine the cause of death of sick SHR rats from stock or experimental groups. In most small rodents, the necropsy remains the first and most useful diagnostic tool in disease identification.

Equally important is the employment of source colony monitoring to establish desirability of supply. Exclusion of disease by obtaining stock from sources free of murine pathogens is the ideal starting point for a disease-control program. A representative group of young adult or retired breeder SHR rats should be obtained from prospective suppliers and examined for serotiters against murine viruses,* *M. pulmonis* and *Salmonella* species by culture methods, and endo- and exoparasites.[74]

DISEASE CONTROL

Isolation and Quarantine

Although acclimatization to new physical environments ordinarily occurs within a few days after arrival, a longer period of observation (2 weeks) is recommended in an isolation or quarantine area. This is to preclude the possibility of introducing diseases that might be incubative at the time of arrival and to allow equilibration to the new environment when receiving animals from outside sources. Shipment of animals intramurally usually permits closer control of environmental conditions during movement and is much less likely to result in detectable disturbance in blood pressure (S. Spector, personal communication, 1975). However, it is recommended that animals shipped intramurally be held for several days before they are used for experimentation. During quarantine it may be desirable to screen samples of the group for specific pathogens or parasites, as well as subjectively observe the general health status of the animals. The quarantine period also provides an excellent opportunity to screen blood pressures in rats that are old enough.

It is most important that the rats be protected from direct or indirect contact with other small rodents during the quarantine period. Separate quarters should be provided during quarantine and for subsequent storage. Only those personnel assigned to the quarantine area should have access to the rats during this period, and their work schedules should be arranged so as to minimize contact with other rodents prior to attending the rats.

*In the United States, serotesting for murine viruses is available by prior arrangement through: Dr. Bernard A. Briody, Mouse Pox Service Laboratory, Department of Microbiology, New Jersey College of Medicine and Dentistry, 100 Bergen Street, Room 917, Newark, New Jersey 07103. Commercial sources of serotesting are also available.

Protection during Experimental Use

There are a number of successful methods for protecting the SHR rat during experimental use. The success of these methods depends upon the diligence with which they are applied and an understanding of basic principles by those using them. Acute and chronic respiratory infections are the most debilitating and consequential over extended periods, so it is especially important to prevent contact with these agents. Since many rodents infected with these agents serve as sources of infection within a colony, the following rules should be observed by the investigator in caring for the SHR rat:

- Avoid housing in rooms with other rodents.
- Avoid housing in open wire caging. Most of the important infections are airborne, and the open wire cage is ideal for rapid, incessant dissemination of these infections.
- Employ cage protective devices such as filter caps. These devices have proved highly successful in protecting both production colonies and experimental animals in marginal situations; however, it is necessary to sanitize cages more frequently to prevent odor buildup. Isolators and laminar flow cage racks may also be considered for housing the SHR rat.
- Limit the human traffic in experimental rooms to those persons essential to accomplish the experimental tasks. Where possible, keep the caretaking personnel for each group of animals to one person.

WATER, BEDDING, AND FEED

Water

Unless it interferes with experimental procedures, one of the following treatments should be applied to the water supply, especially if bottles are used:

- Sodium hypochlorite or gaseous chlorine (difficult to handle) to obtain free chlorine levels of up to 10 ppm.
- Hydrochloric acid in quantities sufficient to lower pH to approximately 2.5. If the acidified water is transported through pipes, stainless steel or plastic piping is required.

Any of these agents can be applied to the water supply through proportionating devices. These agents are well tolerated by rats and effectively sterilize the water initially. Bacterial counts in water bottles remain negligible for several days when these methods are used.

Bedding

Materials discussed in *Rodents: Standards and Guidelines for the Breeding, Care, and Management of Laboratory Animals* are acceptable for use with the SHR rat.[70]* Where contact

*Document currently under revision; projected availability late 1977.

bedding is employed, consideration should be given to sterilization prior to use if autoclave facilities are available.

Feed

Commercial diets presently available appear to be adequate to produce and maintain the SHR rat without additional supplementation, although little specific information is available. If facilities are available, consideration should be given to pasteurization of an autoclavable diet to minimize the possibility of disease introduction through this route.

Breeding colonies have been maintained successfully with normal regimens of 50 percent relative humidity and 12:12 light-dark cycling (C. Hansen, personal communication). There have been reports of special requirements of dietary supplements (e.g., honey) and temperatures for breeding SHR rats. There is no evidence that SHR rats require extraordinary conditions.

References

1. Okamoto, K., ed. 1972. Spontaneous hypertension. Its pathogenesis and complications. Igaku Shoin, Ltd., Tokyo, Japan. 266 pp.
2. Okamoto, K. 1962. Internal secretion in hypertension. Studies on experimental hypertension. Folia Endocrinol. Jap. 38:782-794.
3. Okamoto, K., and K. Aoki. 1963. Development of a strain of spontaneously hypertensive rats. Jap. Circ. J. 27:282-293.
4. Okamoto, K. 1969. Spontaneous hypertension in rats. Pages 227-270 *in* G. W. Richter and M. A. Epstein, eds. International review of experimental pathology, vol. 7. Academic Press, New York.
5. Okamoto, K., Y. Yamori, A. Ooshima, C. Park, H. Haebara, and M. Matsumoto. 1972. Establishment of the inbred strain of the spontaneously hypertensive rat and genetic factors involved in hypertension. Pages 1-8 *in* K. Okamoto, ed. Spontaneous hypertension. Its pathogenesis and complications. Igaku Shoin, Ltd., Tokyo, Japan.
6. Okamoto, K., Y. Yamori, and A. Nagaoka. 1974. Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circ. Res. 34-35 (Suppl. 1):143-153.
7. Yamori, Y., K. Tomimoto, A. Ooshima, F. Hazama, and K. Okamoto. 1974. Developmental course of hypertension in the SHR-substrains susceptible to hypertensive cerebrovascular lesions. Jap. Heart J. 15:209-210.
8. Yamori, Y., and K. Okamoto. 1970. Zymogram analysis of various organs from spontaneously hypertensive rats. Lab. Invest. 22:206-211.
9. Rapp, J. P., and L. Dahl. 1972. Arylesterase isozymes in rats bred for susceptibility and resistance to the hypertensive effect of salt. Proc. Soc. Exp. Biol. Med. 139:349-352.
10. Okamoto, K., Y. Yamori, A. Ooshima, and T. Tanaka. 1972. Development of substrains in spontaneously hypertensive rats: Genealogy, isozymes and effect of hypercholesterolemic diet. Jap. Circ. J. 36:461-470.
11. National Institutes of Health. 1974. Catalogue of NIH rodents: Strains and stocks of laboratory rodents and rabbits provided by the NIH Laboratory Animal Genetic Center, p. 113. DHEW Publication No. (NIH) 74-606. National Institutes of Health, Bethesda, Maryland.
12. Koletsky, S. 1972. New type of spontaneously hypertensive rats with hyperlipemia and endocrine gland defects. Pages 194-202 *in* K. Okamoto, ed. Spontaneous hypertension. Its pathogenesis and complications. Igaku Shoin, Ltd., Tokyo, Japan.
13. Page, I. H. 1960. The mosaic theory of hypertension. Pages 1-5 *in* K. D. Bock and P. T. Cottier, eds. Essential hypertension. An international symposium, June 7-10, 1960, Bern, Switzerland. Springer-Verlag, New York.
14. Pickering, G. 1968. Is there a dividing line between normal and raised arterial pressure? Pages 229-235 *in* High blood pressure, 2d ed. Grune & Stratton, New York.
15. Pickering, G. 1968. The inheritance of arterial pressure. Pages 236-273 *in* High blood pressure, 2d ed. Grune & Stratton, New York.
16. Tanase, H., Y. Suzuki, A. Ooshima, Y. Yamori, and K. Okamoto. 1972. Further genetic analysis of blood pressure in spontaneously hypertensive rats. Pages 9-11 *in* K. Okamoto, ed. Spontaneous hypertension. Its pathogenesis and complications. Igaku Shoin, Ltd., Tokyo, Japan.
17. Yamori, Y., Y. Hamashima, R. Horie, H. Handa, and M. Sato. 1975. Pathology of acute arterial fat deposition in spontaneously hypertensive rats. Jap. Circ. J. 39:593-600.
18. Freis, E. 1960. Hemodynamics of hypertension. Physiol. Rev. 40: 27-54.
19. Pfeffer, M. A., and E. D. Frohlich. 1973. Hemodynamic and myocardial function in young and old normotensive and spontaneously hypertensive rats. Circ. Res. 32 (Suppl. 1):28-38.
20. Dahl, L. K. 1961. Possible role of chronic excess salt consumption in the pathogenesis of essential hypertension. Am. J. Cardiol. 8:571-575.
21. Louis, W. J., and S. Spector. 1971. Effects of sodium intake on inherited hypertension in the rat. Lancet 2:1283-1286.
22. Freis, E. D. 1975. Effectiveness of drug therapy in hypertension: Present status. Circ. Res. 28-29 (Suppl. 2):1170-1175.
23. Freis, E. D., D. Ragan, H. Pillsbury, III, and M. Mathews. 1972. Alteration of the course of hypertension in the spontaneously hypertensive rat. Circ. Res. 31:1-7.
24. Tabei, R., S. Spector, W. J. Louis, and A. Sjoerdsma. 1970. Effects of antihypertensive drugs in the spontaneously hypertensive rat. Clin. Pharmacol. Ther. 11:269-274.
25. Lovenberg, W., H. Yamabe, W. DeJong, and C. T. Hansen. 1973. Genetic variation of the catecholamine biosynthetic enzyme activities in various strains of rats including the spontaneously hypertensive rat. Pages 891-895 *in* E. Usdin and S. Snyder, eds. Frontiers in catecholamine research. Pergamon Press, Great Britain.
26. Yamabe, H., W. DeJong, and W. Lovenberg. 1973. Further studies on catecholamine synthesis in the spontaneously hypertensive rat: Catecholamine synthesis in the central nervous system. Eur. J. Pharmacol. 22:91-98.