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Heredity and Development: Second Edition

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HEREDITY AND DEVELOPMENT

Heredity and Development

SECOND EDITION

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To
Sally Hughes-Schrader

Preface

Heredity and Development is concerned with concepts in the subsciences of genetics and embryology. The presentation emphasizes the manner in which hypotheses and observations lead to the conceptual schemes that allow us to think in an orderly and satisfying way about the problems involved. I hope that I have written for all persons who are genuinely interested in these matters even though their knowledge of biology may be scant. Experience has shown, however, that *Heredity and Development* is used primarily in first-year courses in biology in the universities and in one-semester courses in genetics.

This second edition differs in many ways from the first. The original 22 chapters have been combined in 12. A new chapter, The Genetics of Man,' has been added. Those chapters concerned with recent events have been extensively revised. Questions and problems have been provided for many of the chapters.

The most important change is that there is now a companion volume, *Readings in Heredity and Development*. The two books are closely integrated but there is a clear division of materials between them. *Heredity and Development* deals basically with observations, experiments, and interpretations. *Readings* consists largely of the great synthetic papers. For example, you can read of E.B.Wilson's discoveries about sex chromosomes in *Heredity and Development* and *Readings* presents his famous lecture to the Royal Society, The Bearing of Cytological Research on Heredity.'

I wish to record once again my indebtedness to those who helped with earlier versions of these chapters: Betty Moore, John R.Gregg, Donald McPherson, Th.Dobzhansky, E.P.Volpe, Lester Barth, Lucena Barth, Francis Ryan, Sally Hughes-Schrader, and Franz Schrader.

The new material for this edition has been read in part by Betty Moore and Crellin Pauling. William L.Belser, Kenneth Cooper, and Sally Gall have helped in other ways.

<div align="right">John A.Moore</div>

Riverside
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INTRODUCTION

An individual is a product of his heredity and of his development. His heredity is the substance he receives from his parents—his biological inheritance. An ovum and a sperm, the hereditary substance of man, unite to form a fertilized ovum, the zygote. The essence of this substance is the set of instructions that it contains. The zygote contains all the instructions required to produce another human being.

Shortly after its formation, the zygote undergoes a series of changes that leads, if its luck holds, to an adult. These changes are its development. Development is species-specific, that is, the sequence from zygote to adult is controlled by the nature of the hereditary instructions. For one zygote the instructions may be "make a man," for another "make a mouse." If all goes well, one will in due time be a man, the other a mouse. Development, then, may be thought of as a carrying out of the hereditary instructions contained in the zygote.

The problems of heredity and development have been central to biology since this field began, in the mid-nineteenth century, to become a rigorous science. The problems of heredity and development will always be central to biology. This is inevitable since they are closely associated with the unique feature of life itself—the ability to replicate.

This book is concerned with heredity and development. Its basic purpose is to show how ideas in these two fields were first formulated and then studied. The intellectual history of the two has been quite different. Genetics, the science of heredity, effectively began in 1900. By 1930 the general laws of inheritance seemed to be well established,

in the sense that the rules for the transmission of genes from parent to off-spring were understood. Furthermore the rules, with only slight modifications, were found to apply to all animals and plants that geneticists studied.

Classical genetics was essentially complete by 1930. It was in a state similar to that of physics in 1899, when A.A.Michelson said,

> The more important fundamental laws and facts of physical science have all been discovered, and these are now so firmly established that the possibility of their ever being supplanted in consequence of new discoveries is exceedingly remote…. "Our future discoveries must be looked for in the sixth place of decimals."

For both sciences the period of intellectual calm was brief. Physics was soon revolutionized by studies of the nucleus of physical matter; genetics was revolutionized by studies of the nucleus of living matter. In the decade beginning with 1944, geneticists discovered the chemical substances of which genes are composed, and from this base they have gone on to show how the chemical structure of the gene is responsible for its specific activity.

It is hard to exaggerate the importance of these discoveries. So far as biology is concerned, they are matched in importance only by the theory of evolution. For science in general they represent one of the great intellectual achievements of the twentieth century.

Embryology, the science of development, has not had such an intellectually satisfying history. There was a vigorous beginning and the main events and problems were soon demarcated. But explanation has come hard and the fundamental causes of events in development are still poorly understood. It was inevitable that this be so. There could be no satisfactory explanations in embryology until there was a reasonable comprehension of the action of genes. Now that we seem close to such an understanding, greater progress becomes possible. Embryology is a science of tomorrow.

The science of biology has few stories more interesting than the history of man's attempts to explain heredity and development. We will begin with heredity.

Some of the most mysterious aspects of heredity are taken for granted. We 'naturally expect' the offspring of human beings to be other human beings; of pine trees to be pine trees; of Amoeba to be Amoeba. But a scientist cannot 'naturally expect' anything. Natural events must have as their basis laws that are either known or knowable. At least, a belief of this sort is the working philosophy of scientists.

Establishing the laws that explain why it is that offspring resemble

their parents has been one of the most exciting chapters in modern biology. A bewildering mass of observations has been united in a conceptual scheme that is rational and concise. This is the science of genetics.

In all probability men have speculated about the nature of heredity since the dawn of history. It can be said safely, however, that their speculations led to nothing that could be called science until quite recently.

Our method of approach to the field of genetics will be a historical one. We shall begin with the year 1868, when Charles Darwin published *The Variation of Animals and Plants under Domestication,* and trace the increase in man's understanding of genetic phenomena down to the present day. This method of approach will illustrate how a science develops.

It is possible that this approach will reveal some things about science that you never realized before. We are so accustomed to a succession of triumphs in science that we fail to realize that this is not an inevitable consequence of the application of the 'scientific method.' Perhaps you have some knowledge of the way scientific understanding is thought to come about. The explanation is usually given as follows:

1. A scientist is confronted with a natural phenomenon that he wishes to explain.
2. He invents a hypothesis to explain the unknown phenomenon in terms of known phenomena.
3. Deductions are made from the hypothesis.
4. The deductions are tested. If they are found to be true it becomes more probable that the hypothesis is true. The more deductions that are tested and found to be true the more probable it is that the hypothesis is true.
5. Eventually the scientist convinces himself and his contemporaries that his hypothesis adequately explains the phenomenon.
6. In the course of explaining the first unknown phenomenon other unknown phenomena will be encountered. The same procedure is followed to explain these and so successive phenomena become understandable and organized into the ever-growing body of scientific knowledge.

At least that is the way that science is thought to work. To the non-scientist it may appear that if one has the equipment, time, intelligence, and abides by the rules, scientific progress is inevitable. To those working in science, this view is clearly a distortion. Progress is not inevitable. In our survey of genetics, for example, we shall find that many blind alleys have been entered and, in addition, we shall learn that an 'estab-

lished fact' of one period may turn out to be an 'erroneous conclusion' in the light of later discoveries. The idea of straight-line progress is a distortion due to looking back on the history of science. When we do this we select the experiments, observations, and ideas that subsequent events have shown to be correct and, not surprisingly, we ignore the inadequate experiments, incorrect observations, and faulty ideas.

We shall attempt, therefore, to describe the progress of genetics as it took place and in so doing try to understand some of the problems facing scientists who are working on unknown phenomena. It is most important that we make the attempt; we are living in an age when scientific knowledge is of the utmost concern to all mankind. The proper use of scientific knowledge can result in unparalleled benefits to mankind and a misuse can lead to unimaginable disasters. It is essential that those who will make the decisions, and they will be the non-scientists primarily, have as much knowledge of the nature of scientific methods as possible.

Our purpose, therefore, in studying genetics is twofold. We should gain some appreciation of the data and concepts in the field of genetics and, in addition, an understanding of the manner in which science develops.

1 Darwin's Theory of Pangenesis

BACKGROUND FOR THE THEORY OF PANGENESIS

Charles Darwin's (1809–82) interest in genetics was a consequence of his studies of evolution. It will be necessary, therefore, to give a brief statement of his theory of evolution in order to show its relation to genetics.

Darwin imagined that evolution occurred in this manner: Among the individuals of any species there would be many differences. For example, some might be slightly larger than the average, or have longer legs, or have a thicker coat of fur. If any of these variations made their possessors better adapted to survive, those with the better characteristics would have a greater chance of leaving offspring ('survival of the fittest,' as Spencer later described it). With the passage of time the original population would change, its individuals gradually becoming larger, or developing longer legs or a thicker coat of fur, or whatever characteristic was of value for survival. In this way one species could evolve into another or give rise to two or more different species.

We cannot discuss in detail Darwin's theory of evolution. For the present, we should merely note the importance of variations. Evolution cannot occur unless there are differences among the individuals of the same species. If all individuals are identical and remain so generation after generation, obviously there is no evolution. So variation is essential and, furthermore, to be of importance in evolution it must be inherited. A thick coat of fur might be advantageous for a mammal living in the Arctic, but unless this variation is inherited it is unimportant for evolution.

Darwin fully realized that his theory of evolution must be based on a sound understanding of the mechanism of inheritance. There was no such understanding in his day. He attempted, therefore, to assemble data and ideas from individuals who had speculated about inheritance and those who had been concerned with the practical aspects of animal and plant breeding. He added some observations of his own, thought deeply about the problems, and developed the first comprehensive theory of heredity, or as we now call it, genetics. This appeared in 1868 as a two-volume work entitled *The Variation of Animals and Plants under Domestication.*

Darwin's work in this field was of major interest in the last half of the nineteenth century. He was, of course, the outstanding biologist of his time, so anything he did attracted attention. We shall examine his theory briefly, not only for its historical interest but to see how the problems were stated, what data were available, and finally, to what extent the theory contributed to an understanding of natural events.

Let us constantly keep in mind that our purpose is twofold: first, learning genetics, and second, learning how scientific theories develop and change. For this second purpose, it will be important for us to keep an open mind and, if possible, not to be prejudiced by what we may have read or learned before. It is difficult not to be influenced by what we may know of genetics, but if possible this knowledge should be ignored. If we are discussing the state of genetics in 1868 our approach should be this: Given the data available in 1868, how would we view the problems of inheritance?

First, something of the background. Knowledge of cell structure, which in later years was to form a foundation for genetic concepts, was in a rudimentary state. It was known that animals and plants were composed of cells, but little was known about the internal structure of cells. The nucleus was thought to be a universal cell constituent, although its role in the life of the cell was unknown. It was generally believed, as we believe today, that cells arise solely from pre-existing cells and not *de novo*. Opinions on heredity were vague and varied. The crossing of varieties in both animals and plants had been practiced for centuries, but no general laws or rules to explain the results had been discovered. In fact, the data were so confusing that some doubted that they could be scientifically explained.

One type of observation that convinced Darwin of the 'force of inheritance' was that 'with man and the domestic animals, certain peculiarities have appeared in an individual, at rare intervals, or only once or twice in the history of the world, but have reappeared in several of the children or grandchildren.' One of the most spectacular instances of this

was the porcupine man, whose skin was covered by warty projections (Fig. 1–1). Six of his children and two of his grandchildren showed this same defect. Another instance was found in some domestic pigs, entirely lacking hind legs, whose abnormality was carried through three generations.

To some biologists of the mid-nineteenth century, such instances seemed to be the result of mere chance, or of environmental influence,

1–1 The hand of the porcupine man.

but to Darwin they were evidence that 'something' was transmitted from parent to offspring. The following quotation illustrates the way he reasoned.

> When we reflect that certain extraordinary peculiarities have thus appeared in a single individual out of many millions, all exposed in the same country to the same general conditions of life, and again, that the same extraordinary peculiarity has sometimes appeared in individuals living under widely different conditions of life, we are driven to conclude that such peculiarities are not directly due to the action of the surrounding conditions, but to unknown laws acting on the organisation or constitution of the individual;—that their production stands in hardly closer relation to the conditions than does life itself. If this be so, and the occurrence of the same unusual character in the child and parent cannot be attributed to both having been exposed to the same unusual conditions, then the following problem is worth consideration, as showing that the result cannot be due, as some authors have supposed, to mere coincidence, but must be consequent on the members of the same family inheriting something in common in their constitution. Let it be assumed that, in a large population, a particular affection occurs on an average in one out of a million, so that the *a priori* chance that an individual taken at random will be so affected is only one in a million. Let the population consist of sixty millions, composed, we will assume, of ten million families, each containing six members. On these data, Professor Stokes has calculated for me that the odds will be no less than 8333 millions to 1 that in the ten million families there will not be even a single family in which one parent and two children will be affected by the peculiarity in question. But numerous cases could be given, in which several children have been affected by the same rare peculiarity with one of their parents; and in this case, more especially if the grandchildren be included in the calculation, the odds against mere coincidence become something prodigious, almost beyond enumeration.

Even today, it would be hard to supply better reasons for the belief that 'something' was transmitted from the first porcupine man to his son. Darwin ruled out the possibility of the external environment having any causal relation to the appearance of the defect: If something in the environment was the stimulus, why did just these few persons and no others have the defect? If there had been some unusual feature of the environment, such as rare climatic conditions or a peculiar substance in the diet, many individuals might be expected to have a 'porcupine skin.' Neither could it be due to chance. It was inconceivable that a defect, so rare as never to be recorded before, should affect a father, son, and grandson merely by chance.

Darwin concluded that the best explanation was that the son had inherited his father's defect. This, in turn, means that something is

transmitted from father to son. If this is the case, it should be possible to obtain information on the laws governing this transmission. If these laws could be formulated, not only would this represent a tremendous advance for genetics, but a firm foundation would be provided for the theory of evolution.

The Data To Be Explained. Darwin's procedure, that is, his 'scientific method,' was as follows: First, he assembled all the information he could find that seemed to have a bearing on heredity. Second, he proposed a theory to account for all of the information he had assembled. The mass of data contained in his two-volume work is considerable, but it can be combined into a small number of categories. These were the types of data that Darwin felt must be explained by any comprehensive theory of inheritance.

1. *Transmission of Characters from Parent to Offspring.* Darwin summarized a tremendous mass of observations on this topic. Most of the characters known to him were morphological, such as differences in body, size, type of feathers, or hair and color patterns. Others were physiological; examples are the inheritance of profuse bleeding in man, and peculiar tics or nervous defects. The inherited characters might be large or small, important or unimportant. He concluded, 'When a new character arises, whatever its nature may be, it generally tends to be inherited, at least in a temporary and sometimes in a most persistent manner.' Darwin had no conception of an orderly or predictable transmission of characters from parent to offspring. Inheritance to him was a capricious phenomenon, sometimes temporary and sometimes persistent.

2. *Mutilations.* Some races of man habitually knock out their teeth, cut off parts of their fingers, or perforate their ears or nostrils, yet their children do not show corresponding defects. There were other cases where mutilations appeared to be inherited and they were given on such good authority that Darwin found it 'difficult not to believe them.' One of these was 'a cow that had lost a horn from an accident with consequent suppuration, produced three calves which were hornless on the same side of the head.' Once again the situation was complex. Mutilations appeared to be inherited in some instances but not in others.

3. *Atavism (or Reversion).* This is the presence in an individual of some peculiar characteristic not expressed in its immediate parents, but resembling a remote ancestral condition. Children occasionally resemble their grandparents or more distant ancestors more closely than they do their parents. Domestic animals may have peculiar features not characteristic of their breed, but resembling the wild species from which the domestic forms were derived. For example, it was believed that the wild

ancestors of the domesticated sheep had been black. Thus, when a black sheep suddenly appeared in a flock of carefully bred white sheep, it was explained as a consequence of the persistence of a long-dormant hereditary feature. Instances are reported of reversion during the life of a single individual. Darwin crossed white hens with black cocks. Some of the individual chicks were white the first year but black the second.

4. *Sex.* Most characters appeared to be inherited with equal facility from either the mother or the father, but Darwin knew of a few instances in which the sex of the parent was important. Cases are quoted on traits being transmitted from father to son but never to daughters, or from mother to daughter but never to sons. In color blindness, males are much more commonly affected than females, yet the defect can be transmitted through normal females. In fact, it seemed probable to Darwin that fathers can never transmit color blindness to their sons. Daughters of color-blind fathers, on the other hand, though normal themselves, transmit color blindness to their sons. Thus, the father, grandson, and great-great-grandson will exhibit a peculiarity—the grandmother, daughters, and great-granddaughter having transmitted it in a latent state.' From observation of this sort Darwin states, 'We thus learn, and the fact is an important one, that transmission and development are distinct powers.'

5. *Inbreeding and Inheritance.* If two organisms are crossed and their offspring bred with each other generation after generation, we speak of this as inbreeding. The data available to Darwin suggested that inbreeding would result in a relatively homogeneous population in which there is a blending of characteristics. Darwin regarded this as the general rule, but he adds (in small print!) that in other cases 'some characters refuse to blend, and are transmitted in an unmodified state either from both parents or from one. When gray and white mice are paired, the young are not piebald nor of an intermediate tint, but are pure white or the ordinary gray color.'

6. *Selection and Inheritance.* Selection is a breeding method that has been employed since the early days of agriculture. If a farmer is interested in increasing the size of his chickens, he selects the largest individuals and breeds them. From their offspring he selects the largest and breeds from them. With this procedure, it is usually possible to increase the average size of the descendants in a few generations. One of the most puzzling aspects of selection was the fact that frequently it was possible to produce an organism with characteristics not even remotely suggested in the original stock. For example, continued selection produced the most bizarre varieties of pigeons with characteristics not occurring in the ancestors. In short, selection could create something new. This will be considered next.

7. *Origin of Variability.* All domestic and wild species familiar to Darwin were variable. Many varieties bred true, indicating the hereditary nature of the special features. In some cases a variety was known to have originated from a single exceptional individual. In many cases it appeared that the new variety was 'new' in the sense of never having occurred before. To Darwin the cause of variability was 'an obscure one; but it may be useful to probe our ignorance.' He favored the view that 'variations of all kinds and degrees are directly or indirectly caused by the conditions of life to which each being, and more especially its ancestors, have been exposed.' The great importance of the 'conditions of life' can be brought out by the following quotation: '…if it were possible to expose all the individuals of a species during many generations to absolutely uniform conditions of life, there would be no variability,' The actual conditions of life that were thought to cause variability included excess food (probably the most important), climate, hybridization, grafting in plants, and in fact 'a change of almost any kind in the conditions of life.'

8. *Regeneration.* When the tail or the legs of a salamander are cut off, the lost structures are replaced perfectly by regeneration. The ability to regenerate lost parts is of widespread occurrence and appears to be similar to events occurring in embryonic development. Darwin felt that both the formation of a structure during the course of normal development and its replacement following injury to the adult were due to the workings of inheritance.

9. *Inheritance and Mode of Reproduction.* There are two main types of reproduction, sexual and asexual. An animal like Hydra is capable of both. Sexual reproduction consists of the fertilization of an ovum by a spermatozoon. Asexual reproduction in Hydra is by budding. In this process a small protuberance forms on the side of the Hydra. This grows and eventually detaches as a small individual. A Hydra that originates from a fertilized ovum is identical with a Hydra developing from a bud. Thus, to Darwin inheritance is the same whether by sexual or asexual means.

10. *Delayed-Action Inheritance.* Darwin listed several cases, which he believed to be well substantiated, of the male gametes having an effect on the female organs. One of these was published by Lord Morton. An Arabian chestnut mare was crossed to a quagga (a wild African species belonging to the horse genus and closely resembling the zebra). The first offspring was intermediate in form and color. The mare was subsequently crossed to a black Arabian horse. One filly and one colt were produced. In coloration and type of mane these two offspring showed a striking resemblance to the quagga. For example, dark bars were present on the hind part of the body and the mane was stiff and erect. Darwin

concluded, 'Hence, there can be no doubt that the quagga affected the character of the offspring subsequently begot by the black Arabian horse.' He felt that the quagga sperm had acted directly on the reproductive organs of the female in such a way as to affect the characteristics of future offspring sired by other males.

THE THEORY OF PANGENESIS

These ten categories represent the types of data that Darwin felt must be explained by any comprehensive theory of inheritance and he set about to formulate a theory to explain them. The result was '…the hypothesis of Pangenesis, which implies that the whole organization, in the sense of every separate atom or unit, reproduces itself.' He began by postulating the existence of gemmules, which determine all characteristics of the organism. The properties that gemmules were assumed to possess were these: Each and every cell of an organism, and even parts of cells, produce gemmules of a specific type corresponding to the cell or part. These are able to circulate throughout the body and enter the sex cells. Every sperm and every egg will contain gemmules of all sorts and so they are transmitted to the next generation. During development they unite with partially formed cells or with other gemmules, and in this way produce new cells of the type from which they were formed. We should think of a liver cell as producing gemmules for every part of that cell, enough kinds to produce the identical cell type in the next generation. All other parts of the body would also be producing their own specific gemmules. These must be present in tremendous numbers, since every sperm and ovum will have some of all types produced in the body. In some instances the gemmules could remain dormant for generations.

Today we might wonder about the space problem. If every part of the body produced a specific gemmule, would it not be difficult for all of them to fit into an ovum of microscopic dimensions, or into the even smaller sperm? Thus, if gemmules exist, obviously they must be very small. Darwin did not think this difficulty was fatal to his hypothesis. Biologists, especially those working on disease or with cells, realized that very small things could be extremely important.

The basic postulate of his theory was the existence of the gemmules and their production by cells. Was there any evidence? At the time Darwin wrote we must remember that the 'cell theory' was in the process of being accepted. Darwin reasoned this way: If cells can divide and produce other cells, perhaps they can produce other bodies with the assumed characteristics of gemmules by a similar process. In his own words, 'The existence of free gemmules is a gratuitous assumption, yet can hardly be considered as very improbable, seeing that cells have the

power of multiplication through the self-division of their contents.'

Darwin's Theory of Pangenesis was based on gemmules but he had no real evidence for their existence. They were invented to account for the observed events in inheritance. This is legitimate scientific procedure. Atoms were invented to account for the data of chemistry. The planet that was later named Pluto was invented to account for irregularities in the orbits of known planets. Atoms and Pluto were useful hypotheses long before one could be certain of their reality.

   The Theory Explains the Data. The Theory of Pangenesis can be applied to the ten categories of data requiring explanation.

1. *Transmission of Characters from Parent to Offspring.* The appearance of the same characters in parent and offspring was made possible by the production of gemmules by all parts of the parent's body. These entered the ova and sperm and were transmitted to the offspring where they caused their specific effects. This was true, as well, for those special characters such as those of the porcupine man. The skin cells of the porcupine man produced 'porcupine' gemmules. These reached his children by way of the sperm.

2. *Mutilations.* Mutilations are usually not inherited because normal gemmules would have been produced by the structure before the mutilation occurred. These gemmules would enter the gametes and be passed to the next generation. The few cases in which mutilations appeared to be inherited usually involved diseased parts. Darwin explained this as follows: 'In this case it may be conjectured that the gemmules of the lost part were gradually all attracted by the partially diseased surface, and thus perished.'

3. *Atavism.* Atavism, according to the Theory of Pangenesis, was due to the ancestral gemmules remaining in a dormant condition for many generations and then suddenly developing.

4. *Sex.* Both sexes transmit inherited characters with equal facility, since both transmit gemmules representing every cell of the body. In the case of color blindness in man, and similar instances of inheritance modified by sex, it was assumed that gemmules were dormant in one sex. A color-blind man transmits gemmules of color blindness to his daughter (in whose body they are dormant) and she may in turn transmit them to her sons. In the sons they develop and the sons are color-blind.

5. *Inbreeding and Inheritance.* The blending in the offspring of characteristics of the parents is due to the mixing of the parental gemmules. Those cases in which the characteristics of one parent predominate indicate that the predominating ones 'have some advantage in number, affinity, or vigour over those derived from the other parent.'

6. *Selection and Inheritance.* It is possible to influence the inherited

characteristics of organisms through selection in this manner: The farmer choosing the largest chickens from his flock is choosing the ones that will produce gemmules for large size. If this is repeated every generation, the gemmules for large size will be retained, those for small size will be eliminated, and the chickens will reach their maximum possible size.

7. *Origin of Variability.* According to Darwin, new structures appear as a result of some environmental influence. The new or changed structure will produce new types of gemmules. These will be transmitted to the next generation, and thus the new character will reappear.

8. *Regeneration.* Regeneration of lost parts is possible because the gemmules for the lost parts were produced prior to the loss and are present in the rest of the body. If, for example, the leg of a salamander has been removed, the leg gemmules, which are present in the body, can migrate to the cut surface and develop into a new limb, identical to the old one.

9. *Inheritance and Mode of Reproduction.* Inheritance is the same, whether via sexual or asexual means, since the basis is identical—the transmission of gemmules. In the case of our specific example, Hydra, every cell of the body would produce gemmules. These would move to all parts, including the gametes and the cells that form the buds. Thus, the new individual would receive the same gemmules irrespective of whether they came from a fertilized ovum or from a bud.

10. *Delayed-Action Inheritance.* In those peculiar cases where the male gametes were thought to have a lasting effect on the reproductive organs of the females (as in Lord Morton's mare) a ready explanation was possible. Some of the gemmules from the male gametes entered the reproductive organs of the female and were included in ova produced long afterwards.

Darwin's Theory of Pangenesis, like all great theories, involved a great simplification in man's view of his universe. By assuming the existence of gemmules with definite properties he was able to 'make sense' out of a previously bewildering mass of data. Inheritance is the transmission of the physical entities that are the basis of development in succeeding generations. But to postulate is not to prove. The facts available to Darwin were not sufficient to decide whether his theory was 'right' or 'wrong.' His main contribution was the collection of a tremendous amount of genetic data, and an attempt to provide a theoretical framework for its interpretation. He was most modest about his efforts: 'I am aware that my view is merely a provisional hypothesis or speculation; but until a better one be advanced, it may be serviceable by bringing

together a multitude of facts which are at present left disconnected by any efficient cause. As Whewell, the historian of the inductive sciences, remarks: "Hypotheses may often be of service to science, when they involve a certain portion of incompleteness, and even of error." Under this point of view I venture to advance the hypothesis of Pangenesis, which implies that the whole organization, in the sense of every separate atom or unit, reproduces itself.'

We should now pause to ask a few questions: Did Darwin's Theory of Pangenesis explain the data of heredity? If you are aware of later developments in this field you will probably answer 'no,' but if you can repress the bias of the knowledge of what was to come, you will probably conclude that the answer is 'yes.' If the answer is 'yes,' does this mean that the theory is correct?

Darwin's approach to the study of inheritance was one of two possible methods of attack. He was concerned nearly entirely with the *results* of inheritance, i.e. the kind of offspring obtained when parents of different types were crossed. From the results he attempted to reconstruct the basis of inheritance. As we have already seen, he concluded that every cell produces gemmules and that these are the basis of inheritance. In the thirty years following the presentation of the Theory of Pangenesis little or no advance, based on breeding experiments, was made in our understanding of the mechanism of inheritance.

The second possible method of investigation involves the study of ova and sperm. These gametes are the sole physical link between the parents and offspring, so presumably they would be responsible for the transmission of any inherited characteristics. A careful study of the gametes might be expected to throw some light on inheritance. The branch of biology that is concerned with the study of cells, including the ova and sperm of course, is *cytology*.

These two approaches to inheritance, studying offspring or studying cells, together were eventually to give us a sound theory of inheritance. The major advances during the last half of the nineteenth century were made almost entirely in cytology: they will be considered in Chapter 2. In Chapter 3 we return to the other approach, the study of the characteristics of the offspring. This sister discipline came to be called genetics.

Suggested Readings

Charles Darwin was only one of many early workers interested in inheritance. Excerpts from the writings of others, such as Hippocrates, Aristotle, and Galton are given in Chapter 1 of the companion volume

Readings in Heredity and Development. You will also find there a longer list of references.

DARLINGTON, C.D. 1969. *Genetics and Man.* New York: Schocken Books. The breadth of approach makes this book especially appealing to non-science students.

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Questions

1. On the basis of your knowledge of biology, how would you account for the ten types of data that Darwin felt must be explained by any comprehensive theory of inheritance?

2. What sorts of experiments or observations could you suggest to test Darwin's theory of pangenesis? Were Galton's experiments (*Readings,* Chapter 1) an adequate test?

3. Compare Darwin's theory of pangenesis with that of Hippocrates (*Readings,* Chapter 1).

4. To what extent can the arguments that Aristotle used to refute pangenesis (*Readings,* Chapter 1) be used against Darwin's theory?

5. Why did Aristotle believe that the male and female make qualitatively different contributions to inheritance (*Readings,* Chapter 1)?

6. Evaluate this quotation from Aristotle (*Readings,* Chapter 1) in terms of what you may know of current theories of inheritance: "…why not admit straight away that the semen at the onset is such that out of it blood and flesh can be formed, instead of maintaining that the semen is itself both blood and flesh?".

7. What precautions did Galton (*Readings,* Chapter 1) take in selecting the rabbits that were to be transfused with blood? Why were these precautions necessary? Why did Galton think it important to estimate the amount of blood transfused? Evaluate his experiments in relation to what you know about immunology and infection.

8. Do you find Darwin's criticism of Galton's experiments convincing (*Readings,* Chapter 1)?

2 The Cellular Basis of Inheritance

The development of cytology, like that of all fields of science, depends partly on tools. The compound microscope, which is the basic tool in cytology, appears to have been invented about 1590 by two Dutch spectacles makers. The birthday of cytology was postponed, however, for three-quarters of a century. It was not until 1665 that Robert Hooke first described 'cells' in a piece of cork (Fig. 2–1). He observed that cork and some other plant materials were composed of box-like structures, so small that eleven hundred arranged in length would equal no more than an inch. In living tissues they were filled with vegetable juices but were empty in the dried cork. When the cork was thinly sliced for microscopic examination, these boxes, or cells, appeared as rectangles, as the illustration shows. And interestingly enough he found these same structures in pieces of petrified wood.

This discovery of cells in cork could have been an important advance in knowledge or an unimportant one. If it had turned out subsequently that cells were found only in cork, Hooke would certainly not be widely remembered for his discovery. But the work of many scientists showed that cells were a general phenomenon and, therefore, of some importance. We might conclude that Hooke did not make a discovery that *was* important but, instead, a discovery that *became* important.

The Cell Theory. Following the realization that some cells are present in some organisms, the next conceptual advance was to establish the hypothesis that all organisms are composed solely of cells or cell products. This took nearly a century and a half. In the early years of the

Observ. XVIII. *Of the* Schematisme *or* Texture *of* Cork, *and of the Cells and Pores of some other such frothy Bodies*.
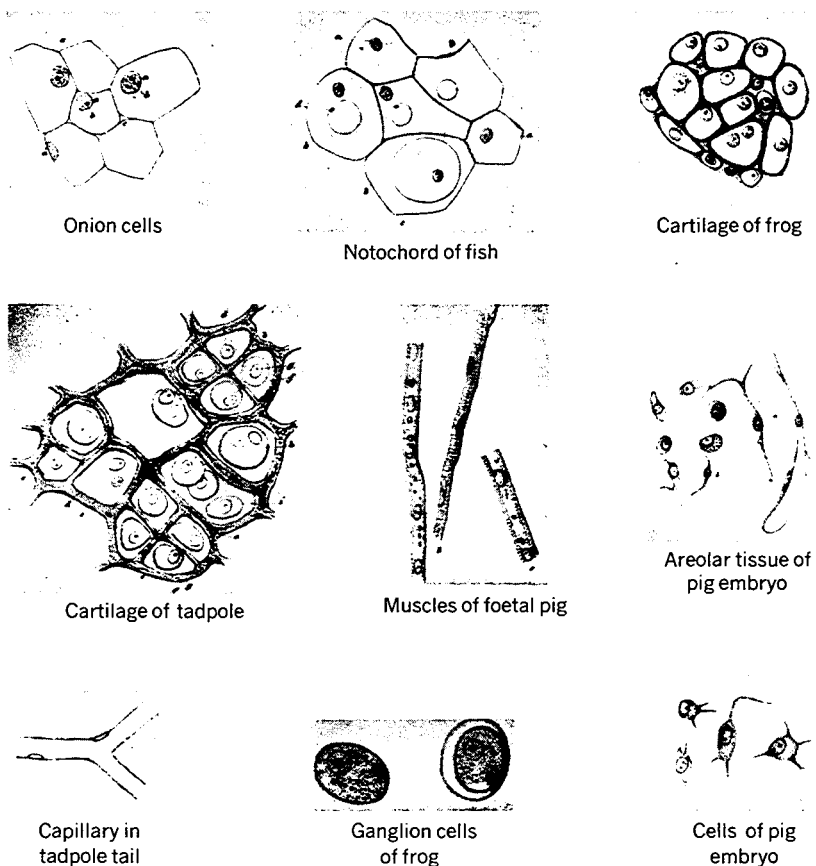
I Took a good clear piece of Cork, and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and thereby left the surface of it exceeding smooth, then examining it very diligently with a *Microscope,* me thought I could perceive it to appear a little porous; but I could not so plainly distinguish them, as to be sure that they were pores, much less what Figure they were of: But judging from the lightness and yielding quality of the Cork, that certainly the texture could not be so curious, but that possibly, if I could use some further diligence, I might find it to be discernable with a *Microscope,* I with the same sharp Penknife, cut off from the former smooth surface an exceeding thin piece of it, and placing it on a black object Plate, because it was it self a white body, and casting the light on it with a deep *plano-convex Glass,* I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars.
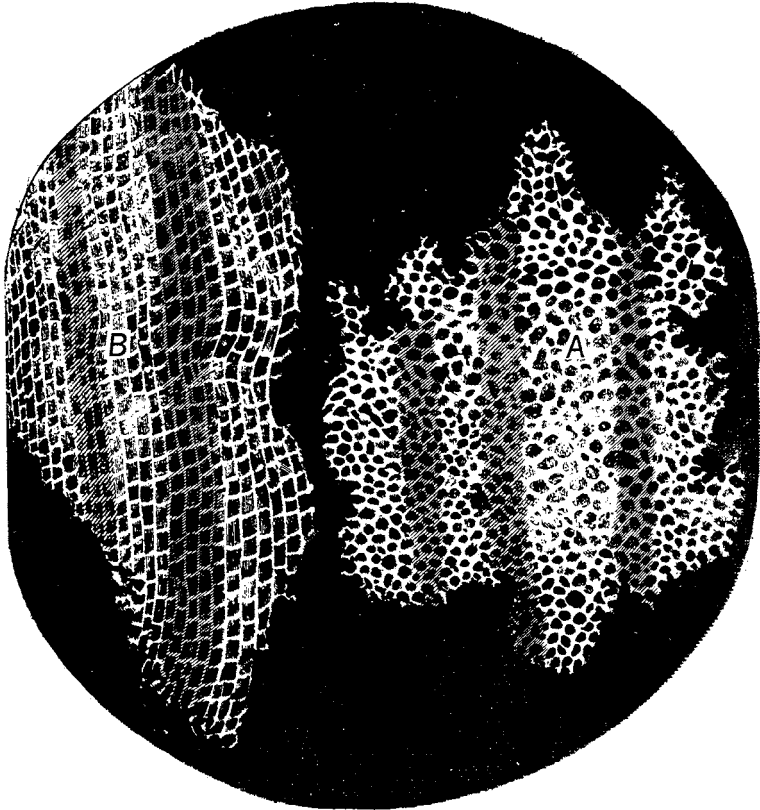
2–1 The drawings and part of the text from Hooke's observations on cork (R. Hooke, *Micrographia,* London, 1667).

nineteenth century a number of cytologists came more and more to adopt this second concept, which we know as the 'cell theory.'

Three names are generally associated with the final formulation of the cell theory. They are R.J.H.Dutrochet, Matthias Jacob Schleiden, and Theodor Schwann. The last-named published his treatise on the microscopic structure of organisms in 1839, when he was 29 years old. In it he summarized his findings on a variety of tissues. His general conclusion was that the bodies of organisms are composed of cells. Figure 2–2 reproduces some of his drawings.

Onion cells

Notochord of fish

Cartilage of frog

Cartilage of tadpole

Muscles of foetal pig

Areolar tissue of pig embryo

Capillary in tadpole tail

Ganglion cells of frog

Cells of pig embryo

2–2 Some of Schwann's drawings of cells (Th.Schwann, *Mikroscopische Untersuchungen über die Uebereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen,* Berlin, 1839).

The following quotations from a translation of Schwann's treatise will reveal some of his views:

> Though the variety in the external structure of plants is great, their internal structure is very simple. This extraordinary range of form is due only to a variation in the fitting together of elementary structures which, indeed, are subject to modification but are essentially identical—that is, they are cells. The entire class of cellular plants is composed solely of cells which can readily be recognized as such; some of them are composed merely of a series of similar or even only of a single cell.

> Animals being subject to a much greater range of variation in their external form than is found in plants also show (especially in the higher species) a much greater range of structure in their different tissues. A muscle differs greatly from a nerve, the latter from a cellular tissue (which shares only its name with the cellular tissue of plants), or elastic tissue, or horny tissue, etc. If, however, we go back to the development of these tissues, then it will appear that all these many forms of tissue are constituted solely of cells that are quite analogous to plant cells…. The purpose of the present treatise is to prove the foregoing by observations.

Much of Schwann's success was due to the fact that he adopted a definite criterion for the recognition of cells, namely, the presence or absence of a nucleus. The latter structure was apparently first recognized as an important and characteristic cell structure by Robert Brown in 1833. It had been observed much earlier, however. Schwann took full advantage of this recent (for him) discovery.

> The most frequent and important basis for recognizing the existence of a cell is the presence or absence of the nucleus. Its sharp outline and its darker color make it easily recognizable in most cases and its characteristic shape, especially if it contains nucleoli…identify the structure as a cell nucleus and make it analogous with the nucleus of the young cells contained in cartilage and plant cells…. More than nine-tenths of the structures thought to be cells show such a nucleus and in many of these a distinct cell membrane can be made out and in most it is more or less distinct. Under these circumstances it is perhaps permissible to conclude that in those spheres where no cell membrane be distinguished, but where a nucleus characteristic of its position and form is encountered, that a cell membrane is actually present but invisible.

Although Schwann's work established the cell as a unit of structure, his views on the origin of cells precluded these elementary bodies from having any importance in inheritance, as the following quotation shows.

> The general principles in the formation of cells may be given as follows. At first there is a structureless substance which may be either quite liquid or more or less gelatinous. This, depending on its chemical constitution and

degree of vitality, has the inherent ability to bring about the formation of cells. It seems that usually the nucleus is formed first and then the cell around it. Cell formation is in the organic world what crystallization represents in the inorganic world. The cell, once formed, grows through its inherent energy, but in doing so it is guided by the organism as a whole in the way that conforms to the general organization. This is the phenomenon basic to all animal and plant growth. It is applicable to cases where the young cells originate in the mother cell, as well as those where they are formed outside of them. In both instances the origin of cells occurs in a liquid or in a structureless substance. We will call this substance, in which cells are formed, a cell germinative substance or Cytoblastema. It can be compared figuratively, but only figuratively, with a solution from which crystals are precipitated.

The Continuity of Cells. Gradually this view was replaced by the realization that cells are formed solely by the division of pre-existing cells. Even before 1839 cell division had been observed, but it was during the following decade that more and more investigators—Robert Remak and Karl Wilhelm von Nägeli, for example—came to the conclusion that cells never originate from a structureless 'cytoblastema,' but always by cell division. In 1855 Rudolf Virchow formulated his well-known statement *omnis cellula e cellula,* which means 'all cells from cells.' The cell then took on a new significance and greater importance. No longer was it a matter of the organism forming cells *de novo,* but instead cells, by their divisions, formed the organism. If existing cells have arisen from pre-existing cells, then there must be a continuity of these elementary structures that goes back to the very beginnings of life. The connection between generations was shown to be cells. Schwann was of the belief that the ovum was a cell. Therefore a cell produced in the ovary was the link between parent and offspring. (It had been known since 1824 that spermatozoa, and not the fluid in which they are found, were the important agents in fertilization, but the realization that spermatozoa are cells did not come until 1865.)

   Nuclear Division. With the gradual accumulation of knowledge, improvement in microscopes, and development of techniques, cytologists were able to see more and more detail in cells. It was assumed that the area surrounding the nucleus, the *cytoplasm,* was the site of the more important life processes. The nucleus, being the most characteristic structure within the cell, came in for a good deal of attention. Its role in cell division was not understood at first. Some observers held that the nucleus disappears during cell division and each daughter cell produces a new one. If this is so, there can be no connection between the nuclei of different cell generations. Others believed that during cell division the

nucleus was constricted and pinched in two with one part going into each daughter cell. It goes without saying that the nucleus would be without importance in the transmission of hereditary factors if its existence terminated at each cell division.

It should be kept in mind that, when living cells are examined with an ordinary light microscope, it is frequently difficult to see the nucleus. During cell division this rather indistinct body *does* disappear, especially if one is looking through a crude microscope. A new nucleus appears to form in each of the daughter cells. The difficulty in making observations, coupled with the fact that methods of fixation and staining were poorly developed, makes it easy to understand why cytologists believed what their eyes told them: that the nucleus disappears during cell division. Nevertheless there were many others who *believed* that the nucleus was not completely dissolved, but that in some manner a portion of the original nucleus persisted to give rise to the daughter nuclei.

In the year 1873 three biologists independently described complex nuclear changes which occurred during cell division and which are now termed *mitosis*. (Simultaneous, though independent, discovery is common in science. We shall have more examples of it.) They were A.Schneider, Otto Bütschli, and Hermann Fol. Schneider's paper appeared first (a 'paper' is an article appearing in a scientific journal). It was not concerned with cell division, but with the morphology of a flatworm, Mesostoma. The bulk of the paper is taken up with details of the structure but, being a careful observer, he described everything he saw, including cell division in the eggs (Fig. 2–3). These develop within the uterus. The uncleaved egg has a large fluid-filled nucleus, which contains a nucleolus (Fig. 2–3*a*). Shortly before the first cell division, the outline of the nucleus becomes indistinct, but by adding a little acetic acid it again becomes visible, though folded and wrinkled. Later the nucleolus disappears. All that remains is a mass of delicate, curved fibers, and these are seen only if acetic acid is added. Next thick 'strands' appear and become oriented in an equatorial plane (Fig. 2–3*b*). The granules of the egg become arranged in a regular manner, best seen after acetic acid treatment. The 'strands' increase in number and when the cell divides they pass into the daughter cells (Fig. 2–3*c, d*). You have probably identified Schneider's 'strands' with chromosomes and that is exactly what they were. The term 'chromosome' did not come into usage until 1888 but from now on we shall use it to avoid confusion.

If Schneider realized the importance of his observations on chromosomes in cell division, he certainly did not stress the point. The discussion in his paper is concerned with the morphology of flatworms and the relation of these animals with other groups in the animal kingdom.
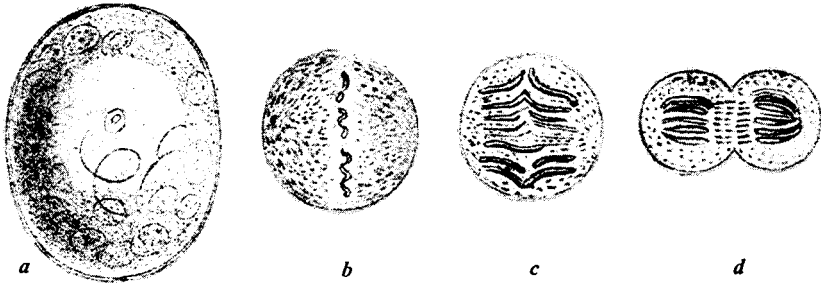
2–3 Nuclear changes during cleavage in Mesostoma embryos. *a* is an uncleaved ovum. The large clear area is the ovum itself, which contains a nucleus and nucleolus. The surrounding structures are follicle cells, which the embryo uses for food. They are not shown in *b, c,* and *d.* The spiral structures are sperm. *b, c,* and *d* show the 'strands,' which we now realize are chromosomes, and their movements during cell division. (A.Schneider, 'Untersuchungen über Plathelminthen.' *Oberhessischen Gesellschaft für Natur- und Heilkunde.* 14:69–140. 1873.)

It remained for others to interpret and show the importance of the phenomena that Schneider had observed.

Schneider was of the opinion that the nucleus persisted during division, though we must remember that he used acetic acid to establish this point. One could always question Schneider's interpretation, since the acetic acid treatment might have produced artifacts (abnormal structures) and the 'strands' could be so interpreted.

Bütschli, in the same year, described cell division in a roundworm, Rhabditis. He agreed with Schneider that the nucleus persisted during cell division.

Fol, the third investigator to describe cell division in 1873, thought that the nucleus entirely disappeared during division and was re-formed in the daughter cells. This view was shared by Flemming and Auerbach who published observations on cell division in 1874. It should be emphasized that these observers based their descriptions wholly or largely on what they observed in living eggs.

The problem of cell division was immediately recognized as being of considerable importance, and numerous investigators followed Schneider, Bütschli, and Fol. A review article on cell division and related topics was published by Professor Mark of Harvard in 1881. He quoted 194 papers (by 86 authors) which appeared in the five years from 1874 through 1878. This period was one of more or less blind experimentation and exploration. The animal and plant kingdoms were combed for favorable material. Some of the investigators observed living cells, and others

those that had been chemically treated. Interpretations of the observed phenomena were numerous and varied.

Some order was brought out of chaos by Walther Flemming in 1878 (and more especially in his monograph of 1882). He was outstanding, first in selecting excellent material, namely, the epidermal cells of larval salamanders; second, in being careful to check in living cells all things that he observed in fixed and stained preparations; and third, in employing hitherto unsurpassed technical methods.

Techniques and Instruments. Before Flemming's contribution is considered in detail, we shall digress to discuss the development of techniques for preparing cells for microscopic observation. Many earlier workers used dyes in a more or less haphazard way, but in 1858 Gerlach described an adequate staining method. He found that the nuclei of preserved cells take up the dye from a dilute solution of carmine, while the rest of the cell remains unstained or becomes only slightly stained. This became a vastly improved method for observing nuclei, most of which, it must be remembered, are seen with great difficulty in the living state. Gerlach did not discover carmine; he merely perfected its use in cytology. This dye was well known to the Indians of Mexico long before the coming of the Spanish. They obtained it from the crushed and dried bodies of cochineal insects reared especially for this purpose. Later the commercial use of carmine spread to Europe. In all probability Gerlach tried it as a 'hunch.' It happened to work.

Another dye, hematoxylin, was first used successfully by Böhmer in 1865. Commercial preparations were available, derived from a tropical American tree known as logwood. This dye, like carmine, stains the nucleus.

The first synthetic aniline dye was made by Perkin. The date of this discovery is generally given as 1856, when Perkin was a lad of 18 trying to synthesize quinine. Many different aniline dyes were made later, and soon they became the principal ones used commercially. They were tried by cytologists from time to time, but it was not until the period of 1875–80 that their use was perfected. It was found that some aniline dyes, such as eosin, would stain parts of the cell not affected by carmine or hematoxylin. It was then possible to use the double-stain methods that are now standard. The nucleus could be stained deep blue with hematoxylin and the cytoplasm a pale pink with eosin. This gave a much improved picture of cell structure.

Technical advances in still another field were providing an aid to cytologists. Rapid improvement was being made in microscopes. In the 1870s Abbe, the greatest microscope designer of recent times, began his

association with the Carl Zeiss optical works in Germany and increasingly fine instruments were turned out by this concern. In 1878 Abbe's oil-immersion objective was first produced (one of the initial users was the famous bacteriologist Koch). The oil-immersion lens enabled one to obtain a good image of cell structures at magnifications as much as 2,500 diameters. Technical advances in this field continued with the invention of Abbe's sub-stage condenser, and in 1886 Zeiss produced the apochromatic objective. This is the finest lens so far developed for the light microscope.

These advances meant that cytology had reached a point in its development where a person like Flemming could make a culminating advance in our understanding of cell division. He certainly did not discover mitosis (neither did any single person) but we owe to him more than to any other the concept of mitosis that we hold today. After Flemming, only details were added.

Flemming's Description of Mitosis. It was well known to Flemming and his contemporaries that the structures observed in living cells might be quite different in appearance from those seen in preserved cells. In some types of living cells no nuclei could be seen, yet after staining typical nuclei were visible. In a situation of this sort the question arises 'Are nuclei present in all normal cells or, in some cells, can they be artifacts resulting from the treatment used in preparing the cells for study?' Flemming reasoned his answer this way. In some types of living cells, which we can call type 1, nuclei can be seen. When these cells are fixed and stained, a nucleus of characteristic shape and color appears. In other types of living cells, which we can call type 2, no nucleus can be seen. Nevertheless when type 2 cells are fixed and stained, a nucleus that in all respects is identical in appearance to the nuclei of fixed and stained cells of type 1, can be seen. Since the stained nuclei of cell types 1 and 2 have the same appearance, and the treatment is the same in both cases, the most reasonable hypothesis is that a nucleus is present, though invisible, in living cells of type 2. It seems most unlikely that cells of type 2 could be without a nucleus in the living state and that fixation and staining could produce an artifact that was identical to the nuclei of fixed and stained type 1 cells. Flemming made an attempt to apply this type of reasoning to all cell structures, and in every case he tried to use the living cell as the basis of reference. Structures that could never be seen in living cells and that made their appearance only after fixation and staining must be regarded as questionable.

The Resting Stage. Flemmings' studies led to this concept of mitosis (Figs. 2–4 and 2–5). A resting stage cell is one not in mitosis. The
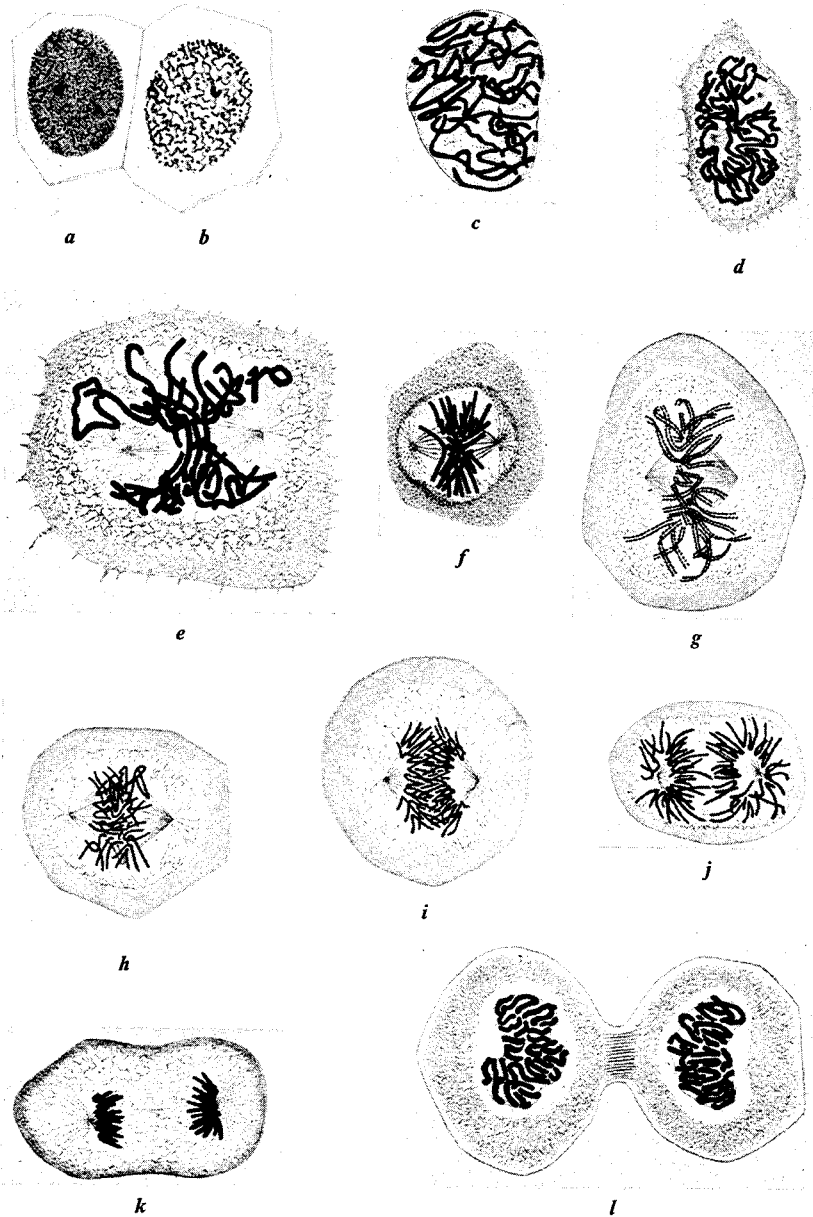
2–4 Flemming's drawing of mitosis in fixed and stained cells of the salamander embryo. The figures are arranged in sequence beginning with a resting stage in *a*. (W.Flemming, *Zellsubstanz, Kern und Zelltheilung,* 1882.) *a*. Resting stage. The chromosomes are invisible. The nucleus contains chromatin and two nucleoli. The

nucleus is spherical and generally occupies the central region of the cell. A nuclear membrane is present. In living resting stage cells the nucleus does not seem to have any internal structure. After fixation and staining, an irregular network of strands and granules, named chromatin, can be detected. In addition, one or more large spherical granules, the nucleoli, are present. Chromosomes cannot be seen in either the living or the preserved resting stage nucleus with the light microscope.

Prophase. Changes in the nucleus are the first indications that mitosis is under way. Long, delicate threads, the chromosomes, make their appearance. At first they are not easy to see, but with the passage of time they become increasingly distinct. Mitosis is a continuous process, but for descriptive purposes it can be divided into a number of stages. When chromosomes first become visible we say that the prophase stage has begun. If prophase chromosomes are examined carefully, they are seen to be double structures, each chromosome being composed of two long strands, the *chromatids,* lying side by side. It should be emphasized that only in the very best preparations is it possible to see the chromatids. In most instances, and this is true today, only the entire chromosome is seen. Flemming was able to see the duplicate prophase chromosomes both in living and preserved salamander cells. During prophase the nucleoli become smaller and eventually they disappear.

Metaphase. Prophase ends and the next stage, metaphase, begins with the disappearance of the nuclear membrane. By this time the chromosomes have become very distinct. In stained preparations they are prominent cell structures, grouped together in the center of the cell. Early in metaphase the spindle and asters become prominent. The spindle is given this name because of its shape. In the living cell the spindle appears as a transparent body. In fixed and stained cells there are one or more tiny

---

nuclear membrane is present. *b.* Late resting stage. The chromosomes are forming. The nucleoli are disappearing. *c.* Prophase. The chromosomes have formed. No nucleoli. The nuclear membrane is still present. (The cytoplasm is not shown.) *d.* Metaphase. The nuclear membrane has disappeared. Two centrioles, each with a tiny aster, are shown. *e.* Metaphase. The centrioles and the astral rays surrounding them are distinct. The chromosomes are moving to the middle of the cell. *f.* Metaphase. The spindle has formed between the two centrioles. *g.* Metaphase. This is an exceptionally good preparation. Each chromosome is seen to be double, that is, each composed of two chromatids. *h–j.* Anaphase. The chromosomes are moving apart and one group is approaching each centriole. *k.* Telophase. The chromosomes have separated into two groups. The spindle has nearly disappeared and the astral rays are becoming indistinct. *l.* The cell is dividing. The chromosomes are being surrounded by a nuclear membrane and shortly each will be in the resting stage.
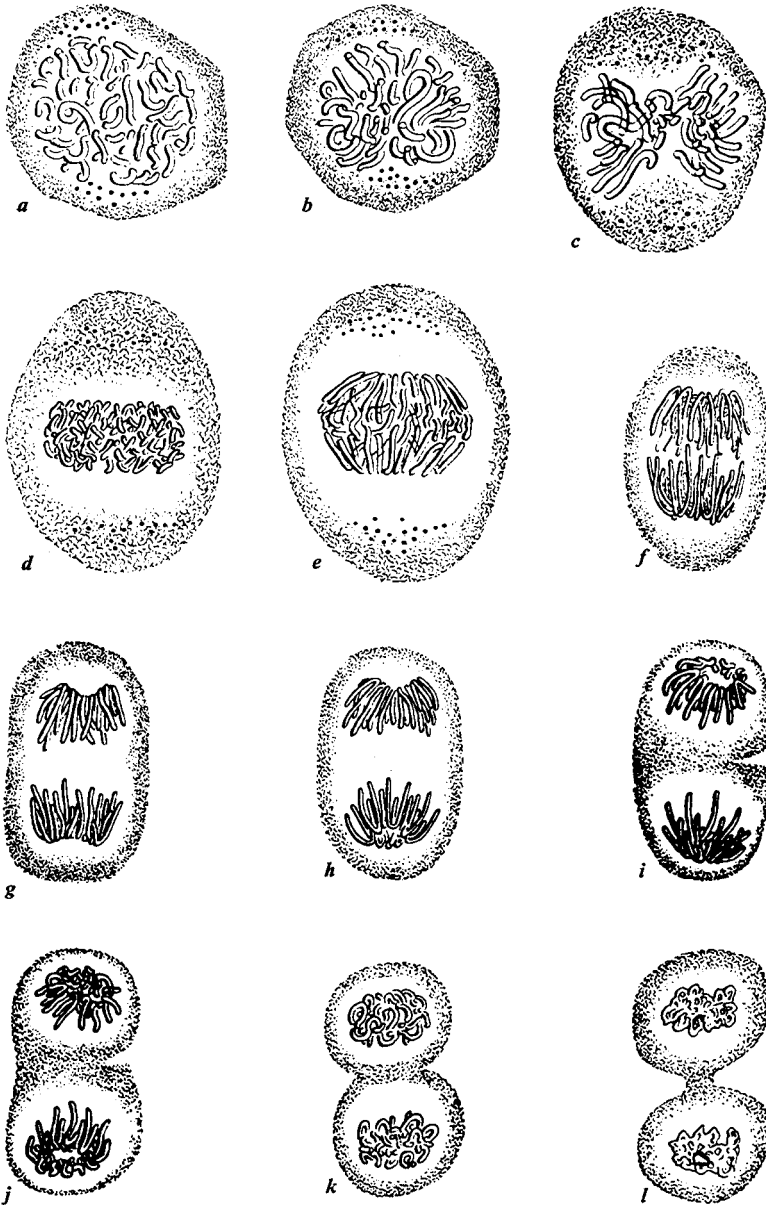
2–5 Flemming's drawings of mitosis in living epidermal cells of a salamander larva. The drawings are arranged in sequence, beginning with a prophase in *a* and ending with the two daughter nuclei in *l*. The nuclear membrane, asters, spindle, and centrioles are not shown (W.Flemming, *Zellsubstanz, Kern und Zelltheilung,* 1882).

granules, the *centrioles,* at each end. One can also see long strands, the *spindle fibers,* connecting the two centriole regions. At metaphase the chromosomes become arranged in a plate perpendicular to the long axis of the spindle. The *asters* are observed in fixed and stained cells as a series of fibers radiating out from the centrioles.

Anaphase. Metaphase ends and the next stage, anaphase, begins with the separation of the chromosomes into two groups. One group goes to each pole of the spindle. Flemming thought it possible that the double nature of the prophase chromosomes might be of significance in this respect. Could it be that each chromosome duplicates itself, forming two chromatids, and that at anaphase one chromatid goes to one pole and the other chromatid to the opposite pole of the spindle? (Flemming's belief was found to be true five years later by van Beneden.)

Telophase. The two groups of chromosomes move to the poles of the spindle. When they arrive there, telophase, the last stage in mitosis, begins. The chromosomes become increasingly less distinct and the nuclear membrane is re-formed. The spindle and asters begin to disappear. The cell as a whole now divides into two daughter cells with the plane of division cutting across the spindle at the equator. As a result, each daughter cell contains a group of chromosomes. Eventually it becomes impossible to see the chromosomes; the cell has entered the resting stage once more. It should be emphasized that the term 'resting' means only that the nucleus is not in mitosis. It does not signify a lack of metabolic activity.

These nuclear changes, known as mitosis, were observed in so many different kinds of animal cells that Flemming believed that they must be a universal feature of living organisms. The nuclei of plants were found to behave in an almost identical manner. Figure 2–6 shows mitosis in a lily. The chromosome stages are identical with those in the salamander but the lily, like most plants, differs from animals in lacking centrioles and asters.

Our general conclusions based on the work of cytologists up to 1882 are these: cells come from pre-existing cells, nuclei from pre-existing nuclei, and chromosomes from pre-existing chromosomes.

At the time when some cytologists were studying the chromosomal events during mitosis, others were investigating fertilization and the formation of ova and sperm. These studies were to contribute greatly to the understanding of heredity, which was to come in the early years of the twentieth century.
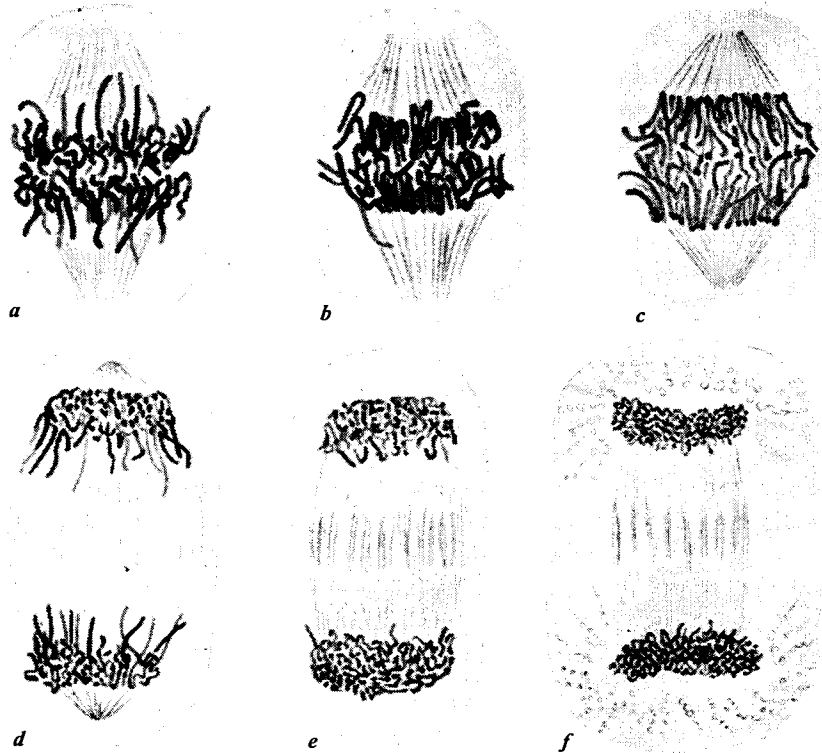
2–6 Mitosis in the lily (W.Flemming, *Zellsubstanz, Kern und Zelltheilung,* 1882).
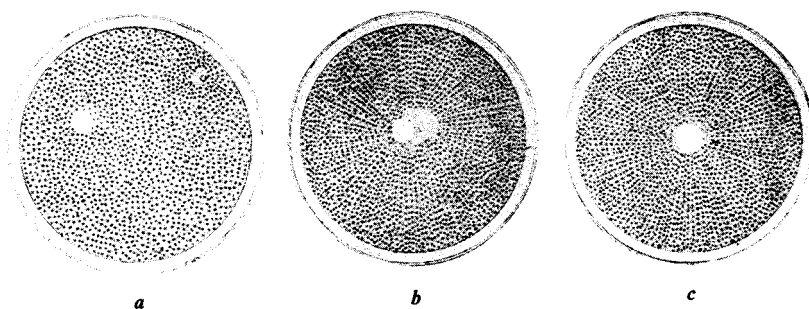
FERTILIZATION

The elementary fact of fertilization, namely, that a sperm rather than the seminal fluid is required to initiate development of the ovum, was discovered by J.L.Prevost and J.B.Dumas in 1824. At this time the precise role of the sperm was not understood. In 1854, George Newport conducted experiments that suggested the sperm actually penetrates the ovum. A full understanding of this event had to wait until it was realized that both the ovum and the sperm are cells. Schwann's belief that the ovum was a cell was not shared by many cytologists, but the work of Carl Gegenbauer in 1861 seemed to show that it was. Several years later it was also established that the sperm was a single cell. Inheritance, then, must be based on the transmission of cells—an ovum from the mother and a sperm from the father.

Fertilization in the Sea Urchin. In 1873–4 several investigators reported that two nuclei could be seen in the ovum soon after fertilization and

before cell division had begun. It remained for Oskar Hertwig (1876) to demonstrate for the sea urchin that one of these nuclei was the nucleus of the ovum and the other was derived from the sperm. He found that these two nuclei approached each other, made contact, and in a slightly later stage only one nucleus was present (Fig. 2–7). In Hertwig's opinion this single nucleus was the result of fusion of a maternal nucleus of the ovum and a paternal nucleus of the sperm. Almost immediately some other workers came to the same conclusion. Observations were made on eggs of many different species, and it was realized that the formation of the zygote nucleus through the fusion of a *paternal pronucleus* derived from the sperm and a *maternal pronucleus* from the ovum is a general phenomenon.

It is necessary to emphasize that an attempt is being made only to trace the sequence of key ideas, observations, and experiments that led to an improved understanding of inheritance. This account is not 'true' history. Hertwig's observations and interpretations were important steps in coming to this understanding. One must not assume, however, that all other scientists immediately recognized that this was so. Many doubted his observations and interpretations and it took several years before he was generally believed to have been correct. New discoveries are rarely accepted when they are proposed. They must be repeated by others before they are accepted as part of scientific knowledge.

Two types of material proved of the greatest usefulness in studies of

a *b* *c*

2–7 Hertwig's figures of sea-urchin embryos showing the nuclear events in fertilization. *a* shows an embryo 5 minutes after ova and sperm were mixed. The egg nucleus is the clear area on the left side of the embryo. The sperm nucleus is in the upper right portion of the embryo. *b* is an embryo 10 minutes after the ova and sperm were mixed. The two nuclei are in contact near the center of the embryo. *c* is an embryo 15 minutes after the ova and sperm were mixed. A single nucleus is present. This is the zygote nucleus that will undergo a series of mitotic divisions to form all of the nuclei of the individual (O.Hertwig, 'Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies,' *Morph. Jahrb.* 1:347–434. 1876).

fertilization: the sea urchin (a marine animal related to the starfish) and Ascaris (a parasitic worm found in the intestine of man and other mammals). The sea urchin was especially suitable because it was easy to obtain the ova and sperm, because fertilization could be carried out under the controlled conditions of the laboratory, and because of the transparency of the ova and early embryos. The adults were collected in the ocean, usually by dredging, and in the laboratory both males and females could be stimulated to shed their gametes. The ova could be collected in one dish and the sperm in another. These would number in the millions. When the two were mixed, fertilization occurred in a matter of seconds.

One of the most striking things about fertilization and early development in the sea urchin is the fact that events are synchronous in all the zygotes fertilized at one time. Thus, if one preserves embryos at successive five-minute intervals after fertilization, the sequence of nuclear events can be worked out with precision. Shortly after fertilization, the paternal pronucleus would be noticed close to the outer membrane of the ovum. At later times it would be found progressively closer to the maternal pronucleus, and eventually fused with it.

Fertilization in Ascaris. As cytological material the sea urchin has one serious defect: its chromosomes are small and numerous. One can observe the general events in fertilization, but the details of chromosome movements and changes could not be determined with ease. On the other hand, the parasitic worm Ascaris provides excellent material for studying the behavior of chromosomes since it has only four chromosomes and these are large and stain successfully. As a consequence the detailed nuclear events in fertilization were first observed in Ascaris.

The process of fertilization in Ascaris was described by Edouard van Beneden (1846–1912) in 1883 and by others such as Theodor Boveri (1862–1915) in 1888. Boveri's figures, as reproduced in Figure 2–8, will be the basis of our account of fertilization. (For the present please ignore the legend for this figure, since it cannot be fully understood until the entire chapter has been read.) The first figure, *a,* shows a section of the entire ovum shortly after fertilization. The paternal pronucleus is in the lower right-hand quadrant. It contains two chromosomes. The structure forming a wrinkled cap immediately above it is the acrosome, which is the portion of the sperm head composed of Golgi material. In the center of the ovum there is a dark granular area. This is the centrosome, which was formed by a part of the sperm lying immediately behind the sperm nucleus. There are two structures near the top of the figure. The one within the ovum is the maternal pronucleus. It contains two chromo-
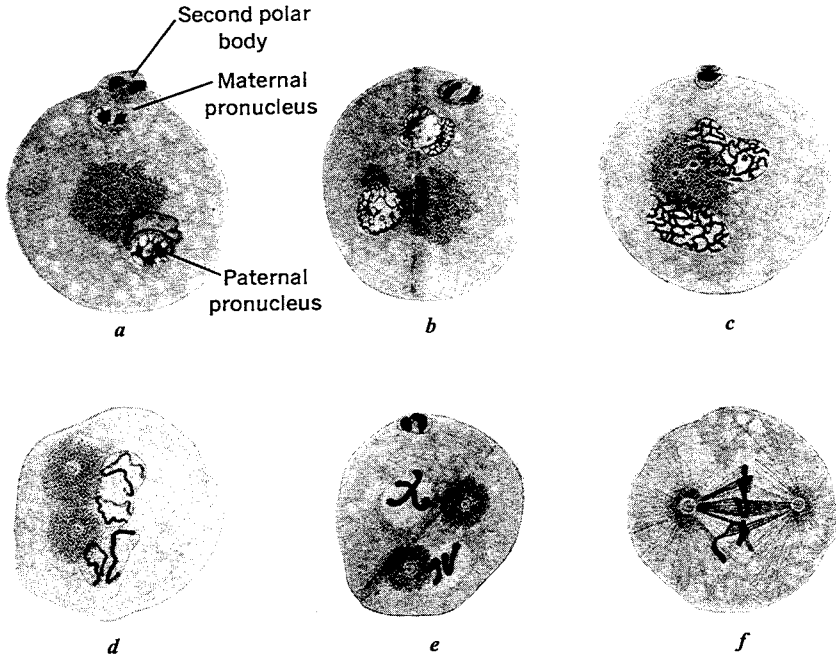
2–8 Fertilization in Ascaris. In *a* the sperm has entered the ovum and formed the paternal pronucleus. The maternal chromosomes have undergone the second meiotic division. This resulted in a maternal pronucleus with 2 chromosomes and a second polar body with 2 chromosomes. The dark structure in the center of the egg is the centrosome brought in by the sperm. In *b* the maternal and paternal pronuclei have enlarged and are approaching each other. In *c* the pronuclei are continuing to enlarge. Notice the two granules between the pronuclei and in the centrosome substance. These are the centrioles. In *d* it can be seen that each pronucleus has 2 chromosomes. The centrioles are moving apart and the surrounding centrosome substance is dividing into two portions. In *e* the centrioles and the associated centrosomes have nearly reached opposite sides of the egg. The 2 chromosomes of each pronucleus are more prominent than before. The second polar body is still attached to the top of the egg. In *f* the first mitotic division of the embryo has begun. Four chromosomes, the diploid number for this species, can be seen on the spindle. These were derived from the 2 pronuclei. At this division each of these 4 chromosomes will split and the two cells that result from the division will each receive 4 chromosomes (Th.Boveri, 'Die Befruchtung und Teilung des Eies von Ascaris megalocephala,' *Jenaische Zeit.* 22:685–882. 1888).

somes. The other structure, which is attached to the top of the ovum, is a polar body. It can be seen in *b, c,* and *e* as well. For the present we shall disregard it since it is concerned with meiosis—a subject to be considered in the last part of the chapter. In *b* the maternal and paternal pronuclei have moved somewhat closer and their chromosomes have become indistinct. In *c* the chromosomes in both pronuclei have become elongated and coiled. Two centrioles have appeared in the centrosome material. In *d* the centrosome itself has divided, half being centered around each centriole. The two centrioles, with their associated centrosomes, move farther apart in *e*. In *f* they are on opposite sides of the cell with a spindle between them and an aster radiating out from each. During this period considerable changes have been occurring in the pronuclei. In *d* the chromosomes have shortened and it can be seen that each pronucleus contains two. A further shortening of the chromosomes is apparent in *e*. During the interval between *e* and *f* the membranes around both the maternal and paternal pronuclei disappear and in *f* the four chromosomes have entered the spindle. The mitotic stage shown in *f* is an early metaphase. Somewhat later each of these four chromosomes will become double to make a total of eight, and at anaphase these will separate and four chromosomes will move to each pole of the spindle.

The chromosome number of the zygote, therefore, is four. Half of this total is provided by the paternal pronucleus and half by the maternal pronucleus. The number of chromosomes in a pronucleus is spoken of as the *haploid* (or *monoploid*) number and the number in the zygote is the *diploid* number. It was clear from the work of van Beneden, Boveri, and others that each parent transmits an equal number of chromosomes to the zygote. So far as one could tell the chromosomes in the maternal pronucleus were morphologically the same as those in the paternal pronucleus.

Further study revealed that throughout the animal kingdom similar events are observed with only a few exceptions. Fertilization involves the combination of a haploid pronucleus derived from the sperm and a haploid pronucleus derived from the ovum. Their pooled chromosomes form the diploid number in the zygote. Since the increase in cell number during embryonic development involves mitosis, all the cells of the embryo and adult should be expected to contain the diploid number of chromosomes. Research has shown this to be true with only a few exceptions.

THE FORMATION OF GAMETES
An important problem was raised by these discoveries of the chromosomal events during fertilization: if the nuclei of embryonic and adult

cells are diploid, how do the nuclei of ova and sperm become haploid? Ascaris provided excellent material for the study of this problem and the observations of van Beneden, Boveri, and Hertwig established the essential points during the 1880s, first solving the problem in the ovum and later in the sperm. They discovered that there are two unusual cell divisions during the formation of gametes. As a result of these divisions diploid cells have their chromosome numbers reduced to the haploid condition. These two divisions are highly modified mitotic divisions; they are known as the *meiotic divisions.* The process itself is *meiosis.* The relation between mitosis and meiosis can be brought out by a description of the chromosomal changes during the formation of the ovary and of mature ova.

Mitosis in the Early Ovarian Cells. The ovary of Ascaris begins to form early in development. At first it consists of a few cells and in the course of time these divide to form the tremendous number comprising the ovary of the adult. *This increase in the number of cells is brought about by mitosis.* In mitosis each chromosome duplicates itself at every cell division so the number of chromosomes remains constant from one cell generation to the next. So far as individual cells are concerned this is what occurs: the Ascaris nucleus contains four chromosomes as the diploid number; before every cell division there is a duplication of each of these four chromosomes to give a total of eight chromatids; at anaphase the chromatids are separated, four going to each daughter cell. This process is repeated with the result that all the cells of the ovary are diploid.

Meiosis in the Female. Many of these diploid ovarian cells become enlarged and form ova. The ovum of Ascaris remains diploid until it has been released from the ovary and entered by a sperm. The ovum nucleus then undergoes a series of two meiotic divisions that leads to each of the resulting cells having the haploid number of chromosomes. The process of meiosis in the Ascaris ovum is shown in Figure 2–9, which is reproduced from the work of Boveri.

The First Meiotic Division of the Ova. At the onset of meiosis each of the four long chromosomes (as shown in Figure 2–8*f*) becomes condensed to form a tiny sphere. Next the chromosomes come together in pairs, a process that is known as *synapsis.* The chromosomes do not fuse during synapsis, they merely come close to one another. Next, each chromosome becomes duplicate. Thus, each of the two pairs of synapsed chromosomes becomes a group of four, such a group being known as a *tetrad.* The first of Boveri's figures, namely 2–9*a*, shows an ovum in this condition, which is the metaphase of the first meiotic division. In it we see the chromosomes grouped into two tetrads. In *b* the tetrads are being
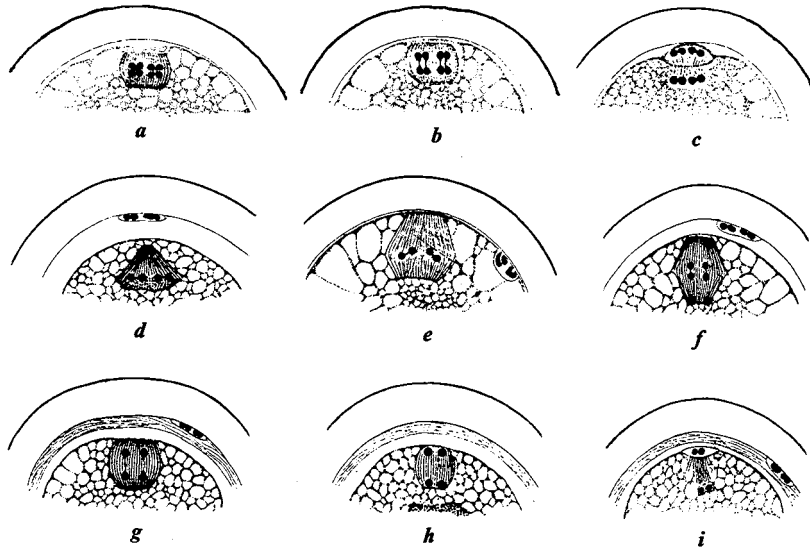
2–9 Meiosis in Ascaris eggs. *a* shows the upper portion of the egg and its nucleus. Previously the 4 chromosomes have undergone synapsis to form two pairs. Each chromosome then duplicated itself. The result is 2 tetrads, each composed of 4 chromatids. In this figure the tetrads are in the metaphase of the first meiotic division. *b* is the anaphase of the first meiotic division. Each tetrad has divided into 2 dyads. *c* the first meiotic division is complete. The first polar body has pinched off from the egg. It contains 2 dyads. The egg likewise contains two dyads. *d* the second meiotic division has begun and the 2 dyads are in the spindle. The first polar body with its chromosomes is beneath one of the egg membranes. It can be seen in all of the remaining figures except *h*. In *e* the dyads are rotating prior to their separation. *f* is a metaphase of the second meiotic division. *g* is the anaphase of the second meiotic division. *h* is the telophase of the second meiotic division. *i* the second meiotic division is complete. The second polar body has formed and it contains 2 chromosomes. The egg nucleus also contains 2 chromosomes (Th.Boveri, 'Die Bildung der Richtungskörper bei Ascaris megalocephala und Ascaris lumbricoides,' *Jenaische Zeit.* 21:423–515. 1887).

divided and in *c* they have separated completely. Half of each tetrad, or a *dyad,* goes to each pole of the spindle. It will be noticed that the spindle is not in the center of the cell but instead it is at the periphery. Inasmuch as the cell will divide across the equator of the spindle, the result will be two cells of very unequal sizes. The large cell resulting from the division is the ovum and the small cell is the *first polar body*. The chromosomes that enter the first polar body are morphologically and numerically equivalent to those that remain in the ovum.

The Second Meiotic Division of the Ova. In *d* the first polar body is well separated from the ovum and the two dyads within the ovum are

on the spindle of the second meiotic division. *At this division the chromosomes do not duplicate themselves.* Consequently the dyads are divided and as a result two chromosomes go to each pole of the spindle. This second meiotic division divides the cell unequally, as did the first, the result being a large ovum and a tiny second polar body. At the end of the second, and last, meiotic division there are only two chromosomes in the Ascaris ovum. The nuclear membrane forms around these two chromosomes, the haploid number, and in this manner the maternal pronucleus is produced.

The subsequent history of the maternal pronucleus has been discussed as an aspect of fertilization and Figure 2–8 should be re-studied (the maternal pronucleus in 2–9*i* is in the same stage as in 2–8*a*).

Meiosis in the Male. The observation that the paternal pronucleus was haploid, yet the male diploid in its body cells suggested that a process similar to that just described must also occur in the male. A study of sperm formation in Ascaris showed this to be the case (Fig. 2–10). The last two cell divisions before a sperm forms are meiotic divisions. As in the egg, the four chromosomes form two pairs and each chromosome duplicates itself. The result is two tetrads each composed of four chromatids. During the first meiotic division the tetrads are divided and half of each goes into each of the daughter cells. Not only is nuclear division equal but cell division is also equal, which is in contrast to the situation in the ova. At the next division the dyads are divided between the two daughter cells, which are again of equal size. Thus, from one cell with four chromosomes, and by means of two meiotic divisions, four cells each with two chromosomes are formed. Each of these four haploid cells develops without further division into a sperm cell.

The essential difference between meiosis and mitosis is this: in mitosis there is one duplication of every chromosome for each cell division; in meiosis there is only one duplication of every chromosome for the two meiotic divisions. As a consequence, in mitosis the chromosome number remains constant from one cell generation to the next; in meiosis the two meiotic divisions form cells with the haploid number of chromosomes.

With full realization that the nuclear events associated with maturation and fertilization were important biological phenomena, cytologists examined many species of animals and plants. It was found that the reduction divisions leading to haploid pronuclei occur throughout the animal and plant kingdoms. In short, another principle of almost universal application (a few exceptions were found) had been discovered. The facts as outlined in this section were generally, though not universally, believed by 1890.

a *b* *c* *d*

e

f *g*

h *i*

j *k*

l *m* *n* *o*
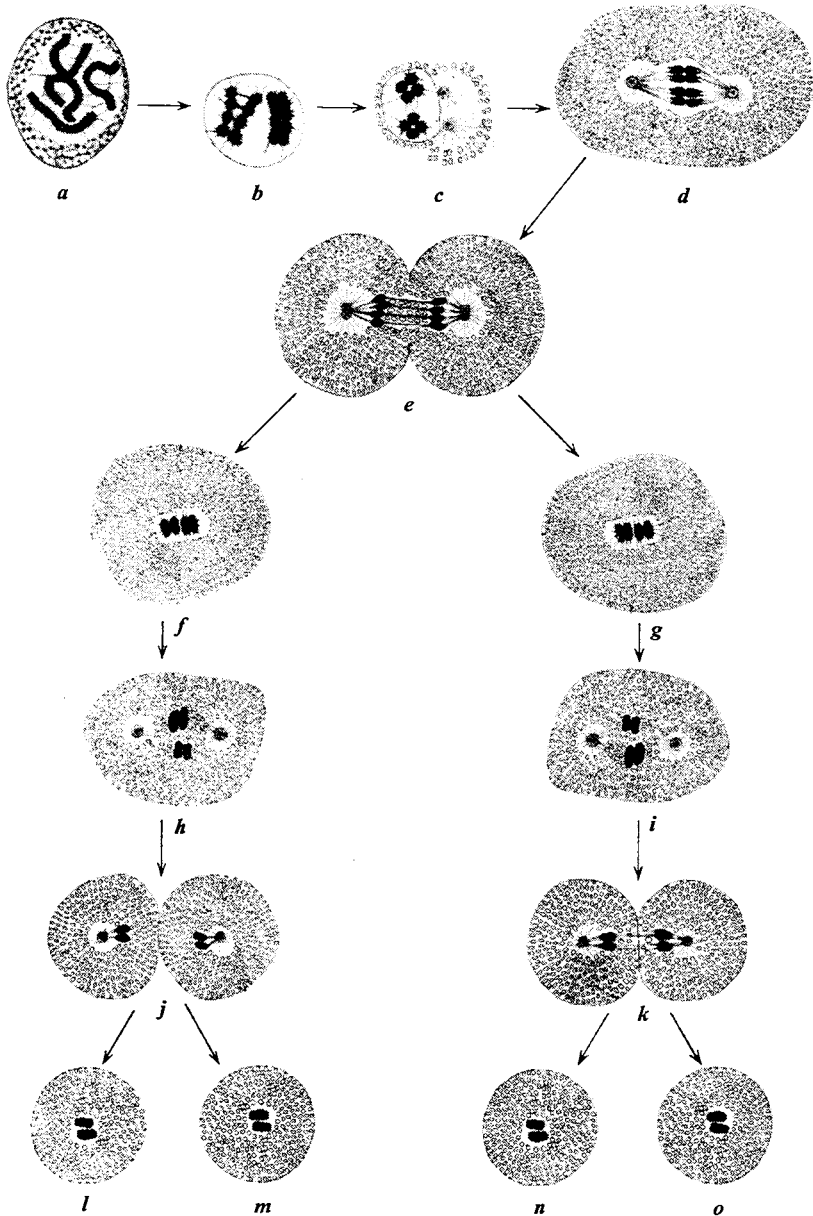
2–10 Meiosis in Ascaris males. The diploid chromosome number in Ascaris is 4. The cells of the testis that will later form the sperm are diploid as shown in *a. b* shows a nucleus near the beginning of meiosis. The 4 chromosomes are undergoing synapsis. As meiosis continues each chromosome becomes shortened until it forms a tiny sphere. During this process each chromosome splits. As a result each

A summary of meiosis and fertilization in Ascaris is given in Figure 2–11.

These observations are interesting and important in their own right but it is not immediately obvious how they relate to the mechanism of inheritance—neither was it obvious to the early cytologists. The conceptual gap between observing an orderly sequence of chromosomal behavior and a general theory of inheritance is awesome. Few minds are capable of being the first to bridge a gap of this magnitude.

As we have learned, Darwin's theory of pangenesis was not generally accepted. Various alternatives were proposed, were subjected to the merciless scrutiny of the scientific community, and judged inadequate. But at least some progress was made: by the end of the nineteenth century, it was generally agreed that inheritance, however it was effected, was related to the nucleus.
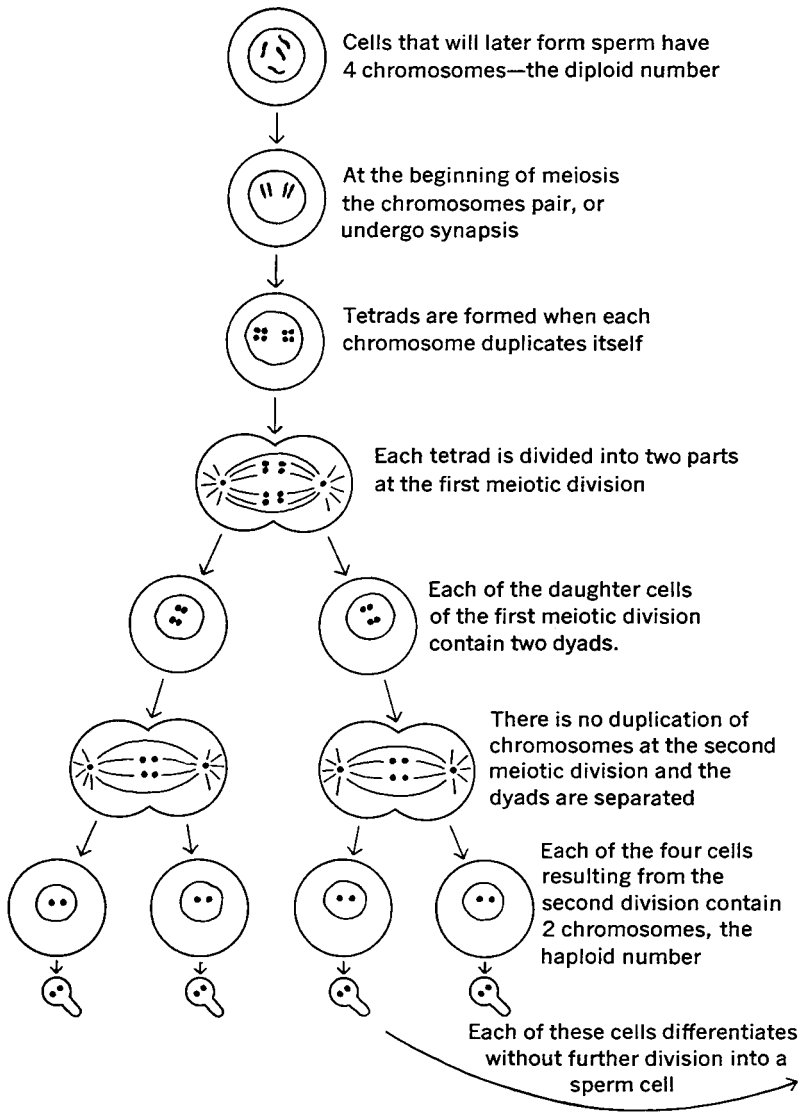
THE NUCLEUS AND HEREDITY

The middle years of the 1880s witnessed several attempts to see if inheritance was controlled by some definite part of the cell. We might have expected this to be the case when we realize that cytologists, in a decade of uparalleled discovery, had worked out the essentials of mitosis, fertilization, and meiosis.

Haeckel's Hypothesis of the Nuclear Control of Inheritance. An effort to find a cytological basis for inheritance was made as early as 1866 by Ernst Haeckel (1834–1919), who postulated that the nucleus was responsible for the transmission of the inherited features of an organism. The data available to Haeckel in 1866 were not sufficient to test this hypothesis. As E.B.Wilson (1856–1939), the great American cytologist, was to remark some years later, it was a lucky guess. If a lucky guess of this sort had been made by some obscure scientist, it is probable that its influence on subsequent events would have been negligible. But Haeckel was a leader in the field of biology in his day. An idea of his, no matter how slight the factual basis, would have been noticed. It is conceivable, therefore, that Haeckel's hypothesis of nuclear control of
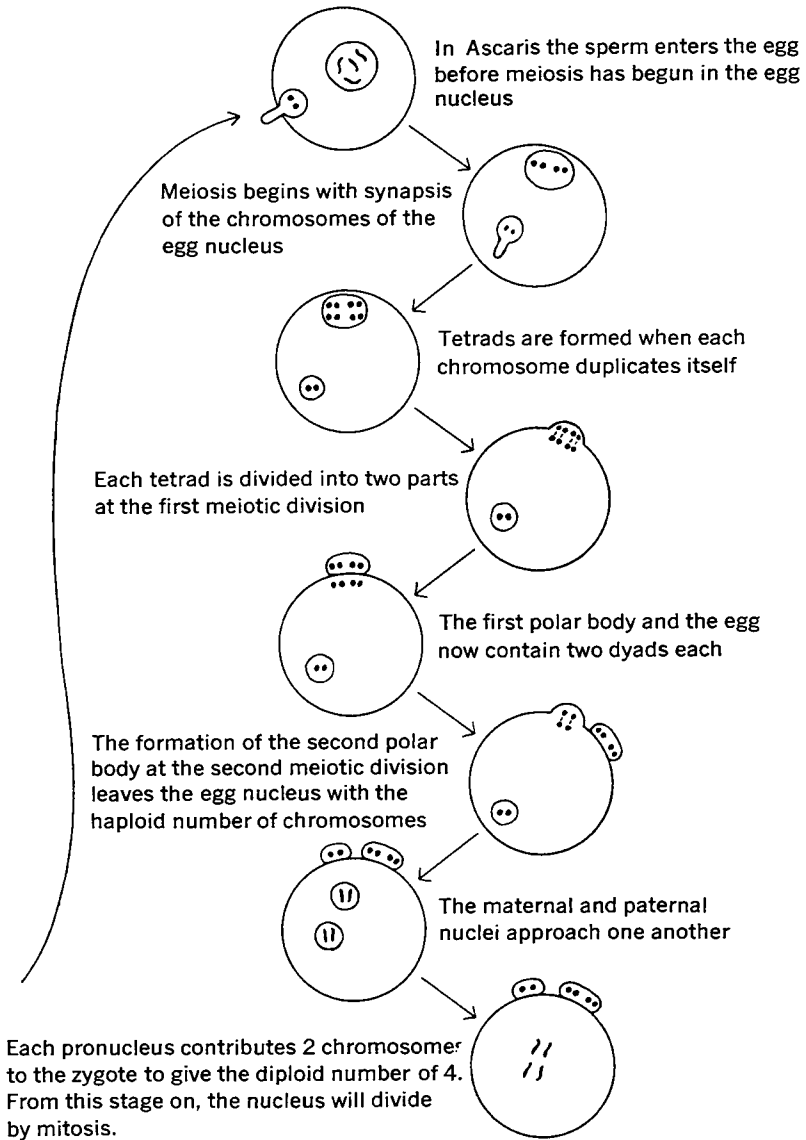
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of the 2 pairs of synapsed chromosomes forms a tetrad, as shown in *c*. At the first meiotic division the 2 tetrads enter the spindle *(d)* and are divided, half of each tetrad (a dyad) going to each pole as shown in *e*. As a result of the first meiotic division 2 cells are formed *(f, g)*. Each of these contains 2 dyads. In the second meiotic division *(h, i, j, k)* the dyads of the 2 cells are pulled apart. At the end of this division there are 4 cells *(l, m, n, o)*. Each of these contains 2 chromosomes, the haploid number. There is no further division of these 4 cells and they develop directly into sperm (A.Brauer, 'Zur Kenntniss der Spermatogenese von Ascaris megalocephala,' *Arch. Mikr. Anat.* 42:153–213. 1893).

OUTLINE OF MEIOSIS AND

Cells that will later form sperm have
4 chromosomes—the diploid number

At the beginning of meiosis
the chromosomes pair, or
undergo synapsis

Tetrads are formed when each
chromosome duplicates itself

Each tetrad is divided into two parts
at the first meiotic division

Each of the daughter cells
of the first meiotic division
contain two dyads.

There is no duplication of
chromosomes at the second
meiotic division and the
dyads are separated

Each of the four cells
resulting from the
second division contain
2 chromosomes, the
haploid number

Each of these cells differentiates
without further division into a
sperm cell

2–11

FERTILIZATION IN ASCARIS

In Ascaris the sperm enters the egg before meiosis has begun in the egg nucleus

Meiosis begins with synapsis of the chromosomes of the egg nucleus

Tetrads are formed when each chromosome duplicates itself

Each tetrad is divided into two parts at the first meiotic division

The first polar body and the egg now contain two dyads each

The formation of the second polar body at the second meiotic division leaves the egg nucleus with the haploid number of chromosomes

The maternal and paternal nuclei approach one another

Each pronucleus contributes 2 chromosomes to the zygote to give the diploid number of 4. From this stage on, the nucleus will divide by mitosis.

inheritance helped to prepare others for thinking and experimenting along these lines.

Nägeli's Idioplasm Theory. In 1884 Carl Wilhelm von Nägeli (1817–1891) suggested that a substance which he called the *idioplasm* was responsible for inheritance. The idioplasm was thought to be an invisible chemical network that extended throughout the cell and from cell to cell. Nägeli did not observe the idioplasm in cells. He invented it to account for inheritance. He did not regard it as a highly stable material, but as one that might change during development, or as the result of nutrition or other external conditions. In any event it must return to the original condition in the embryo. Nägeli did considerable theorizing on the subject of inheritance, but his concept of possible mechanisms was extremely vague. His hypothesis was nearly impossible to test, and hence it could be of no real usefulness in directing efforts to profitable experimentation.

Early Evidence for the Nuclear Control of Inheritance. In 1884–85 four German scientists, working independently, came to the conclusion that the physical basis of inheritance must lie in the chromosomes. They were Oskar Hertwig, Edouard Strasburger, Rudolf Kölliker, and August Weismann (1834–1914). The first three were primarily laboratory scientists. For at least a decade they had been leaders in the analysis of problems concerned with the nucleus. Weismann, on the contrary, is remembered largely for his theoretical work.

These four men believed that the chromosomes were the physical basis of inheritance for the following reasons.

1. Even though inheritance was not well understood, it seemed that both parents have an equal share in transmitting their characteristics to the offspring. The soundest support for this belief came from work on plant hybrids. In the 1760s, Joseph Gottlieb Kölreuter crossed *Nicotiana paniculata* and *Nicotiana rustica,* two species of the tobacco genus that differ in many ways. So far as he could tell, the hybrid offspring were the same whether the cross was *paniculata* ♀×*rustica* ♂ *or rustica* ♀ ×*paniculata* ♂. The obvious conclusion was that both parents contributed equally to the characteristics of the offspring.

   What is the physical basis of this equality? It was know, of course, that the only links between parent and offspring are the ovum and sperm. These two cells are about as different as any two cells could be. Usually the ovum has a mass thousands or millions of times the mass of the sperm. Ova usually contain a large quantity of cytoplasm, whereas sperm contain almost none. This would suggest that the cytoplasm was not the basis of inheritance because, if it were, it might be expected that the

female's contribution would be much greater than the male's. The only parts of the sperm and ova that seemed to these four scientists to be equivalent were the nuclei. The sperm pronucleus and the egg pronucleus were identical so far as one could tell. *Perhaps this equivalence of structure was the basis of the equivalent importance of the two gametes in inheritance.* Van Beneden's description of the pronuclei in Ascaris, each with two chromosomes, seemed most suggestive.

2. During cell division, the cytoplasm and its formed structures seem to be divided passively. The chromosomes, on the other hand, go through a complicated mitosis which results in each of the daughter cells receiving exactly the same number of chromosomes. It seemed to Hertwig and the others that the significance of this complicated process might be that the nucleus was the basis of inheritance: why should the chromosomes, alone among the cell structures, be duplicated and then divided equally unless they were of great importance in inheritance?

3. The complex chromosomal changes during meiosis were understandable in terms of keeping the chromosomes constant from generation to generation. There was no similar phenomenon for any other cell structure. Since inheritance was an intergeneration phenomenon and the chromosomes seemed to be the only cell structures that were transmitted in an exact way from one generation to another, perhaps the chromosomes were of importance in inheritance.

4. Finally, there was a more direct test of nuclear function in regenerating protozoa. The forms selected for this work were single-celled organisms with one nucleus. It was possible to cut the animals into two parts, one part containing cytoplasm and the other cytoplasm and the nucleus. Both parts healed. The part without a nucleus lived for some time, but it was unable to regenerate to form a whole animal, and it was incapable of reproduction. The part with the nucleus could regenerate a whole animal and could reproduce normally.

These observations were suggestive, but they did not 'prove' that the nucleus was the physical basis of inheritance. The fact that chromosomes appeared to be the only cell structure that remained constant from cell to cell, and from generation to generation, *could* mean that inheritance was by way of the chromosomes. Many famous cytologists believed that a good working hypothesis was 'The nucleus is important in heredity.'

In the next chapter, we shall learn that in the year 1900 the rediscovery of a scientific paper written much earlier by Mendel put the subject of inheritance in an entirely new light. It is of interest, therefore, to summarize the advances that those cytologists interested in heredity

had made up to the year Mendel's results became generally known. Such a summary was given retrospectively by E.B.Wilson in 1914:

> The work of cytology in its period of foundation laid a broad and substantial basis for our more general conceptions of heredity and its physical substratum. It demonstrated the basic fact that heredity is a consequence of the genetic continuity of cells by division, and that the germ-cells are the vehicle of transmission from one generation to another. It accumulated strong evidence that the cell-nucleus plays an important role in heredity. It made known the significant fact that in all the ordinary forms of cell-division the nucleus does not divide *en masse* but first resolves itself into a definite number of chromosomes; that these bodies, originally formed as long threads, split lengthwise so as to effect a meristic division of the entire nuclear substance. It proved that fertilization of the egg everywhere involves the union or close association of two nuclei, one of maternal and one of paternal origin. It established the fact, sometimes designated as 'Van Beneden's law' in honor of its discoverer, that these primary germ-nuclei give rise to similar groups of chromosomes, each containing half the number found in the body-cells. It demonstrated that when new germ-cells are formed each again receives only half the number characteristic of the body-cells. It steadily accumulated evidence, especially through the admirable studies of Boveri, that the chromosomes of successive generations of cells, though commonly lost to view in the resting nucleus, do not really lose their individuality, or that in some less obvious way they conform to the principle of genetic continuity. From these facts followed the far-reaching conclusion that the nuclei of the body-cells are diploid or duplex structures, descended equally from the original maternal and paternal chromosome-groups of the fertilized egg. Continually receiving confirmation by the labours of later years, this result gradually took a central place in cytology; and about it all more specific discoveries relating to the chromosomes naturally group themselves.

> All this had been made known at a time when the experimental study of heredity was not yet sufficiently advanced for a full appreciation of its significance; but some very interesting theoretical suggestions had been offered by Roux, Weismann, de Vries, and other writers. While most of these hardly admitted of actual verification, two nevertheless proved to be of especial importance to later research. One was the pregnant suggestion of Roux (1883), that the formation of chromosomes from long threads brings about an alignment in linear series of different materials or 'qualities.' By longitudinal splitting of the threads all the 'qualities' are equally divided, or otherwise definitely distributed, between the daughter-nuclei. The other was Weismann's far-seeing prediction of the reduction division, that is to say, of a form of division involving the separation of undivided whole chromosomes instead of the division-products of single chromosomes. This fruitful suggestion (1887) pointed out a way that was

destined to lead years afterwards to the probable explanation of Mendel's law of heredity.

> Such, in bird's-eye view, were the most essential conclusions of our science down to the close of the nineteenth century.*

Suggested Readings

Readings in Heredity and Development contains selections from Grew (1682), Roget (1836), Virchow (1858), Weismann (1885, 1887, 1891), and Wilson (1900) as well as an extensive bibliography. The article by Coleman, listed below, provides an excellent introduction to the history of cytology as related to inheritance.

BRADBURY, S. 1967. *The Evolution of the Microscope.* New York: Pergamon Press.
COLEMAN, WILLIAM. 1965. 'Cell, nucleus, and inheritance: an historical study.' *Proceedings of the American Philosophical Society 109:*124–58.
HUGHES, ARTHUR. 1959. *A History of Cytology.* New York: Abelard-Schuman.
MARK, E.L. 1881. 'Maturation, fecundation, and segmentation of *Limax campestris* Binney.' *Bulletin Museum of Comparative Zoology 6:*173–625.
WILSON, E.B. 1914. Croonian Lecture: The bearing of cytological research on heredity.' *Proceedings of the Royal Society of London.* B. *88:*333–52.

Questions

1. Examine Figure 2–2 carefully. Do you detect any element of sameness among these diverse objects that suggests all should be put into the same class, that is, be identified as cells? Had Hooke seen these objects do you believe that he would have regarded them as equivalent to the structures he observed in cork?
2. The nucleus of a cell does disappear before cell division. What could have been the reason, therefore, why some cytologists believed that the nucleus had an unbroken continuity from one cell generation to another?
3. In most instances it is far easier to see cell structures in fixed and stained cells than in living cells. That being the case, why did Flemming lay such stress on the importance of observing structures in living cells?
4. After studying Flemming's illustrations of chromosomes, would you have thought that all of the chromosomes of a cell are more or less alike, or that each is unique?
5. Would it be possible to work out the sequence of events in mitosis from a study of fixed and stained material only? Would it be necessary to assume any relation between a nucleus in the resting stage and a metaphase?
6. Prevost and Dumas performed experiments that led them to believe that the sperm and not the seminal fluid is the active agent in fertilization. Can you suggest how such experiments might have been done?

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* This quotation is from Wilson's Croonian Lecture, which is given in its entirety in *Readings,* Chapter 4.

7. The meiotic mechanisms described for Ascaris are almost universal in animals and very similar in plants. Can you devise other ways of halving the number of chromosomes?

8. You probably already know something about DNA and its role in inheritance. Study Figures 2–4, 2–9, and 2–10 and try to image what is happening to the DNA.

9. Haeckel's hypothesis of nuclear control of inheritance was shown to be correct—a generation after he proposed it. Why cannot scientific ideas be accepted more promptly?

10. Compare Darwin's Theory of Pangenesis with Nägeli's Theory of Idioplasm.

11. In terms of what you know of cell biology, how would you evaluate these statements of Virchow (*Readings,* Chapter 2)?

    a. 'Every animal presents itself as a sum of vital unities, every one of which manifests all the characteristics of life.'

    b. After saying that cells are the basis for all the phenomena of life, he writes, 'According to my ideas, this is the only possible starting point for all biological doctrines.'

    c. '…the cell is really the ultimate morphological element in which there is any manifestation of life and…we must not transfer the seat of real action beyond the cell.'

12. How could one prove the hypothesis *'omnis cellula e cellula'*?

13. Can you suggest why the phenomenon of mitosis was not discovered in the eighteenth century?

14. Do you believe that the chromosomes maintain their essential structure during the resting stage?

3 Mendelism

During the entire period from Darwin's attempted synthesis of the facts of inheritance down to 1900, a scientific paper that was to revolutionize our understanding of heredity lay unappreciated on the shelves of many libraries. The article itself had been published in 1866. In it the author, Gregor Mendel, presented some of the results of his experiments in crossing varieties of garden peas.

The Discovery of Mendel's Paper. The 'discovery' and appreciation of the importance of Mendel's paper is a very dramatic incident in the history of science. Three individuals in the year 1900 realized the great importance of Mendel's work.

During the 1890s there had been renewed interest in plant hybridization. The three scientists who 'discovered' Mendel—Hugo de Vries, Carl Correns, and Erik von Tschermak—were doing breeding experiments of their own. Each of them independently came to more or less the same conclusions that Mendel had expressed in 1866 *before they knew of Mendel's paper*. This is another example of a frequent happening in science. When the field is 'ready,' the discovery is certain to be made. If Mendel had never lived, the history of genetics would not have been greatly different. About the year 1900, either he would be rediscovered or, had he never lived, others would reach essentially the same conclusions as he had in 1866. His work was unappreciated in his own lifetime, for biologists in 1866 had neither the background nor the prescience to understand the significance of what he had accomplished.

Gregor Mendel's famous article is not a scientific paper in the usual

sense, but a lecture presented to the Natural History Society of Brünn in 1865. The full results of his research were never published, but the portion that he did include, coupled with an extraordinary analysis of the data, makes his paper one of the landmarks of science.

Mendel was fully aware that experiments in plant breeding had been conducted by many famous men. It was true, nevertheless, that no general principles had emerged from previous studies. To Mendel this was a serious affair, since an understanding of inheritance was essential for an understanding of evolution and he was deeply interested in Darwin's work (*The Origin of Species* appeared during the period he was conducting his experiments). He began experiments that were intended to give information on inheritance and evolution.

Peas as Experimental Material. Mendel selected peas for his experiments because they possessed many desirable features:

1. Numerous varieties of peas were available commercially. They provided the material that he studied.
2. The plants were easy to cultivate and the generation time was short.
3. The offspring of the crosses between the varieties were fertile.
4. The structure of the pea flower is such that accidental pollination was thought not to occur. The anthers that produce the pollen and the stigma where the pollen grains germinate are completely enclosed by the petals. Normally, pollen from a flower falls on the stigma of the same flower and self-fertilization results. In those cases where crosses between varieties are desired, it is possible to remove the anthers before they mature and somewhat later, when the stigma is mature, to cover it with pollen from another flower.

Mendel's approach to the problem of inheritance was different from that of previous workers. His predecessors had concentrated on the whole organism. Usually they had crossed varieties that differed in many characters and the offspring were found to be intermediate or in rare cases more like one parent. Mendel focused his attention on specific differences and studied how these were inherited generation after generation. Some of his varieties had *round* seeds; others had *wrinkled* seeds. In all he studied seven different characters of the pea, and for each he had two varieties, as shown in the following lists:

CHARACTER AFFECTED	VARIETIES
Seed shape	*round* or *wrinkled*
Seed color	*yellow* or *green*
Seed coat color	*colored* or *white*
Pod shape	*inflated* or *wrinkled*

Pod color	*green* or *yellow*
Flower position	*axial* or *terminal*
Stem length	*long* or *short*

Crosses of Plants with Contrasting Characters. First, he made sure that all of his varieties would breed true. Once this was established he made crosses between all of the pairs just listed. The results were most unexpected in the light of earlier experiments by other plant breeders: the offspring were never intermediate but were always like one of the parents. When peas with *round* seeds were crossed with peas with *wrinkled* seeds, for example, the offspring were plants with *round* seeds. Mendel spoke of the form that appeared in the offspring as *dominant* in comparison to the form that did not appear, which he called *recessive*. Dominance can be determined only by making a cross and observing the type of offspring obtained; it could not be predicted before the experiment was performed merely by examining the parent plants. The varieties that Mendel used were found to have these relationships:

DOMINANT	RECESSIVE
round seed	*wrinkled* seed
yellow seed	*green* seed
colored seed coat	*white* seed coat
inflated pod	*wrinkled* pod
green pod	*yellow* pod
axial flowers	*terminal* flowers
long stem	*short* stem

Over the course of years, geneticists have introduced some terms that make it easier to discuss crosses. The original parental generation is abbreviated P. The offspring of the P generation is the first filial or F_1 generation. The offspring of the F_1 is the second filial generation or F_2, the third is the F_3, and so on. It is also customary to describe crosses in terms of the character. Thus, the cross of a plant with round seeds with a plant with wrinkled seeds is shortened to *round×wrinkled*.

The F_2 Generation. Some plant breeders might have stopped the experiments after a single cross had determined dominant and recessive characteristics. The results were clear cut. The F_1 plants were always like one of the parents. Mendel, however, continued his crosses and was careful to realize that although an F_1 of the *round×wrinkled* might be identical with the *round* parent, its parentage was different. Perhaps its offspring would reflect the different origin.

Since peas are self-fertilizing, the F_1 plants pollinated their own ovules and gave the F_2, and when Mendel studied the F_2 plants he found that both dominant and recessive characters were present. Now he did a

simple though revolutionary thing: he counted the number of individuals of each type. In every cross there was a ratio of 3 dominant to 1 recessive. His results can be summarized as follows:

P	F_1	F_2	COUNTS	RATIO
round×wrinkled	round		5,474 round	
			1,850 wrinkled	2.96:1
yellow×green	yellow		6,022 yellow	
			2,001 green	3.01:1
colored×white	colored		705 colored	
			224 white	3.15:1
inflated×wrinkled	inflated		882 inflated	
			299 wrinkled	2.95:1
green pods×yellow pods	green		428 green	
			152 yellow	2.82:1
axial×terminal	axial		651 axial	
			207 terminal	3.14:1
long×short	long		787 long	
			277 short	2.84:1

These results would suggest that the rules of inheritance were the same, irrespective of the varieties being crossed. The F_1 plants were always of one type, which resembled one of the parents. In the F_2, two classes appeared and the frequency was 75 per cent dominants and 25 per cent recessives, or a ratio of 3:1.

The F_3 Generation. Mendel continued his experiments and obtained an F_3 generation. We may take as an example the *round×wrinkled* cross, which in the F_2 gave 75 per cent *round* and 25 per cent *wrinkled*. He allowed a number of the *wrinkled* plants to self-fertilize to give an F_3, and found that all bred true, that is, only *wrinkled* plants were obtained in the F_3. The F_2 *round* plants gave two results:

1. One-third (193 of 565 plants) bred true, giving *round* plants in the F_3.
2. Two-thirds (372 of 565 plants) gave *round* and *wrinkled* in a ratio of 3:1. On the basis of their genetic behavior these were like the F_1 plants.

Mendel's Hypothesis. Mendel explained these results in this way: Let us assume that the *round* variety is round because it has a *gene* **R,** and the *wrinkled* variety is wrinkled because it has gene **r**. (Mendel did not use the term gene but spoke of 'factors' or 'traits.' It will be simpler for us to use the modern term gene from the very beginning and note the gradual change in its meaning. For the present we shall understand a gene to be the basis of an inherited character. We will not commit our-

selves further to what it is or where it is.) If a cross is made between the *round* and *wrinkled* varieties, the gametes of the *round* plant will have **R** and the gametes of the *wrinkled* plant will have **r**. Fertilization will produce an F_1 with both genes, **Rr**. In appearance this plant is *round,* **R** being dominant and **r** recessive. We speak of the appearance of the individual as its *phenotype* and the genetic composition of the individual as its *genotype*. Thus, the phenotype of this F_1 plant is *round* and its genotype is **Rr**. Plants of the **Rr** type will produce gametes and Mendel assumed that any single gamete would contain either **R** or **r** *but never both*. He also assumed that gametes containing **R** and gametes containing **r** are produced in equal numbers. If the union of F_1 gametes is at random, then we will obtain a 3:1 ratio of *round* to *wrinkled*. The entire cross could be presented in this schematic manner:

P_1	phenotype	*round*	×	*wrinkled*
	genotype	**R**		**r**
	gamete genotype	**R**		**r**
F_1	phenotype	*round*		*round*
	genotype	**Rr**		**Rr**
	gamete genotype	**R** **r**		**R** **r**
F_2	phenotype	*round* *round*	*round*	*wrinkled*
	genotype	**RR** **Rr**	**Rr**	**rr**
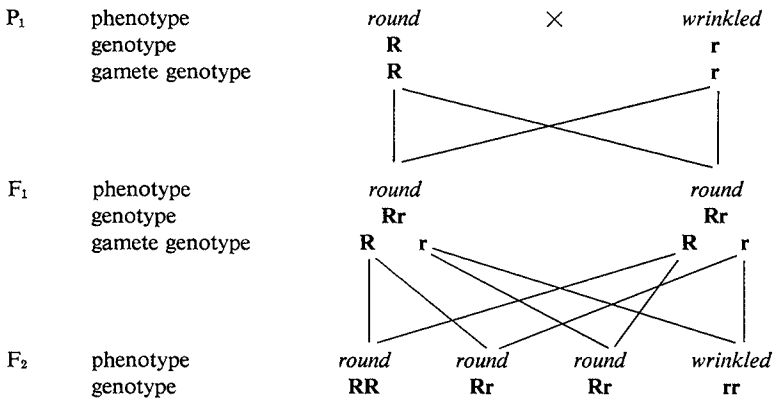
It is actually unnecessary to show two plants in the F_1 since both are the same. Two are used in order to make it easier to visualize the cross that gives the F_2.

This scheme provides a formal explanation of the results, namely, the origin of the 3:1 ratio. It also shows that the F_2 *round* plants are of two types. One in three of the *round* plants is pure *round*. If self-fertilized, it would breed true. The remaining 2/3 of the *round* plants are **Rr**. If these are allowed to self-fertilize there will result an F_3 ratio of 3 *round* to 1 *wrinkled*. It will be recalled that Mendel made these tests of the F_2 and the theoretical and actual results are the same.

This schematic interpretation applies to all of Mendel's crosses involving one pair of genes. Several important conclusions can be reached if the interpretation is correct:

1. Dominant and recessive genes do not affect one another. In the F_1 of the cross discussed, the genotype was **Rr**. There was no visible

effect of the **r** gene, the seeds being just as round as in the pure *round* parent. When the **Rr** plant was allowed to self-fertilize both *round* and *wrinkled* seeds were obtained. These F_2 *wrinkled* seeds were identical in appearance to the P generation *wrinkled* seeds.

2. The gametes produced by an F_1 plant of the **Rr** constitution will contain either **R** or **r,** never both.
3. The **R** and **r** types of gametes will be produced in equal numbers by an **Rr** plant.
4. Combination between gametes is a chance affair, and the frequency of different classes of offspring will depend on the frequencies of gametes. Since an F_1 plant having the **Rr** constitution will produce 50 per cent gametes of the **R** type and 50 per cent gametes of the **r** type, the mathematical basis of the F_2 frequencies will be as follows:

POLLEN

	50% **R**	50% **r**
50% **R**	25% **RR**	25% **Rr**
50% **r**	25% **Rr**	25% **rr**

OVULES

Crosses Involving Two Pairs of Genes. Mendel's next step was to see if the conceptual scheme devised for crosses involving one pair of genes could be applied to crosses involving two pairs of genes. For this he used *round* and *wrinkled* as one pair and *yellow* and *green* as the other. Previous work had shown that the cross between *yellow* and *green* produced *yellow* in the F_1 and a ratio of 3 *yellow* to 1 *green* in the F_2.

When a cross was made between a plant with *round-yellow* seeds and a plant with *wrinkled-green* seeds all of the F_1 plants had *round-yellow* seeds. In the F_2 the following seed types were obtained:

315 *round-yellow*
108 *round-green*
101 *wrinkled-yellow*
32 *wrinkled-green*

One interesting thing brought out by these data is the appearance of two new seed types that were not present in either the P or the F_1 generation. These new types are *round-green* and *wrinkled-yellow*. This and the other results, however, fit perfectly into the Mendelian scheme, if we assume the complete independence in inheritance of the two pairs of genes. The cross would be as follows:

P	phenotype	*round-yellow*	×	*wrinkled-green*
	genotype	R Y		r y
	gamete genotype	R Y		r y

F₁	phenotype	*round-yellow*
	genotype	Rr Yy
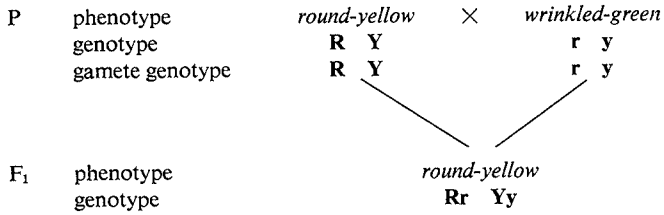
In the formation of gametes by the F_1 plant, Mendel assumed that a gamete would have only one member of a pair of genes. Thus, a gamete would have either **R** *or* **r** and in addition either **Y** *or* **y**. Four classes of gametes would be produced and these in equal frequency. The classes would be **RY, Ry, rY, and ry**. If an F_1 plant is allowed to self-fertilize there will be these four types of pollen and the same four types of ovules. This will give 16 possible combinations, as shown below:

POLLEN

		RY	Ry	rY	ry
	RY	RR YY *round-yellow*	RR Yy *round-yellow*	Rr YY *round-yellow*	Rr Yy *round-yellow*
OVULES	**Ry**	RR Yy *round-yellow*	RR yy *round-green*	Rr Yy *round-yellow*	Rr yy *round-green*
	rY	Rr YY *round-yellow*	Rr Yy *round-yellow*	rr YY *wrinkled-yellow*	rr Yy *wrinkled-yellow*
	ry	Rr Yy *round-yellow*	Rr yy *round-green*	rr Yy *wrinkled-yellow*	rr yy *wrinkled-green*

Of the 16 possible F_2 combinations, 9 will be *round-yellow,* 3 will be *round-green,* 3 will be *wrinkled-yellow,* and 1 will be *wrinkled-green*. Here is a comparison of Mendel's data with the theoretical expectation:

	ACTUAL	EXPECTED
Round-yellow	315	313
Round-green	108	104
Wrinkled-yellow	101	104
Wrinkled-green	32	35
	556	556

The figures given in the 'actual' column are those obtained by counting the seeds. The 'expected' values are computed in this manner: If we

have a total of 556 plants and expect 1/16 of them to be *wrinkled-green,* we find 1/16 of 556, which is 35. The other expected classes are 9/16, 3/16, and 3/16 of 556.

Testing the Hypothesis. Mendel made a further test of the adequacy of his hypothesis. He predicted that the F_2 plants would have four phenotypic classes and a total of nine genotypic classes (refer to the checkerboard). Genetic tests would allow him to distinguish among plants of the same phenotype but of different genotypes. His test consisted of allowing all the F_2 plants to self-fertilize to produce an F_3 and then seeing if the actual results of the crosses were the same as were expected on the basis of the hypothesis. This is what he found. *The Breeding Behavior of the F_2 Round-yellow.* It can be seen from the checkerboard that 9/16 of the F_2 plants are *round-yellow.* These plants are listed in the first column of the table that follows, grouped according to genotype. These plants are all of the same phenotype but they belong to four different genotypes, namely **RRYY, RRYy, RrYY,** and **RrYy**. Although of identical appearance, these four genotypes should be distinguishable on the basis of the ratios of the types of offspring they will produce following self-fertilization. These predicted ratios are listed in the third column. The fourth column gives the number of plants that one would expect to give the ratio listed in column 3, if Mendel's hypothesis is correct. For example, Mendel predicted one out of every nine *round-yellow* plants to be **RRYY.** If a plant of this genotype is self-fertilized, it would give only *round-yellow* offspring in the F_3. No other genotype will give this result. Mendel planted 315 of the F_2 *round-yellow* plants and of these 301 gave progeny. He would predict therefore 1/9 of these 301 plants, or 33, to be **RRYY** and give only *round-yellow* seeds. The fifth column gives the actual results. In the example we have been using, Mendel predicted that 33 of the plants would be **RRYY** and he found that 38 were of this genotype.

F_2		FRE-QUENCY	EXPECTED F_3 RATIOS	NUMBER EXPECTED	ACTUAL NUMBER
RRYY		1/9	all *round-yellow*	33	38
RRYy **RRYy**	}	2/9	3 *round-yellow;* 1 round-green	67	65
RrYY **RrYY**	}	2/9	3 *round-yellow;* 1 *wrinkled-yellow*	67	60
RrYy **RrYy** **RrYy** **RrYy**	}	4/9	9 *round-yellow;* 3 *round-green;* 3 *wrinkled-yellow;* 1 *wrinkled-green*	134	138
				301	301

The Breeding Behavior of the F_2 Round-green. Three-sixteenths of the F_2 were of this category. Mendel raised 102 of these plants and these are the results:

F_2		FRE-QUENCY	EXPECTED F_3 RATIOS	NUMBER EXPECTED	ACTUAL NUMBER
RRyy		1/3	all *round-green*	34	35
Rryy **Rryy**	}	2/3	3 *round-green;* 1 *wrinkled-green*	68	67
				102	102

The Breeding Behavior of the F_2 Wrinkled-yellow. The *wrinkled-yellow* comprised 3/16 of the F_2. Mendel raised 96 of these plants and these are the results:

F_2		FRE-QUENCY		NUMBER EXPECTED	ACTUAL NUMBER
rrYY		1/3	all *wrinkled-yellow*	32	28
rrYy **rrYy**	}	2/3	3 *wrinkled-yellow;* 1 *wrinkled-green*	64	68
				96	96

The Breeding Behavior of the F_2 Wrinkled-green. This category comprised 1/16 of the F_2. Mendel raised 30 plants of this type. The results were as follows:

F_2	FREQUENCY	EXPECTED F_3 RATIOS	NUMBER EXPECTED	ACTUAL NUMBER
rryy	1	all *wrinkled-green*	30	30

The fact that the F_2 plants gave an F_3 that did not differ materially from the prediction, indicated that Mendel's conceptual scheme of inheritance was in full accord with his experimental results. In every case the actual values are surprisingly close to those expected. The expected values are based on the probability of the various types of gametes combining in a certain way. The expected and actual values are rarely identical: we should not expect them to be so any more than we should always expect five heads for every ten tosses of a coin.

Mendel went one step farther and crossed plants differing in three contrasting characters. The results were entirely according to expectation but they will not be discussed.

These are some of the conclusions that may be drawn from Mendel's experiments:

1. The most important conclusion is that inheritance appears to follow definite and rather simple rules. Mendel was able to apply the same type of explanation to the results of all of his crosses. He had reached that stage in the development of a scientific theory where results could be predicted with a high degree of accuracy. This is one goal of a scientist.

2. When plants of two different types were crossed, there was no blending of the individual characteristics. Mendel studied seven pairs of contrasting characters. One member of each pair of contrasting characters could be thought of as dominant and the other as recessive. In a hybrid formed from crossing a pure-breeding dominant and a pure-breeding recessive, the appearance of the plant was identical with that of the dominant parent.

3. The factors responsible for the dominant and recessive condition were not modified by their occurrence together in a hybrid. If two hybrids were crossed, both dominant and recessive offspring would appear. Neither the dominant nor the recessive offspring would give any evidence of contamination resulting from hybridization. In short, an F_2 recessive would be identical in genotype and phenotype to the P generation recessive.

4. When a pure-breeding plant exhibiting a dominant characteristic **(A)** is crossed with a recessive **(a)** the F_1 **(Aa)** is like the **A** plant in appearance. *Segregation* occurs in the F_2, which results in a ratio of three plants having the dominant character (one of which will be pure breeding and the other two like the F_1) to one recessive. Segregation is often called *Mendel's First Law*.

5. If two pairs of genes, such as **Aa** and **Bb,** are involved in a cross, each pair acts independently so far as transmission to the next generation is concerned. This phenomenon is known as *independent assortment* and it is often spoken of as *Mendel's Second Law*. Its mode of operation can be understood if we consider the F_2 originating from an F_1 **AaBb** plant. So far as the phenotypes are concerned 3/4 of the F_2 will have the **A** phenotype and 1/4 will have the **a** phenotype. The same is true for the other pair of genes: 3/4 of the plants will have the **B** phenotype and 1/4 will have the **b** phenotype. It is entirely a matter of chance which combination of genes a given F_2 plant will receive. Thus of the 3/4 that will have the **A** phenotype, 3/4 will also have the **B** phenotype and 1/4 will have the **b** phenotype. Of the 1/4 that will have the **a** phenotype, 3/4 will have the **B** phenotype and 1/4 will have the **b** phenotype. So far as both characters are concerned 9/16 of the F_2 (3/4 of 3/4) will have both the **A** and **B** phenotypes, 3/16 will have the **A** and **b** phenotypes, 3/16 will

have the **a** and **B** phenotypes, and 1/16 will have the **a** and **b** phenotypes. The 9:3:3:1 ratio of the F_2 is due to the independent assortment of genes in the gametes of the F_1 plant.

6. The gametes will contain only one type of inherited factor of each contrasting pair. Thus the gametes of an F_1 **Aa** plant will produce gametes containing either **A** or **a**, never both. If two factors are involved, as in an **AaBb** plant the gametes will be **AB**, or **Ab**, or **aB**, or **ab**, never **Aa, Bb, ABb, Aab,** and so on. All possible combinations will be obtained, consisting of one member of each pair of genes. Every type of gamete will be produced in equal frequency.

It must be remembered that, without further work, these conclusions could apply only to the seven pairs of pea genes actually studied by Mendel. The fact that Mendel's rules applied to these would indicate that other genes of peas *might* behave in a similar way. The discovery that rules of inheritance could be established for peas would suggest that the same or similar rules might also apply to other plants (and possibly animals). This, of course, would have to be tested by experimentation. The great worth of Mendel's theory was that its clear and definite formulation made testing by experimentation possible. The same could not be said for any previous theory of inheritance.

Most of the important discoveries in biology turn out in retrospect to be fairly simple. Inevitably we wonder, Why did not that idea occur to someone before? Why had no one discovered these simple relations when varieties were crossed? Why had no one realized the significance of Mendel's approach during the 34 years between 1866 and 1900? These are unanswerable questions, but the following facts are germane to the last one. Mendel was almost unknown among biologists during his day and his results were published in a journal that attracted little attention. This is clearly only part of the story. It is perhaps more correct to say that biologists in 1866 were unable to appreciate the significance of Mendel's work. Their minds were not prepared. There is one interesting bit of information in this connection. Mendel carried on a lengthy correspondence with Nägeli, explaining the results of his experiments. It should be remembered that Nägeli was greatly interested in heredity, he being the proponent of the idioplasm concept. He, of all people, should have seen Mendel's point but he failed to appreciate the significance of the pea experiments.

Still another fact is that Mendel did not believe for long in the universality of his findings. This was the result of an unfortunate choice of material, the hawkweed, which was recommended to him by Nägeli for

some additional experiments. In the hawkweed the ovules are capable of parthenogenetic development. As a result, many of the crosses he believed he was performing were not crosses at all. Mendel did not realize this and was unable to understand why he did not observe the ratios he had previously found in peas. He probably came to believe that his results held for peas alone. Mendel and his ideas were of no importance during the last third of the nineteenth century when students of inheritance concerned themselves mainly with the cycle of chromosomal behavior in meiosis, fertilization, and the mitotic divisions during development.

In the last few years before 1900, the three scientists who were to rediscover Mendel's work were experimenting with crosses of varieties of plants. Two of them, Hugo de Vries (1848–1935) and Carl Correns (1864–1935), independently discovered Mendel's rules of inheritance before they knew of Mendel.

In 1900, de Vries reported the results of hybridizing varieties of 15 species of plants. When one member of the parental generation was dominant and the other recessive, the F_1 generation always resembled the dominant parent. In the F_2 generations, he observed the 3:1 ratio that Mendel had found long before: the number of recessives in the 15 crosses varied from 22 to 28 per cent, averaging 24.93 per cent. De Vries emphasized the critical point that, in the F_1 individuals which have dominant and recessive genes, the gametes are *pure,* that is, they have only one sort of gene, never both. This phenomenon, the Purity of the Gametes, was to be a matter of contention for years. What could the mechanism possibly be? It was difficult to accept any biological phenomenon for which there seemed to be no mechanism.

Carl Correns conducted extensive crosses among varieties of corn and peas and also published in 1900. In most instances he obtained the same results as had Mendel. There were exceptions, however. In a few instances there was no dominance: the F_1 individuals were intermediate in appearance. Correns reported also that Mendel's Law of Segregation did not always apply, though in the majority of cases it did. Since segregation was the usual result, however, he wondered how it might come about. Thus in an F_1 hybrid, which might be symbolized **Aa** how could the gametes come to contain either **A** or **a,** never both? And what could account for the fact that the breeding results indicated that the ratio of **A** to **a** is 1:1? He made the tentative and prophetic suggestion that the chromosomes could be responsible. Thus, segregation could be based on the separation of chromosomes at a nuclear division, quite possibly the one in meiosis that Weismann referred to as the reduction division.

Clearly there was more to Mendel's laws than peas.

Confirmations and Exceptions to Mendel's Scheme. In the first few years after 1900, when Mendel's findings became widely known to biologists, the results of many crosses were reported. The majority of these gave ratios that were nearly identical with Mendel's pea crosses. That is, in a cross of a pure-breeding dominant and a pure-breeding recessive, the F_1 population consisted only of plants with the dominant characteristics. The F_2 of such a cross contained both of the original types, in the ratio of three dominants to one recessive. If one of the F_1 plants was crossed with a pure recessive, the ratio of dominants to recessives in the offspring was 1:1. If two pairs of contrasting characters were involved, the F_2 ratio was 9:3:3:1. If the F_1 of the cross just mentioned was crossed to a double recessive, the ratio was 1:1:1:1.

A few gave slightly different results that could be understood with minor adjustments of the Mendelian scheme. Still others defied explanation at that time. This last group of cases was put aside in the hope that eventually a modified Mendelian theory could explain the results. Subsequent events showed this to be a profitable procedure.

In these early years of the twentieth century, the British geneticist, William Bateson (1861–1926), was the most vigorous disciple of Mendelism. Even before Mendel's work was known to the scientific world at large, Bateson had undertaken an extensive program of breeding. As a result of this preparation, he was in a proper frame of mind to grasp the significance of Mendel's work. In England, Bateson waged a scientific battle to convince his fellow biologists that Mendel's approach to the problem of inheritance was a useful one. This 'battle' was not always waged with the fairness, objectivity, lack of bias, and honest criticism that should be the basis for scientific discussions. In fact, the participants behaved like ordinary human beings. Much of the genetic work of this period was summarized in reports Bateson made to the Royal Society (1902, 1905, 1906, 1908, 1909) and in his book, *Mendel's Principles of Heredity* (1909).

Some Useful Genetic Terms. Bateson and others introduced some terms that are useful in describing genetic events. From Mendel's experiments he 'reached the conception of unit-characters existing in antagonistic pairs. Such characters we propose to call *allelomorphs* [now called *alleles*], and the zygote formed by the union of a pair of opposite allelomorphic gametes, we shall call a *heterozygote*. Similarly, the zygote formed by the union of gametes having similar allelomorphs, may be spoken of as a *homozygote*.'

To use these terms in examples, we might observe that in peas *round* and *wrinkled* genes are alleles; so are *yellow* and *green* genes. A pure-
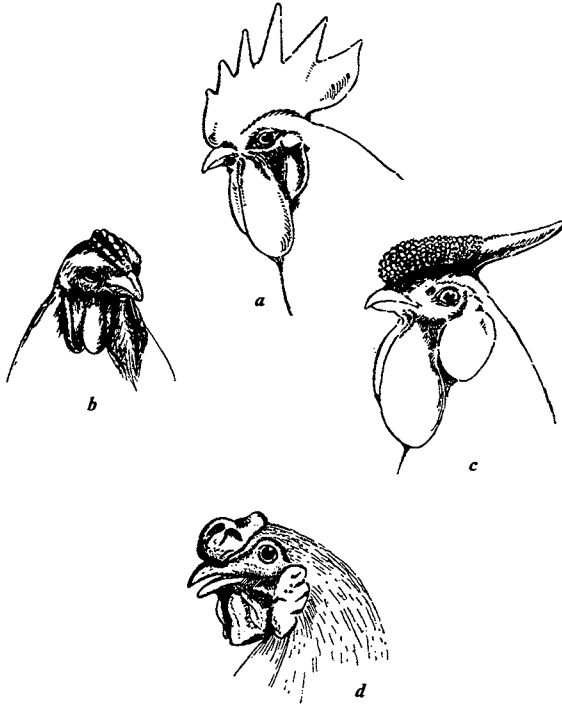
3–1 Combs of fowls. a. *single.* b. *rose.* c. *pea.* d. *walnut.* (From T.H.Morgan. 1919. "The Physical Basis of Inheritance." Lippincott.)
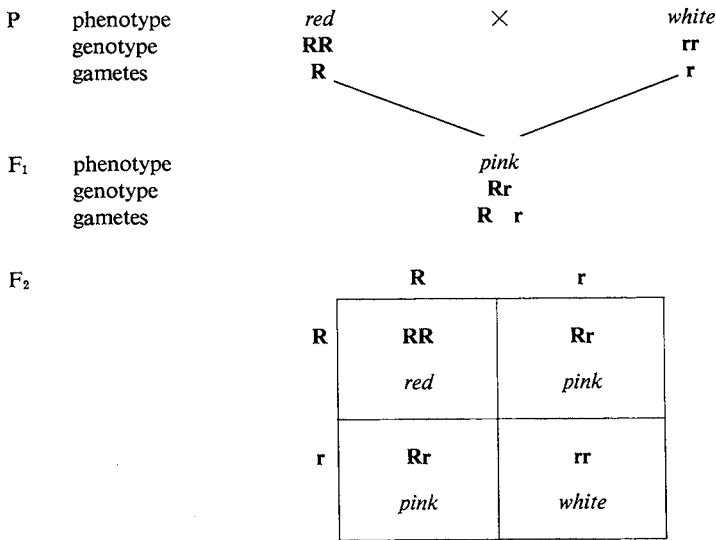
breeding *round* plant, which we have designated **RR,*** is homozygous. A *wrinkled* plant, **rr,** is also homozygous. The F_1 of a cross of these is **Rr** and heterozygous. Recalling that *round* is dominant, we know that a *round* plant can be either homozygous **(RR)** or heterozygous **(Rr),** and that one could not tell by external appearance which was which. It is worth repeating that there is a difference between appearance and genetic make-up. We speak of the appearance of the organism as its *phenotype* (*yellow* or *green*) and the genetic make-up as its *genotype* (**RR, Rr,** or **rr**). The individual factors of inheritance, **R** or **r,** carried on the chromosomes are the *genes*.

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* Heretofore we have been following Mendel's method of notation and used **R,** rather than **RR**. In 1900 both would have meant the same. Other studies, being carried out in the early 1900s, suggested that **RR** is a more accurate way of designating a pure-breeding dominant individual. The reasons will be given in the next chapter.

Returning now to Bateson, these are some of the types of genetic data he gave:

EXCEPTIONS TO THE MENDELIAN RATIOS

1. Blended Character Expression in Heterozygotes. In Mendel's results the heterozygote was always identical in appearance with the homozygous dominant. Even today we recognize this as the most frequent condition, but there are some cases where the heterozygote is intermediate. In such a situation neither allele is dominant or recessive. One example is a common cultivated flower, the four o'clock. When a *red*-flowered four o'clock is crossed with a *white*-flowered one, all of the F_1 plants have *pink* flowers. In the F_2, *red*-, *white*-, and *pink*-flowered plants appear. The genetic basis of this cross is diagrammed below:

P	phenotype	*red*	×	*white*
	genotype	**RR**		**rr**
	gametes	**R**		**r**

F_1	phenotype	*pink*
	genotype	**Rr**
	gametes	**R r**

F_2

	R	**r**
R	**RR** *red*	**Rr** *pink*
r	**Rr** *pink*	**rr** *white*

F_2 ratio: 1 *red*, 2 *pink*, 1 *white*.

2. Two Pairs of Alleles Affecting the Same Character. In all the crosses considered so far, a character has been affected by only one pair of alleles. This may seem to imply that every character of an organism is determined by a single pair of alleles. This is not the case, as shown by some experiments with chickens which were reported by Bateson.

Poultry breeders recognized a number of comb types, usually involving differences in comb shape. Some of these types were called *single, rose, pea,* and *walnut* (Fig. 3–1). A cross between *rose* and *single* gave

rose in the F_1 and 3 *rose* to 1 *single* in the F_2. *Rose* and *single* behaved as ordinary alleles with *rose* the dominant.

		phenotype	rose	$\times$	single
		genotype	RR		rr
		gametes	R		r

F_1	phenotype		rose	
	genotype		Rr	
	gametes		R r	

F_2

	R	r
R	RR *rose*	Rr *rose*
r	Rr *rose*	rr *single*
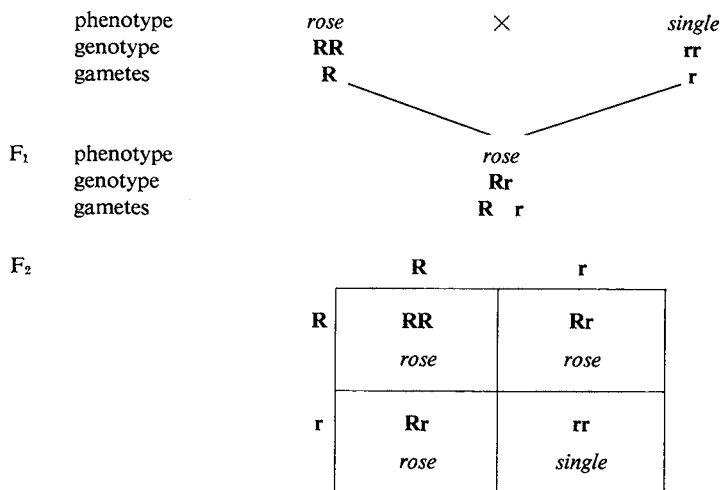
Similarly, a cross between *pea* and *single* gave *pea* in the F_1 and 3 *pea* to 1 *single* in the F_2. *Pea* and *single* behaved as ordinary alleles with *pea* the dominant.

		phenotype	pea	$\times$	single
		genotype	PP		pp
		gametes	P		p

F_1	phenotype		pea	
	genotype		Pp	
	gametes		P p	

F_2

	P	p
P	PP *pea*	Pp *pea*
p	Pp *pea*	pp *single*
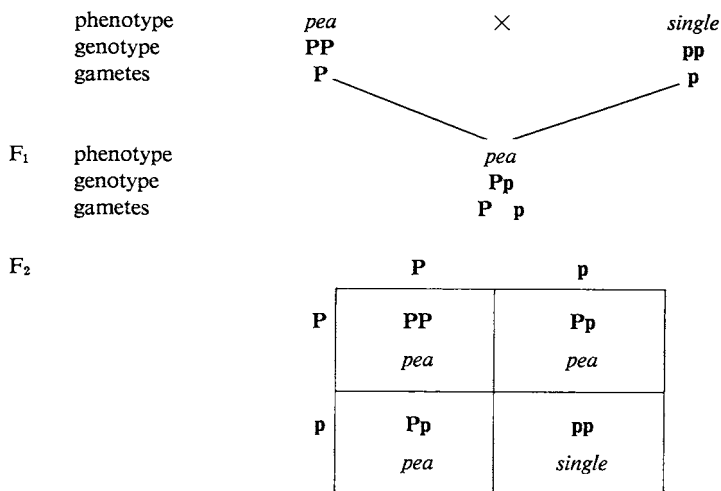
Considered separately there is nothing unusual about these crosses. Considered together there is one puzzle, largely semantic: why is the genotype of *single* written as **rr** in the first cross and as **pp** in the second? The answer to this question hinges on the fact that the *single* comb condition results from the interaction of two different genes. The two

previous crosses and the next one to be described show that both the **r** and **p** genes are involved in the genetic determination of comb shape (subsequent experiments showed that additional genes were concerned). A comb of the *single* type forms in a chicken when both the *pea* and *rose* genes are recessive. The genotype of a *single* comb chicken, therefore, is **pprr.** A *pea* comb chicken would have the genotype **PPrr** or **Pprr** and a *rose* comb chicken would have the genotype **ppRR** or **ppRr.** We might have written the cross of *rose* comb×*single* comb as **ppRR ×pprr.** At that time, however, we were unaware of the existence of the **p** genes. Furthermore, there was no need to do so since both parents are of the same genotype with respect to the **p** gene: by convention geneticists use the symbols only for those cases where the genes differ in the two parents. If the cross involves differences in both the **p** and **r** comb shape genes, then the necessary genetic symbols must be employed. Such a cross will now be described.

Let us cross the two dominants, *rose* and *pea.* The F_1 will be found to have a new type of comb, *walnut,* which might be looked upon as a blending of the two dominant shapes. The F_2 will give a ratio of 9 *walnut,* 3 *rose,* 3 *pea,* and 1 *single.*

P	phenotype	pea	×	rose
	genotype	PP rr		pp RR
	gametes	Pr		pR

F_1	phenotype	walnut
	genotype	Pp Rr
	gametes	PR Pr pR pr

F_2

	PR	Pr	pR	pr
PR	PP RR *walnut*	PP Rr *walnut*	Pp RR *walnut*	Pp Rr *walnut*
Pr	PP Rr *walnut*	PP rr *pea*	Pp Rr *walnut*	Pp rr *pea*
pR	Pp RR *walnut*	Pp Rr *walnut*	pp RR *rose*	pp Rr *rose*
pr	Pp Rr *walnut*	Pp rr *pea*	pp Rr *rose*	pp rr *single*
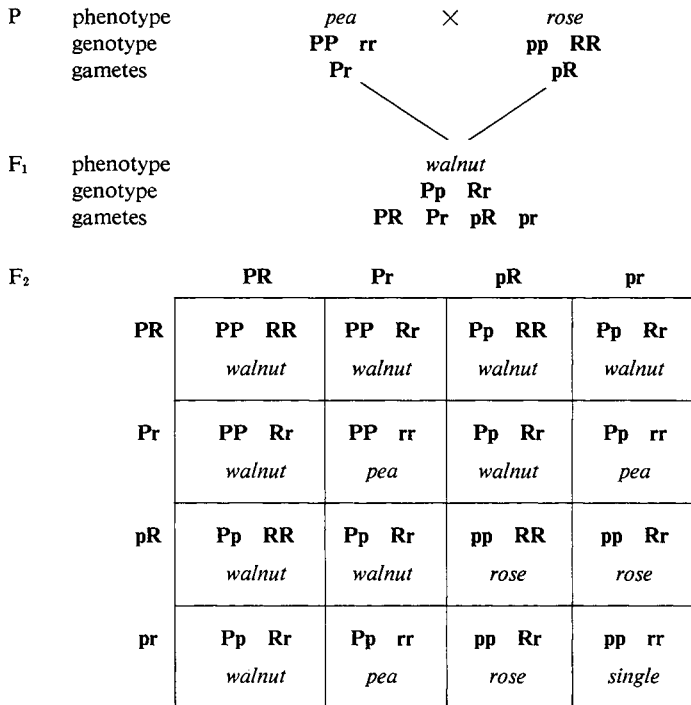
F_2 ratio: 9 *walnut;* 3 *rose;* 3 *pea;* 1 *single.*

The *single* comb is the result of both (recessive) **p** and **r** genes being homozygous. *Rose* is obtained if the animal is homozygous or heterozygous for **R** and homozygous for **p**. *Pea* is obtained if the animal is homozygous or heterozygous for **P** and homozygous for **r**. *Walnut* results when there is at least one **P** gene together with at least one **R** gene.

3. Yellow Mice. Cuénot reported some crosses in mice that neither he nor Bateson could explain in the usual Mendelian manner. He worked with *yellow* and *agouti* hair color genes. These two types of crosses will demonstrate the problem.

1. *yellow×yellow* gives 2 *yellow* to 1 *agouti*.
2. *yellow×agouti* gives 1 *yellow* to 1 *agouti*.

Cuénot found it impossible to obtain a strain of *yellow* mice that would breed true. The *yellow* animals always behaved as though they were heterozygous. His *agouti* strains bred true. During the course of his experiments he observed that the litter size in crosses of *yellow× yellow* was smaller than in the *yellow×agouti* cross.

Can you devise a hypothesis to explain the results? From the data given it is possible to arrive at the explanation that was later found to be correct.

4. Coupling of Genes. Bateson listed another discovery that could not be explained, namely, cases involving two pairs of genes that did not show independent assortment. Let us assume that there are two pairs of genes, **A** and **a** and **B** and **b** and that the phenotypic expression of **A** is *A,* that of **a** is *a,* and so on. In the cross **AABB×aabb** we should expect an F_2 phenotypic ratio of 9 *AB,* 3 *Ab,* 3 *aB,* and 1 *ab* if Mendel's rules applied. In some crosses, however, Bateson found that the *A* and *B* characters appeared to be completely *coupled,* that is, they were inherited together. The same was true for *a* and *b.* In fact, the F_2 ratios might be close to 3 *AB* to 1 *ab* with few or no individuals of the *Ab* or *aB* phenotypes. Results of this sort could not be accounted for by the Mendelian scheme.

Limitations of Mendelism. A biologist who restricted himself solely to a Mendelian approach, that is, to a study of the inheritance of 'unit characters' as expressed in the ratios observed in succeeding generations, could make considerable progress. He could study numerous sorts of animals and plants and observe the instances where Mendel's rules held and when they did not. Many individuals restricted their investigations in this way: Bateson and his coworkers were among them. But one soon realizes that the prospects for a deeper understanding of inheritance are limited.

Mendelism really bothered many biologists: it seemed so at variance with the phenomena they usually observed. They knew of few instances that exhibited the extraordinary mathematical exactness that one could easily observe in Mendelian crosses. Furthermore, biologists seek to identify the physical (i. e. structural) basis of phenomena. What in the organism and its gametes could be responsible for Mendelian inheritance? Could it be gemmules or an idioplasm behaving in some exact manner? How could one possibly account for the 'purity of the gametes,' that is, for an **Rr** individual to produce either **R** or **r** gametes but never **Rr** gametes—and to produce them in such exact ratios?

The answers lay elsewhere.

Suggested Readings

Chapter 3 of *Readings* contains long excerpts from the report that Bateson made to the Royal Society in 1902, in which he gave a detailed account of Mendel's experiments and adds much new data. A paper by T.H.Morgan, questioning the basic tenets of Mendelism, is also reproduced.

DUNN, L.C. 1965. *A Short History of Genetics.* New York: McGraw-Hill.

MENDEL, GREGOR. 1866. 'Experiments on plant hybrids.' (See Stern and Sherwood)

OLBY, ROBERT C. 1966. *Origins of Mendelism*. New York: Schocken Books.

STERN, CURT, and EVA R.SHERWOOD. Editors. 1966. *The Origins of Genetics. A Mendel Source Book.* San Francisco: W.H.Freeman. Translations of papers by Mendel, de Vries, and Correns; Mendel's letters to Nägeli; Fisher's attack on Mendel and Wright's reply ; and references to Mendel's experiments in Focke's monograph on plant hybrids.

STURTEVANT, A.H. 1965. *A History of Genetics.* New York: Harper and Row.

Questions

1. Four desirable features for experimentation are listed for Mendel's peas. Why is each desirable?
2. In a plant of the genotype **Rr Yy,** Mendel assumed that the gametes could only be **RY, Ry, rY,** and **ry**. According to his scheme, **Rr Yy, Rr Y, Rr,** and **Yy** could not be formed. What is the basis of his belief that such gametes could not be formed? Can you devise a mechanism that would allow some types of gametes to form (i.e. **RY, Ry** etc.) but not others (i.e. **Yy, Rr**)? (If you already know what the solution eventually proved to be, ignore it, and try to devise other mechanisms.)
3. Account for the fact that pre-Mendelian hybridizers found blending to be a rule of inheritance but that Mendel did not.
4. What do the phenomena of dominance-recessiveness, segregation, and independent assortment tell us about the Mendelian units of inheritance?

5. How do you suppose Aristotle (*Readings,* Chapter 1) would have reacted to Mendel's experiments?
6. A pea plant that is *yellow* and *round* is crossed with one that is *green* and *wrinkled.* The offspring are:

    1/2 *yellow, round*

    1/2 *green, round*

    What was the genotype of the parents?
7. In another cross of peas the offspring are:

    3/8 *yellow, round*

    3/8 *yellow, wrinkled*

    1/8 *green, round*

    1/8 *green, wrinkled*

    What was the genotype and the phenotype of the parents?
       Hint. When doing genetic problems of this type, it is best to solve for one character at a time. It is necessary, also, to remember how the usual genetic ratios are obtained. Thus, a 3:1 ratio is the consequence of crossing two heterozygotes, **Aa**×**Aa**. A 1:1 ratio is the consequence of crossing a heterozygote and a homozygous recessive, **Aa**×**aa**.
8. In man *brown eyes* **(B)** is dominant over *blue* **(b)**. (The genetic situation is not quite this simple but, in doing the following problems, assume that it is.)
    a. A heterozygous *brown-eyed* woman marries a *blue-eyed* man. What will be the genotypes and phenotypes of the children?
    b. The first child of two *brown-eyed* parents has *blue* eyes. What are the genotypes of the three individuals? What other genotypes, and in what proportion, might be expected in future children of these parents?
    c. In a family of nine children two have *blue eyes* and seven have *brown* eyes. What are the probable genotypes and phenotypes of the parents?
9. How might Mendel have accounted for the pattern of inheritance described by Darwin for color blindness (pages 12, 15)? For the porcupine man (page 9)?
10. What evidence can be cited to show that two alleles are not modified by their occurrence together in a heterozygous individual?
11. Can you modify Darwin's Theory of Pangenesis in such a way that it will account for the results obtained by Mendel?
12. Before Mendel's work was rediscovered in 1900, many biologists held the hypothesis that the nucleus was responsible for inheritance. Do Mendel's results support this hypothesis?
13. A *walnut* rooster, heterozygous for both *rose* and *pea,* is crossed with a hen having a *single* comb. What will be the genotypes and phenotypes of the offspring?

14. A *walnut* rooster is crossed with a *rose* hen. The offspring are:

   3/4 *walnut*

   1/4 *pea*

   What were the genotypes of the parents?
15. Did you formulate a reasonable hypothesis to account for inheritance in *yellow* mice?
16. A Mendelian cross of the type **Aa**×**aa** gives a 1:1 ratio. In man, and most other animals, the ratio of females to males is also 1:1. Evaluate this hypothesis: The inheritance of sex is an example of a simple Mendelian cross.

4 The Chromosomes and Inheritance

BOVERI AND THE SEA URCHIN CHROMOSOMES

In Chapter 2, we learned that in 1900 the hypothesis that 'chromosomes are the physical basis of inheritance' seemed to be reasonable. It was far from being established as true and, in fact, there was a real difficulty in knowing how to test such a hypothesis. Until 1902 no one had a clue as to how this might be done. In that year, Theodore Boveri (1862–1915) carried out an ingenious experiment demonstrating that a complete set of chromosomes is necessary for normal development. Since development is one aspect of inheritance, the relation between chromosomes and inheritance was established.

The significance of Boveri's experiment will be more apparent if we give something of the background from which he worked. At the time of his experiment, most cytologists believed that within any single species one chromosome was about the same as another. Thus, in the sea urchin each cell of the embryo has 36 chromosomes. They were all very small and looked identical in shape; they reacted alike to fixation and staining; and when things look alike there is a natural tendency to believe that they are alike in other respects as well.

Boveri thought otherwise. He believed that chromosomes differed from one another, and that a complete set of 36 was necessary for normal development in the sea urchin. He believed that not just any 36 would suffice: but the very 36 which were present in each cell of the normal embryo were the necessary ones.

Double Fertilization of Sea Urchin Ova. Boveri tested this hypothesis in a clever way. Oskar Hertwig and others had observed previously that

if one used a highly concentrated sperm suspension, it was possible to get two sperm to enter one egg of the sea urchin. Mitosis becomes most confused in these double fertilizations, but it is possible by this method to vary the number of chromosomes distributed to the cells. In order to understand the complications we should first review normal fertilization.

In the case of an embryo entered by a single sperm, the sperm brings in a *centrosome,* which is the region containing the centriole. The centrosome divides into two and the spindle forms between the two new centrosomes. The paternal and maternal nuclei fuse, and then their chromosomes appear in the spindle. Each pronucleus of the sea urchin has 18 chromosomes so the fusion nucleus will have 36. Each of these 36 duplicates itself, thus forming a total of 72 chromosomes. These are divided equally at the first cleavage division, and each daughter cell receives 36.

When *two* sperm enter, not only will there be an additional 18 chromosomes to make a total of 54 but there will be an extra centrosome as well. Boveri observed that one of two things happened:

1. In some embryos the centrosomes of both sperm divided, giving four centrosomes in the single cell. At the time of first cleavage these embryos divided into four instead of into two cells.
2. In other embryos, the centrosome of one sperm divided but the other remained single, giving three centrosomes for the single cell. At first cleavage this type of embryo divided into three cells.

In both classes of double-fertilization embryos, there would be 54 chromosomes (18 from the maternal nucleus and 18 from each of the two paternal nuclei). These 54 will duplicate themselves during the first mitotic division of the embryo, producing a total of 108 chromosomes. In those embryos with four centrosomes, the chromosomes will be divided among four cells. Boveri found this division very unequal. Some cells would get many chromosomes and others only a few. If the apportionment was strictly equal, each cell would receive 27 chromosomes (108/4=27). This is far from the normal complement of 36 per cell, which Boveri believed necessary for regular development. In fact, there is no way in which each of the four cells could get the normal complement of 36. Abnormal development was to be expected, and this Boveri observed in 1,499 out of 1,500 embryos.

The embryos with three centrosomes that divided into three cells at the first division also showed very abnormal distributions of chromosomes. One would expect, however, that they would have a better chance of getting 36 chromosomes in each cell than would the group

that formed four cells. The reason is this: We have seen that there is no way of apportioning 108 chromosomes among four cells so each will receive a normal complement of 36. If the 108 chromosomes are divided equally among three cells, however, the result is 36. The experimental results validated this reasoning. In the group that divided into three cells, 58 in a total of 719 developed normally. We have already seen that only one embryo in 1,500 developed normally among the embryos that divided into four cells at first cleavage.

According to Boveri, these data correspond fairly well with the chance expectation that normal larvae will come from embryos that begin development with a normal set of chromosomes in each of the cells formed at the first division. He interpreted the data to mean that for normal development every cell of the embryo must have the regular set of 36 chromosomes. He believed that each chromosome in the set must be endowed with a specific quality, and that all are necessary for normal development.

These experiments emphasized the importance of chromosomes for normal development, which is one aspect of inheritance. It was a direct approach to the study of the role of chromosomes in inheritance.

SUTTON AND GRASSHOPPER CHROMOSOMES

In the same year that Boveri published the results of his work, a second and much more fruitful approach was made by Walter Stanborough Sutton (1877–1916). At the time, he was a graduate student working at Columbia University with the cytologist Edmund Beecher Wilson (1856–1939). He published two papers on the chromosomal basis of inheritance, the first in 1902 and the second in 1903.

Individuality of the Chromosomes. Sutton's 1902 paper was a study of the chromosomes in the testis of a grasshopper of the genus Brachystola. The chromosomes of this form

> exhibit a chromosome group, the members of which show distinct differences in size. Accordingly one feature of this study has been a critical examination of large numbers of dividing cells (mainly from the testis) in order to determine whether, as has usually been taken for granted, these differences are merely a matter of chance, or whether in accordance with the view recently expressed by Montgomery,…characteristic size relations are a constant attribute of the chromosomes individually considered. With the aid of camera drawings of the chromosome group in the various cell-generations, I will give below a brief account of the evidence which has led me to adopt the latter conclusion.

The cells in the testis undergo a series of mitotic divisions before they begin meiosis. These cells are known as *spermatogonia* and they have the diploid number of chromosomes. The youngest spermatogonia that Sutton could find possessed 23 chromosomes. One of these, the 'accessory' chromosome, had a peculiar behavior and it will be considered separately. The other 22 were of various sizes. When these were measured carefully it was found that there were not 22 different sizes, but only 11. In other words there were two chromosomes of each size class (Fig. 4–1). In addition to the minor size variation, the chromosomes could be divided into two groups that differed strikingly in size. Three of the pairs were very small and the other eight were large.

Sutton found that these early spermatogonia went through eight mitotic divisions. At each metaphase the same 11 pairs of chromosomes were observed. Of these, eight pairs were large and three small. He concluded that constant size was an attribute of the individual chromosomes.

After these eight mitotic divisions, the cells undergo the usual two meiotic divisions. The chromosomes synapse in pairs, each member of the pair being of the same size. As a result, 11 tetrads form; eight are large and three are small. Then the two meiotic divisions occur and each sperm receives one chromosome of each of the 11 sizes.

Sutton found that the diploid number in the female was 22. Further, these had the same size relations as were present in the male, consisting of eight large and three small pairs. (Sutton made an error in this count. Later workers found 24 chromosomes.) He postulated that every ovum after meiosis would contain one chromosome of each of the 11 sizes.

Fertilization of an ovum containing 11 chromosomes with a sperm containing 11 would restore the diploid number of 22. Some of the sperm will have an accessory chromosome in addition to the regular 11.

4–1 A haploid set of chromosomes of Brachystola. The metaphase chromosomes have been redrawn, showing one of each pair, and arranged in groups. The accessory chromosome is at the left, the eight large chromosomes in the center, and the three small chromosomes at the right. A diploid nucleus in a male would have one accessory, two of each of the eight large chromosomes and two of each of the three small ones to make a total of 23. (Modified from W.S.Sutton. Biological Bulletin *4:*24–39. 1902.)

Fertilization with a sperm of this type will result in a zygote with 22 chromosomes plus an accessory. (In 1901 McClung suggested that the accessory chromosome is in some way concerned with sex determination. More will be said about this in Chapter 5.) Sutton continues:

> Taken as a whole, the evidence presented by the cells of Brachystola is such as to lend great weight to the conclusion that a chromosome may exist only by virtue of direct descent by longitudinal division from a pre-existing chromosome and that the members of the daughter group bear to one another the same respective relations as did those of the mother group—in other words, that the chromosome in Brachystola is a distinct morphological individual.

> This conclusion inevitably raises the question whether there is also a physiological individuality, i.e., whether the chromosomes represent respectively different series or groups of qualities or whether they are merely different-sized aggregations of the same material and, therefore, qualitatively alike.

> On this question my observations do not furnish direct evidence. But it is *a priori* improbable that the constant morphological differences we have seen should exist except by virtue of more fundamental differences of which they are an expression; and, further, by the unequal distribution of the accessory chromosome we are enabled to compare the developmental possibilities of cells containing it with those of cells which do not. Granting the normal constitution of the female cells examined and the similarity of the reduction process in the two sexes, such a comparison must show that this particular chromosome does possess a power not inherent in any of the others—the power of impressing on the containing cell the stamp of maleness, in accordance with McClung's hypothesis.

> The evidence advanced in the case of the ordinary chromosomes is obviously more in the nature of suggestion than of proof, but it is offered in this connection as a morphological complement to the beautiful experimental researches of Boveri already referred to. In this paper Boveri shows how he has artificially accomplished for the various chromosomes of the sea-urchin, the same result that nature is constantly giving us in the case of the accessory chromosome of the Orthoptera. He has been able to produce and to study the development of blastomeres lacking certain of the chromosomes of the normal series.

> If, as the facts in Brachystola so strongly suggest, the chromosomes are persistent individuals in the sense that each bears a genetic relation to one only of the previous generation, the probability must be accepted that each represents the same qualities as its parent element. A given relative size may, therefore, be taken as characteristic of the physical basis of a certain definite set of qualities. But each element of the chromosome series of the spermatozoon has a morphological counterpart in that of the mature egg and from this it follows that the two cover the same field in development. When the two copulate, therefore, in synapsis the entire chromatin basis of

a certain set of qualities inherited from the two parents is localized for the first and only time in a single continuous chromatin mass; and when in the second spermatocyte division, the two parts are again separated, one goes entire to each pole contributing to the daughter cells the corresponding group of qualities from the paternal or the maternal stock as the case may be.

> There is, therefore, in Brachystola no qualitative division of chromosomes but only a separation of the two members of a pair which, while coexisting in a single nucleus, may be regarded as jointly controlling certain restricted portions of the development of the individual. By the light of this conception we are enabled to see an explanation of that hitherto problematical process, synapsis, in the provision which it makes that the two chromosomes representing the same specific characters shall in no case enter the nucleus of a single spermatid or mature egg.

> I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity. To this subject I hope soon to return in another place.

The Chromosomes in Heredity. And soon he did in a paper entitled 'The Chromosomes in Heredity,' published in 1903. In this he pointed out that the segregation and recombination of genes as studied by the geneticists showed a striking parallel to the behavior of chromosomes as revealed by the cytologists. The pertinent cytological data, according to Sutton, were as follows:

1. The diploid chromosome group consists of two morphologically similar chromosome sets. Every chromosome type is represented twice. Expressed another way, chromosomes exist in homologous pairs. Strong grounds exist for the belief that one set was derived from the father and one set from the mother at the time of fertilization.
2. Synapsis consists of the pairing of homologous chromosomes.
3. As a result of meiosis every gamete receives only one chromosome of each homologous pair.
4. The chromosomes retain their morphological individuality throughout the various cell divisions.
5. The distribution in meiosis of the members of each homologous pair of chromosomes is independent of that of each other pair. As a result, each gamete receives one of each pair, but *which one* is a matter of chance.

Sutton then made the point that Mendel's results could be explained on the assumption that genes were parts of the chromosomes. The following example will show how this is possible:

Let us assume that the *round* and *wrinkled* genes in peas are carried on a specific pair of chromosomes (Fig. 4–2). If a chromosome has the *round* gene, we shall call it **R** and if it has the *wrinkled* gene, we shall call it **r**. Let us further assume that the *yellow* and *green* genes are carried by a different pair of chromosomes. If the chromosome has the *yellow* gene, we shall designate it **Y** and if it carries the *green* gene, we shall call it **y**. A pure-breeding *round-yellow* plant would be symbolized as **RRYY** indicating that it has a pair of chromosomes carrying the *round* gene and another pair with the *yellow* gene. Similarly, a *wrinkled-green* plant would be **rryy**.

When the reduction divisions occur, the *round-yellow* plant would produce haploid gametes with one **R** and one **Y** chromosome—or **RY**. This alone could result, since a gamete receives only one chromosome of every kind. A **RR** or a **YY** gamete would be impossible in normal meiosis. The *wrinkled-green* plant would produce gametes solely of the **ry** type. A union of gametes of the two plants would result in one type of offspring, namely, **RrYy**. This F$_1$ individual would be diploid and would have received one member of each chromosome type from the male gamete and one from the female gamete.

The gametes of the F$_1$ plant would be of four possible types. The **R** and the **r** would go to different cells during meiosis. The **Y** and **y** chromosomes would likewise be separated and *their separation would not affect the separation of* **R** *and* **r**. Thus, all possible combinations, namely, **RY, Ry, rY,** and **ry** would be produced, and in approximately equal numbers. If you will re-examine Mendel's description of this cross (p. 54), you will note the exact parallel between the scheme for Mendel's breeding experiments and the chromosome movements just described. The F$_2$ chromosomes would be of the type shown in the genetic checkerboard. The sole difference to be noted is that Mendel characterized his pure-breeding peas of the parental generation as **RY** and **ry,** while we have used a diploid chromosome designation **RRYY** and **rryy**. Mendel could have used **RRYY** and **rryy** just as well. *Sutton's Hypothesis*. We may conclude, therefore, that genes of a type postulated by Mendel could be

1. parts of the chromosomes, or
2. parts of some other cell structures that behave in the same way as chromosomes in mitosis, meiosis, and fertilization.

When a scientist is confronted with two hypotheses, one involving known factors and the other invoking unknown factors, the first is usually chosen. In the case under consideration, such a choice would have a great practical advantage: It would be easier to make observa-
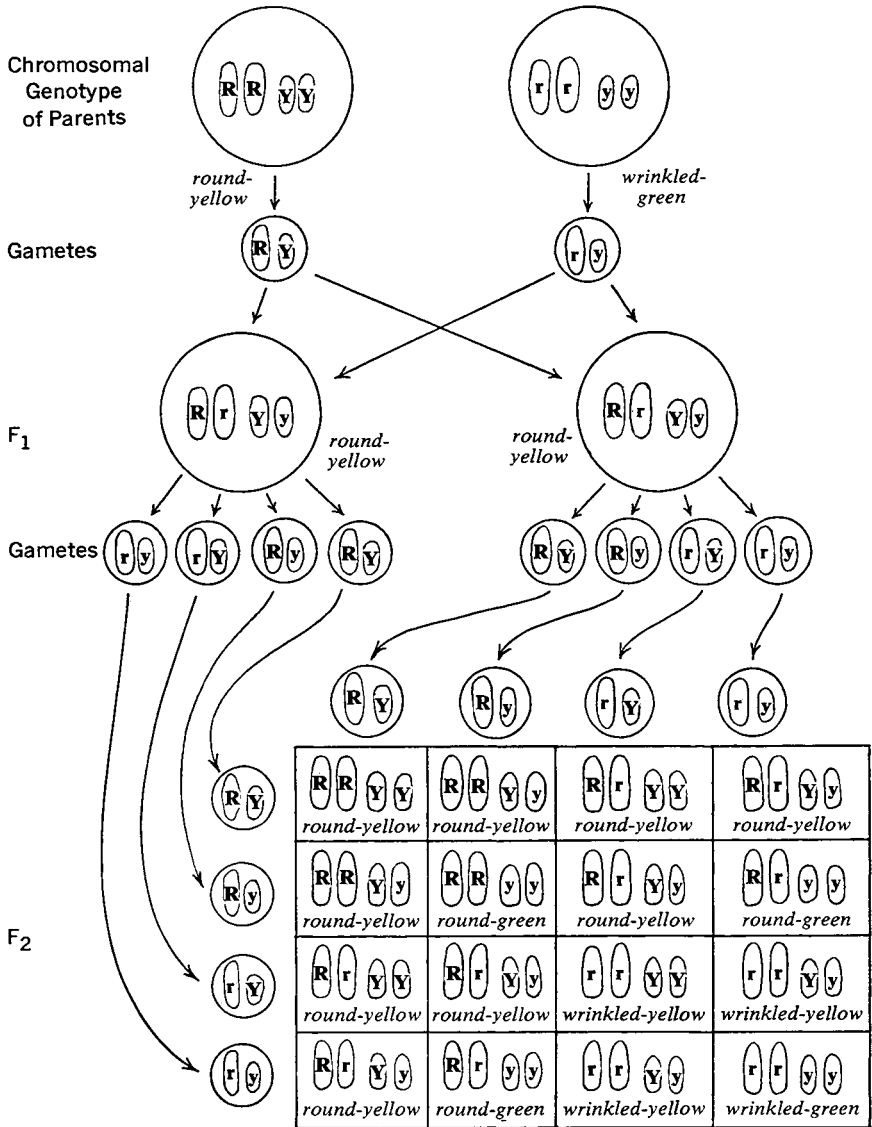
4–2 Diagram of chromosome distributions in Mendel's cross of a *round-yellow ×wrinkled-green* pea on the basis of Sutton's hypothesis.

tions and design experiments to test the role of chromosomes in Mendelian heredity than it would be to investigate the role of some unknown cell structures.

Sutton's general hypothesis was not new. As we have already seen, some cytologists believed, at least as early as 1884, that the chromosomes were involved in inheritance. Sutton pointed out additional reasons for so thinking and, even more important, made a definite link between genetic data and cytological data.

If we are to use the genes-are-parts-of-chromosomes hypothesis, it will be necessary to find a parallel between all types of genetic behavior and chromosome behavior. Any variations in chromosomal phenomena from the usual condition must be reflected in the genetic results. Similarly, if genetic ratios are obtained that cannot be explained in Mendelian terms, one must find a chromosomal basis for the deviation. Sutton indicated one type of genetic behavior that could be expected if his hypothesis was correct.

> We have seen reason, in the foregoing considerations, to believe that there is a definite relation between chromosomes and allelomorphs or unit characters, but we have not before inquired whether an entire chromosome or only a part of one is to be regarded as the basis of a single allelomorph. The answer must unquestionably be in favor of the latter possibility, for otherwise the number of distinct characters possessed by an individual could not exceed the number of chromosomes in the germ-products; which is undoubtedly contrary to fact. We must, therefore, assume that some chromosomes at least are related to a number of different allelomorphs. If then, the chromosomes permanently retain their individuality, it follows that all the allelomorphs represented by any one chromosome must be inherited together.

If Sutton's reasoning is correct, the Mendelian principle of independent assortment could apply only to cases where the two pairs of contrasting factors were carried on separate chromosomes.

Sutton's hypothesis demanded that there be exceptions to Mendel's law of independent assortment. The exceptions would be detected, without fail, when more gene pairs were discovered for an organism than there were pairs of chromosomes. When two pairs of genes were on the same chromosome they would obviously be linked in some manner and tend to be inherited as a unit. Bateson observed such a case (page 66) but he was unable to offer a reasonable explanatory hypothesis.

The importance of Sutton's theoretical considerations can scarcely be overemphasized. Two completely different disciplines were found to have an area in common: cytology and genetics became mutually supporting and stimulating fields. Theories of inheritance could be 'double-checked.'

Still another line of cytological investigation was to reveal a specific relation of chromosomes to inheritance.

SEX CHROMOSOMES

The first impression cytologists gained from a study of chromosomes was a feeling that the behavior of these cell structures was similar in all animals. Whether the form studied was a worm, snail, salamander, or mammal, one observed the same duplication of each chromosome to form two chromatids during mitosis. This was followed by the distribution of one of the two chromatids to each daughter cell. Each chromosome appeared double at the same time, and the movements during metaphase, anaphase, and telophase were synchronous. A similarity of behavior was observed in the events connected with the reduction of chromosome number in meiosis of both male and female gametes. It should be emphasized that this similarity of behavior applied not only to the behavior of the group of chromosomes but to the individual chromosomes in the group as well.

Atypical Chromosomes. It was not long before this concept of uniform behavior was found to have its exceptions. In the last decade of the nineteenth century and the first years of the twentieth, a few cases were reported of one or two of the chromosomes of a set behaving in a manner quite unlike the rest. The unusual behavior might be a difference in reaction of the chromosome to stains, meiotic movements that were not synchronous, or the presence of 'extra' or 'accessory chromosomes.' The term *accessory chromosome* referred to those cases where there was one chromosome without a mate, in contrast to the usual situation of all chromosomes being in morphologically similar pairs.

The analysis of accessory chromosomes led to important results concerning the role of chromosomes in heredity. As is frequently the case in science, these observations were made and recorded before their true significance was understood.

Henking's Description of the **X** *Chromosome*. In 1891 H.Henking published his observations on chromosome behavior during sperm formation in a bug, Pyrrhocoris (Fig. 4–3). This species has 23 chromosomes. Twenty-two of them form 11 pairs, the two members of a pair having the same appearance. The extra chromosome was called **X**. It did not have a mate. During the first meiotic division the 22 chromosomes synapsed to form 11 pairs. Later these formed tetrads.

The behavior of the **X** chromosome was different. Having no mate, it could not synapse and form a tetrad. It did duplicate itself, however, to form a structure like a dyad. At the beginning of meiosis, the cells therefore contained 11 tetrads, plus the **X** in the form of a dyad. At the
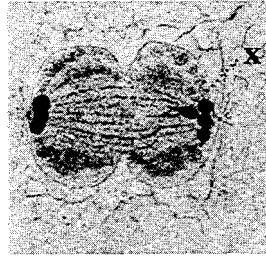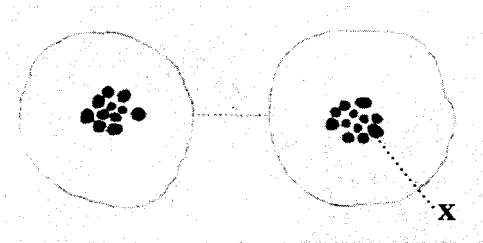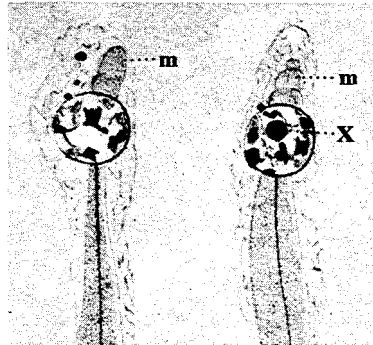
4–3 Meiosis in Pyrrhocoris. *a* shows a cell in the telophase of the second meiotic division. Since this is a lateral view not all of the chromosomes are shown. The peculiar body, indicated as 'X' by Henking, goes to one pole of the spindle and will, therefore, be in only half of the cells formed by this division. Consequently two types of cells result from meiosis. One type, *b,* has 11 chromosomes and the other type, *c,* has 11 chromosomes plus the X. These cells develop directly into sperm. Thus, half of the sperm will have an X *(e)* and the others lack an X *(d)* (H.Henking, 'Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten,' *Zeit. für wiss. Zool.* 51:685–736. 1891).

first meiotic division the 11 tetrads were separated but the **X** went entire to one of the daughter cells. At the end of the first division, one of the daughter cells contained 11 dyads of the usual sort, plus the **X** dyad. The other daughter cell contained only the 11 dyads. At the second meiotic division of the cell with the **X,** the **X** dyad and the 11 regular dyads were divided so each of the resulting cells contained an **X** chromosome plus 11 of the regular chromosomes. In the cell without the **X** dyad, the other dyads were divided so each daughter cell contained 11 regular chromosomes. Therefore, the four cells resulting from the two meiotic divisions consisted of two with 11 chromosomes plus an **X,** and two with the 11 chromosomes alone. So far as the **X** was concerned, half of the sperm contained an **X** and the other half did not.

During the next decade, many workers discovered these chromosomes with atypical behavior. They were given a variety of names such as '**X** chromosomes' and 'accessory chromosomes.' In every case the accessory was unique in some feature such as stainability, time of movement to the poles of the spindle, enclosure in a separate vesicle instead of the nucleus, lack of a mate during synapsis, or distribution to only half of the sperm.

Sex and the Accessory Chromosomes. In 1901 C.E.McClung (1870–1946) suggested that the accessory chromosome was in some way connected with sex determination:

> Being convinced from the behavior in the spermatogonia and the first spermatocytes of the primary importance of the accessory chromosome, and attracted by the unusual method of its participation in the spermatocyte mitoses, I sought an explanation that would be commensurate with the importance of these facts. Upon the assumption that there is a qualitative difference between the various chromosomes of the nucleus, it would necessarily follow that there are formed two kinds of spermatozoa which, by fertilization of the egg, would produce individuals qualitatively different. Since the number of each of these varieties of spermatozoa is the same, it would happen that there would be an approximately equal number of these two kinds of offspring. We know that the only quality which separates the members of a species into these two groups is that of sex. I therefore came to the conclusion that the accessory chromosome is the element which determines that the germ cells of the embryo shall continue their development past the slightly modified egg cell into the highly specialized spermatozoon.

> It would not be desirable in a preliminary paper of this character to extend it by a detail of the discussion by which the problem was considered. Suffice it to say that by this assumption it is possible to reconcile the results of many empirical theories which have proved measurably true upon the general ground that the egg is placed in a delicate adjustment with its en-

vironment, and in response to this, is able to attract that form of spermatozoon which will produce an individual of the sex most desirable to the welfare of the species. The power of selection which pertains to the female organism is thus logically carried to the female element.

Numerous objections to this theory received consideration, but the proof in support of it seemed to overbalance them largely, and I was finally induced to commit myself to its support. I trust that the element here discussed will attract the attention which I am convinced it deserves and can only hope that my investigations will aid in bringing it to the notice of a larger circle of investigators than that now acquainted with it.

McClung's hypothesis was not accepted or even widely believed at first. In part this was due to the type of reasoning displayed in the second paragraph of the quotation. It was difficult to imagine how an unfertilized ovum could select the type of sperm and so produce the 'sex most desirable to the welfare of the species.' Were this possible, it would be a most interesting extension of feminine intuition! The question was all the more confusing when somewhat later it was found that the female had not one less chromosome than the male, but one more. *Clarification of Chromosomal Sex Determination.* In 1905 the situation was clarified by E.B.Wilson and one of his students, Nettie M. Stevens. They studied meiosis in a number of insects and found that **X** chromosomes were the rule, not the exception. Because of the association of **X** chromosomes with sex they were called *sex chromosomes.* All other chromosomes were called *autosomes*. Thus, Henking's bug, Pyrrhocoris, would have one **X** sex chromosome and 22, or 11 pairs, of autosomes.

Stevens and Wilson found two types of sex chromosome behavior, the **X0-XX** type and the **XY-XX** type (Fig. 4–4).

The **X0-XX** *Type.* In the species having this type of sex chromosome behavior, the male has a single **X** chromosome and the female has two **X** chromosomes. The male can be symbolized as **X0,** where **0** signifies the absence of a homologue of the **X**. The female is **XX**. Both sexes will have the same autosomes. A Pyrrhocoris male would have 11 pairs of autosomes and one **X** chromosome. In meiosis two types of sperm will be produced: One type will contain 11 autosomes plus the **X** and the other type will contain 11 autosomes and no **X**. The ova will all be of one type, containing 11 autosomes and one **X**. The union of a sperm of the first type with an ovum will result in a zygote with 22 autosomes and two **X** chromosomes. This individual will be female. Fertilization of an ovum with the second type of sperm will result in a zygote with 22 autosomes and a single **X** (that is, **X0**). The result will be a male.

The **XY-XX** *Type*. In this type, both the male and female possess a

XO-XX TYPE OF SEX DETERMINATION

Autosomes

Sex chromosomes

♂

Diploid chromosome complement of male

♀

Diploid chromosome complement of female

X

X X

Two types of sperm result from meiosis

x

One type of ovum results from meiosis

X

Sex determined by type of sperm entering the ovum

X X

♀

X

♂

XY-XX TYPE OF SEX DETERMINATION

♂

♀

Diploid chromosome complement of male

Diploid chromosome complement of female

X Y

X X

Two types of sperm result from meiosis

x

y

One type of ovum results from meiosis

X

Sex determined by type of sperm entering the ovum

X X

♀

X Y

♂

4–4

pair of sex chromosomes. Once again, the female has a pair that are identical and she is symbolized as **XX**. The male has one chromosome that is identical with the **X** of the female plus another, the **Y,** that is morphologically different. The **Y** might be longer, shorter, or of a different shape. It is similar to the **X** in some way, since synapsis occurs between **X** and **Y**.

The results of meiosis would be these: Every ovum would contain autosomes (the number depending on the species) and one **X**. The sperm would be of two types. One would contain autosomes and one **X,** and the other would contain autosomes and one **Y**. The fertilization of an ovum with an **X**-bearing sperm would give a zygote with the diploid set of autosomes and **XX**. This would be a female. The fertilization of an ovum with a **Y**-bearing sperm would give a zygote with the diploid set of autosomes and **XY**. This would be a male. (Incidentally, man has the **XX-XY** type of sex determination and so does *Drosophila melanogaster,* a small fly much used in genetic work.)

These two types of sex chromosome distribution described by Stevens and Wilson are those most frequently encountered in the animal kingdom. With each type, the male produces two classes of sperm, and sex is determined by the kind of sperm entering the ovum. Additional types were discovered later. In birds, for example, it is the female that produces two classes of gametes and the male only one.

If these observations on sex chromosomes are correct, we may draw some important conclusions:

1. Sex is determined at the time of fertilization.
2. If sex determination is due only to sex chromosomes, we can regard the sex of an individual as irreversible, unless we can alter the chromosomes.
3. The two sexes should be produced in approximately equal numbers.
4. The relation between sex and chromosomes is additional evidence supporting Sutton's hypothesis that chromosomes are the basis of inheritance.

It was becoming even more probable that the physical basis of inheritance was to be sought in the chromosomes. This was a good working hypothesis even before 1900 (page 41) but the observations on the relation of sex to the sex chromosomes, and especially Sutton's remarkable theoretical analysis, made it a far more useful hypothesis. Inheritance was so poorly understood before 1900 that one could not design specific experiments that might uncover its cellular basis.

Mendelism changed all that. It became possible to think in symbolic terms —of organisms having dominant and recessive genes and these

being allocated to the gametes in specific ways. Bateson had emphasized that the essential element of Mendel's hypothesis was the purity of the gametes formed by a heterozygous individual. Sutton had shown how the observable behavior of chromosomes could account for this otherwise obscure phenomenon: the chromosomes of diploid cells exist in pairs and one could carry a dominant gene and the other member of the pair could carry the recessive gene; during meiosis these homologous chromosomes would be separated and half of the gametes would have the dominant gene and the other half the recessive gene; normal meiosis, therefore, could account for the purity of the gametes.

Before 1900, one might find the hypothesis 'the genes are parts of chromosomes' to be probable but it was very difficult to see how it might be tested. The great utility of Sutton's hypothesis was to show how tests could be performed. If one assumed that genes are parts of chromosomes, then one could deduce:

1. The distribution of genes from generation to generation, as determined by the animal or plant breeder, must parallel the distribution of the chromosomes. Every specific aspect of the distribution of genes must have a basis in chromosomal movements.
2. Similarly, if the cytologist observes a peculiar chromosomal behavior in mitosis, meiosis, or fertilization, there must be parallel genetic phenomena.

The synthesis of the modern theory of genetics was made of this basis. It was completed in the laboratory of Thomas Hunt Morgan, in a decade of research devoted to *Drosophila melanogaster*.

Suggested Readings

Chapter 4 of *Readings in Heredity and Development* includes three items by Edmund B.Wilson: the 1902 paper relating Mendel's principles to cytology; his famous Croonian Lecture of 1914 in which he summarized the relation of genetics to cytology; and his reminiscences of Sutton. And, as always, there is a more extensive list of references.

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STURTEVANT, A.H. 1965. *A History of Genetics*. New York: Harper and Row. Chapter 5.
SUTTON, W.S. 1902. 'On the morphology of the chromosome group in Brachystola magna.' *Biological Bulletin 4:*24–39.
SUTTON, W.S. 1903. 'The chromosomes in heredity.' *Biological Bulletin 4:*231–51.
WILSON, EDMUND B. 1928. *The Cell in Development and Heredity*. New York: Macmillan. Chapters 10 and 12.

Questions

1. Evaluate Boveri's experiment on the relation of chromosomes to normal development in the sea urchin embryo. Do you find it convincing? How does it differ from some of the pre-1900 observations suggesting that the nucleus (and chromosomes) were the basis of inheritance (page 41)?

2. Explain why it is legitimate, on the basis of Mendel's work, to give the genotype of a pure breeding *round* pea as **R** but, on the basis of Sutton's work, it is necessary to designate it **RR**?

3. Let us assume that Mendel studied an eighth pair of genes, which we will call **Aa**. Let us also assume that one of the first seven pairs, **Bb,** is on the same pair of homologous chromosomes as **Aa**. Another pair of genes, **Cc,** is on a different pair of homologous chromosomes. What would you expect to be the F_1 and F_2 of these crosses?
   a. **AA CC×aa cc**
   b. **BB CC×bb cc**
   c. **AA BB×aa bb**

4. Using Sutton's hypothesis, can you explain Bateson's unusual data on coupling of genes (page 66)?

5. In species in which the male has **XY** sex chromosomes and the female **XX,** speculate on the relation between the chromosomes and sex. Is the male a male because he has a **Y** or only one **X** or an **X** and a **Y**? Is the female a female because she has no **Y** or because she has two **X's**. How could you test these various possibilities?

6. Imagine yourself a cytologist, circa 1900. Had you observed a peculiar unpaired chromosome, and discovered that half of the gametes formed by this species had the peculiar chromosome and half did not, what are some of the explanatory hypotheses that you could devise?

5 Morgan and Drosophila

During the first ten years following the rediscovery of Mendel's experiments, the progress of genetics was slow though steady. It was found that Mendel's scheme worked for many organisms and not for peas alone. To be sure some crosses gave ratios that were different from those expected in the Mendelian scheme. These proved difficult to analyze. During this same decade Sutton had suggested that the chromosomes might provide the physical basis for inheritance but those biologists concerned with breeding experiments were unable to appreciate the force of his arguments and data.

In 1910 the American geneticist, Thomas Hunt Morgan (1866–1945), together with his associates Alfred H.Sturtevant (1891–1970), Calvin B. Bridges (1889–1938), and Herman J.Muller (1890–1967), began a remarkable series of experiments. In one decade their efforts changed genetics into the most highly conceptual branch of biology.

It sometimes appears that much of the progress in science is due to fortunate accidents. One of these accidents was the choice by Morgan of the small fly, *Drosophila melanogaster* (Fig. 5–1), for genetic work. If Drosophila had never been used, the progress of genetics would have been very much slower. This species is common in nature and it frequents orchards, grocery stores, and other places where there is ripe fruit. It can easily be bred in the laboratory in simple containers such as vials or half-pint milk bottles. A layer of 'fly food,' consisting of cream of wheat, molasses, and yeast, is placed on the bottom of the container. The yeast, which grows on the other substances, is the main food of the Drosophila. If a pair of flies is placed in such a bottle,
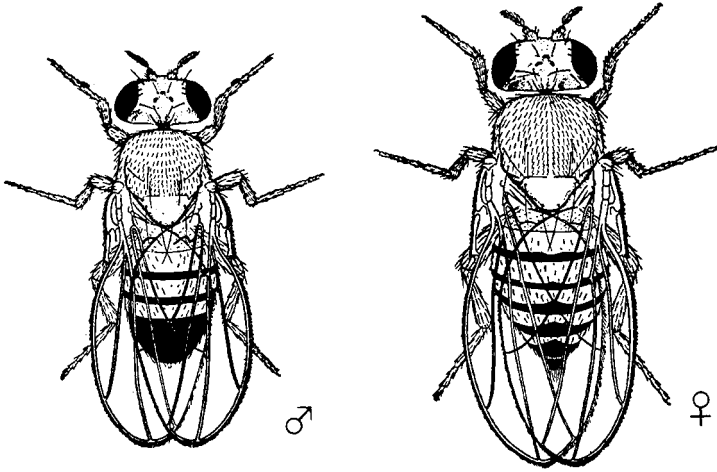
5–1 Drosophila melanogaster. Male left and female right. (From T.H.Morgan. 1919. *The Physical Basis of Inheritance.* Lippincott.)

several hundred young will be produced in about two weeks. Though small, these flies are large enough so that many of the external characteristics can be seen with a hand lens and much of Morgan's early work was done with a no more elaborate magnifying aid. Later it was customary to use low power stereoscopic microscopes to study the flies.

So *Drosophila melanogaster* was easy to collect, simple to maintain in the laboratory, and experimentation was most economical—an important consideration at a period when very little money was available to support scientific work. In fact, Morgan used Drosophila because he was unable to obtain the funds to experiment with rabbits, which are far more expensive to maintain. Whoever refused his request for the funds to work with rabbits, must go down in history as one of the truly great benefactors of modern genetics. Had Morgan studied rabbits rather than Drosophila, great progress in genetics would have probably been delayed by at least a generation.

The Origin of Hereditary Variation. Morgan began his work with Drosophila to answer a question about the origin of hereditary variation in organisms. Up to this point we have discussed alleles without reference to their possible origin. One of the pairs of alleles that Mendel used was **R** and **r,** which determined whether the pea seeds would be *round* or *wrinkled*. Mendel obtained the seeds that he used from seed dealers, who in turn probably obtained them originally from farmers.

What was the origin of the round and wrinkled varieties? Were all peas originally round and then did one suddenly become wrinkled? Or perhaps it was the reverse.

The Mutation Theory of de Vries. In 1901–3 the Dutch botanist, Hugo de Vries, from his studies of the evening primrose (Oenothera) advanced the hypothesis that abrupt changes can occur in the hereditary material of an organism. He believed that these changes were frequent and tended to be inherited. De Vries would have maintained that the gene causing *round* seeds in peas could change to an allele that caused *wrinkled* seeds. He spoke of this process of change as *mutation* and the new variety as a *mutant*.

The White-eyed Mutant. Morgan began raising Drosophila in the hope of observing the origin of mutants. The first mutant that he found was a male with *white* eyes. It suddenly appeared in a culture bottle of *red*-eyed flies. *Red* is the 'normal' or 'wild type' color of the eyes in *Drosophila melanogaster*. He began experiments to determine the mode of inheritance of the *white*-eyed condition.

Morgan mated the *white*-eyed mutant male with a *red*-eyed female (the symbols ♂ and ♀ are used for male and female respectively). These were the results, including the actual numbers of individuals obtained in the F_2.

P	red ♀	×		white ♂
F_1		red ♀	red ♂	
F_2	red ♀ 2,459	white ♀ 0	red ♂ 1,011	white ♂ 782

In addition, the original *white* male was crossed to one of his F_1 daughters.

P	red ♀ (F_1 above)	×	white ♂	
F_1	red ♀ 129	white ♀ 88	red ♂ 132	white ♂ 86

Explaining the Cross: First Hypothesis. These results could not be explained by the usual Mendelian scheme. The peculiar relation of eye color to sex, with the absence of *white* females in the F_2 of the first cross, suggested that sex chromosomes might be involved. Morgan proposed the following hypothesis to explain the data:

Let us call the gene that results in *white* eyes, **w,** and the gene that results in *red* eyes, **W**. The *white*-eyed male will produce sperm, all of

which will carry **w**. Half of these sperm will have, in addition, an **X** chromosome; the other half will not. The genotype of the original *white* male could be written **wwX**. Two types of sperm will be produced, namely, **wX** and **w**. The *red*-eyed female would be **WWXX**. These symbols represent the two *red* genes and the two **X** chromosomes. All of the eggs would be **WX**.

Morgan's first cross could be represented by the following scheme.

P	phenotype	*red* ♀	×	*white* ♂
	genotype	**WWXX**		**wwX**
	gametes	**WX**		**wX** **w**
F₁	phenotype	*red* ♀		*red* ♂
	genotype	**WwXX**		**WwX**
	gametes	**WX** **wX**		**WX** **w**
F₂	phenotype	*red* ♀ *red* ♀		*red* ♂ *white* ♂
	genotype	**WWXX** **WwXX**		**WwX** **wwX**
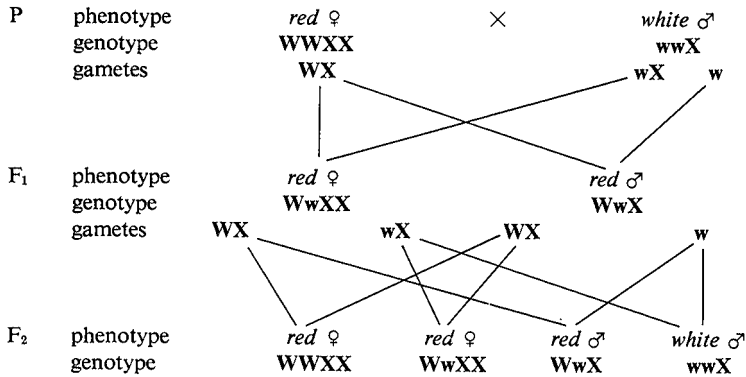
This scheme fits the experimental results, but Morgan pointed out that it is necessary to make one assumption about gamete formation in the F₁ *red* males. In these males, which are heterozygous for *white* eyes, it is necessary to assume that the **W** gene always goes to the same pole of the spindle with the **X** and that the **w** gene never does during the chromosome movements of meiosis. Consequently, there are no **wX** gametes formed by **WwX** males. He adds, 'This all-important point can not be fully discussed in this communication' (Morgan, 1910).

If Morgan's theory was correct, it should have been possible to make deductions about the behavior of the various genotypes and to test these deductions experimentally. Morgan made four such deductions and tested them by making the appropriate crosses.

1. If the genotype of the *white* male is **wwX** and of the *white* female **wwXX,** the following would be expected in a cross of these two types:

P	phenotype	*white* ♀	×	*white* ♂
	genotype	**wwXX**		**wwX**
	gametes	**wX**		**w** **wX**
F₁	phenotype	*white* ♂		*white* ♀
	genotype	**wwX**		**wwXX**
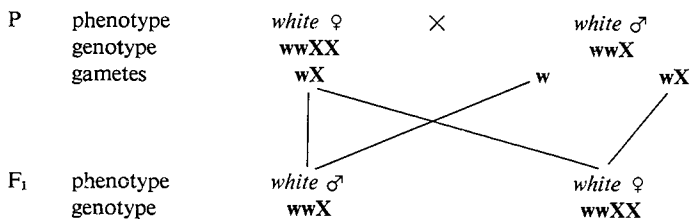
This cross was made and the results were entirely according to expectation, that is, only *white*-eyed flies were obtained.

2. The hypothesis requires that two genotypes be present in the F_2 females, namely, **WWXX** and **WwXX**. The two types could be differentiated by crossing to *white* males. These results would be expected:

a. P phenotype   *red* ♀   ×   *white* ♂
genotype   **WWXX**   **wwX**
gametes   **WX**   **wX**   **w**

F_1 phenotype   *red* ♀   *red* ♂
genotype   **WwXX**   **WwX**

b. P phenotype   *red* ♀   ×   *white* ♂
genotype   **WwXX**   **wwX**
gametes   **WX**   **wX**   **wX**   **w**

F_1 phenotype   *red* ♀   *white* ♀   *red* ♂   *white* ♂
genotype   **WwXX**   **wwXX**   **WwX**   **wwX**
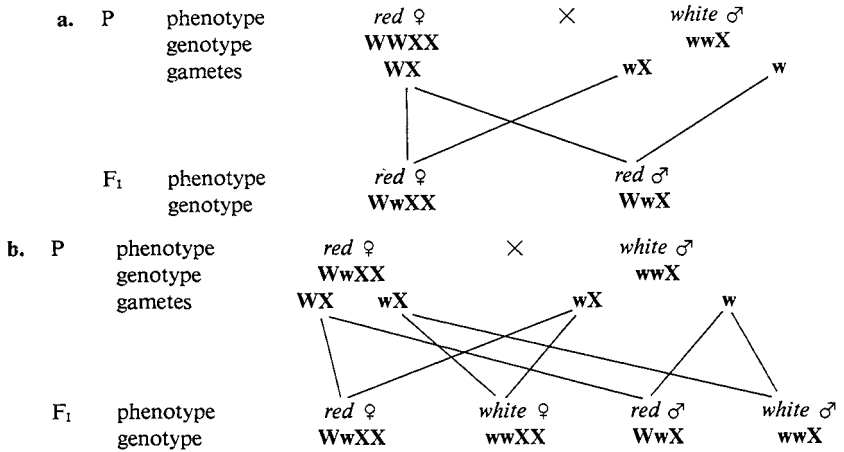
Tests of the F_2 *red* females showed that these two classes exist.

3. The genotype of the F_1 female in the original cross was thought to be **WwXX**. If this was correct, a cross of the F_1 female and a *white* male would give the same results as cross 2b (above). This cross was made and the prediction verified.

4. The hypothesis requires the F_1 male in the original cross to be **WwX**. If such a male is crossed to a *white* female, the following results would be expected:

P phenotype   *red* ♂   ×   *white* ♀
genotype   **WwX**   **wwXX**
gametes   **WX**   **w**   **wX**

F_1 phenotype   *red* ♀   *white* ♂
genotype   **WwXX**   **wwX**
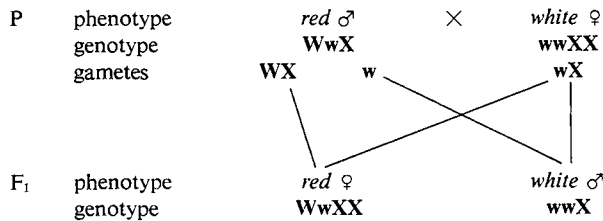
Once again, the actual experiment yielded the expected results. Note, however, the assumption that **W** and **X** were always together in the same sperm and that no **wX** sperm could be formed by **WwX** males.

Nearly all of Morgan's hypothesis was based on facts or on ideas that seemed quite probable. The role of chromosomes in sex determination, the concept of alleles showing dominance and recessiveness, and the concept of segregation had all been part of biological knowledge for years. He made four deductions from his hypothesis and found that every one could be verified experimentally. He predicted the results expected from crosses before they were made and later found his predictions confirmed. Do you consider that his hypothesis was 'established beyond a reasonable doubt'?

Still another cross was made, but the results were most surprising. A *white*-eyed female was crossed with a *red*-eyed male. (The male was from a wild stock that had never been bred with the stock that produced the *white*-eyed male.) All of the females derived from this cross had *red* eyes and all of the males had *white* eyes. One might have expected that all of the F_1 would have been *red*-eyed, since the male should have been of the genotype **WWX**. This was not the case, so Morgan assumed that all males he used were heterozygous for the *red* eye gene and had the genotype **WwX:**

P	phenotype		*white* ♀	×	*red* ♂	
	genotype		**wwXX**		**WwX**	
	gametes		**wX**		**WX**	**w**
F_1	phenotype		*red* ♀		*white* ♂	
	genotype		**WwXX**		**wwX**	
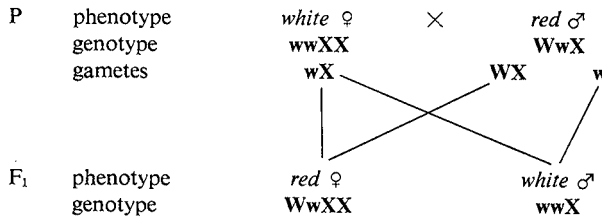
At this point we should pause and consider these questions:

a. Why are there only two classes of sperm, namely **WX** and **w,** formed by the heterozygous *red* males of the genotype **WwX**? Why is no **wX** class produced?

b. Why are all the wild *red*-eyed males heterozygous? Why does the **WWX** type not occur?

Morgan's hypothesis demands that *red*-eyed males are never homozygous and that they show unusual phenomena in sperm formation. If these basic conditions do not hold, then the hypothesis is either wrong or incomplete. Can you devise other hypotheses for explaining the data? *Explaining the Results: Second Hypothesis.* It was not long—in fact, only one year—until Morgan devised a simpler hypothesis to account for the *white* eye case. If one assumes that *the gene for white eyes is part of the* **X** *chromosome,* then the results of all the crosses correspond to what would be expected from the behavior of the **X** chromosome.

There would then be no need for invoking subsidiary assumptions, such as unusual types of meiosis in some males, or requiring all wild males to be heterozygous for eye color. Morgan's second hypothesis has withstood every conceivable test, and there seems to be no reasonable doubt of its correctness.

The symbolic representation for the new scheme will be different from that given before. **W** will continue to mean *red* and **w** *white,* but there will be no need to use **X**. If we assume that **W** and **w** are located on the **X** chromosomes, '**W**' should be interpreted as an **X** chromosome with the **W** allele. In the same manner, **w** will indicate an **X** chromosome with the **w** allele. The **Y** chromosome will be indicated by a **Y,** since by this time it was realized that Drosophila is of the **XX** ♀—**XY** ♂ sex chromosome type. The crosses seemed to indicate that the **Y** chromosomes contain no **W** or **w** alleles. (As later work was to show, the **Y** of *Drosophila melanogaster* is almost entirely without genes.) The following, then, are the correct diagrammatic representations of the crosses through the F_2 generation:
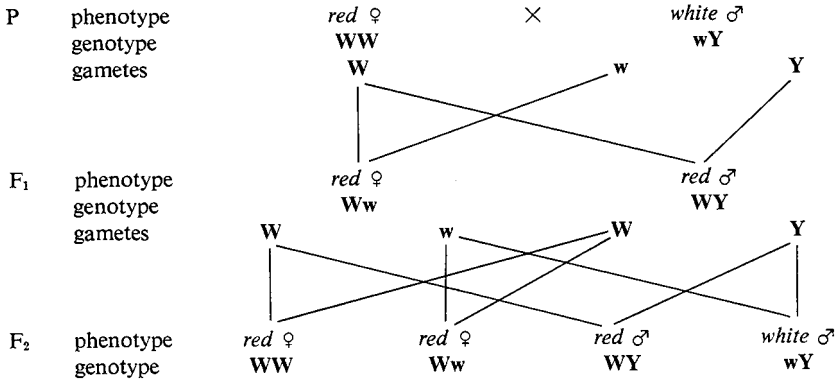
The reciprocal P generation cross would be as follows:
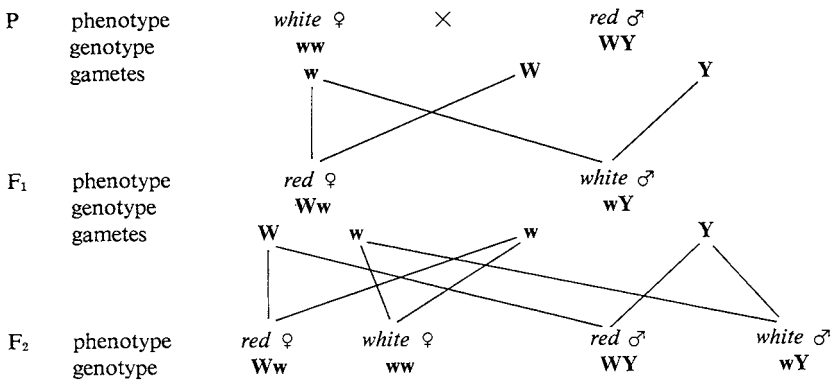
This new hypothesis explains the observed results of the genetic experiments, and it does not invoke any unknown phenomena.

Morgan soon discovered other genes which, from their mode of inheritance, he concluded were carried on the **X** chromosome. All genes that are on the **X** are said to be *sex linked* and they always show the type of inheritance just outlined for the *white* eye crosses.

These experiments with *white*-eyed flies provided additional evidence supporting the hypothesis that chromosomes are the physical basis of inheritance. If some genes are parts of the **X** chromosome, their inheritance must reflect the behavior of the **X** during meiosis and fertilization. The *white* eye genes behaves as though it were part of the **X**. This can mean either that it is part of the **X** or that it is part of some unknown cell structure that behaves exactly like the **X** during meiosis, fertilization, and mitosis.

Crosses with Sex-linked and Autosomal Genes. Morgan and his associates discovered mutant genes by the dozens. Some were sex linked. Those genes carried on any chromosome except the sex chromosomes are said to be *autosomal* genes. The inheritance of autosomal genes followed the usual Mendelian scheme. Sex-linked inheritance follows the scheme that has just been described.

The following example of a cross involving sex-linked genes and autosomal genes will bring out the difference between the two types. We already know that *white* eye is a sex-linked gene recessive to *red*. Our other characteristic in this cross is *vestigial* wing, which is an autosomal gene recessive to *long* wing.

P	phenotype	*red-vestigial* ♂	×	*white-long* ♀
	genotype	WY vv		ww VV
	gametes	Wv Yv		wV

F₁	phenotype	*red-long* ♀		*white-long* ♂
	genotype	Ww Vv		wY Vv
	gametes	WV Wv wV wv		wV wv YV Yv
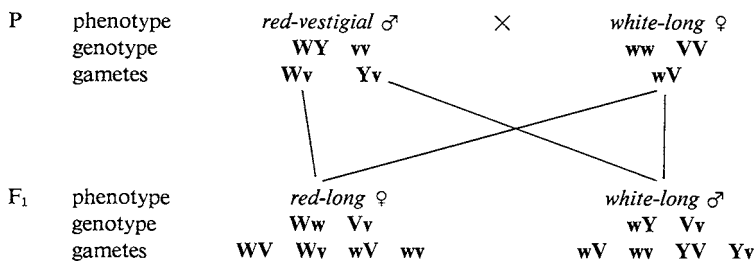
The F₂ which is shown in the checkerboard on the next page consists of the following:

♀ 3/8 *red-long;* 3/8 *white-long;* 1/8 *red-vestigial;* 1/8 *white-vestigial*.
♂ 3/8 *red-long;* 3/8 *white-long;* 1/8 *red-vestigial;* 1/8 *white-vestigial*.

SPERM

	wV	wv	YV	Yv
WV	Ww VV *red-long* ♀	Ww Vv *red-long* ♀	WY VV *red-long* ♂	WY Vv *red-long* ♂
Wv	Ww Vv *red-long* ♀	Ww vv *red-vestigial* ♀	WY Vv *red-long* ♂	WY vv *red-vestigial* ♂
wV	ww VV *white-long* ♀	ww Vv *white-long* ♀	wY VV *white-long* ♂	wY Vv *white-long* ♂
wv	ww Vv *white-long* ♀	ww vv *white-vestigial* ♀	wY Vv *white-long* ♂	wY vv *white-vestigial* ♂

OVA (label at left of rows)

What is the ratio of *long* to *vestigial,* neglecting the eye-color genes? Is there any difference in the ratios of the autosomal genes between the F_2 males and females?

The Importance of Morgan's Work. These first experiments of Morgan are important in several ways. A new experimental animal was introduced to geneticists that was easy to raise in the laboratory and was a producer of large numbers of offspring. In addition, the crosses themselves added considerably to genetic theory in that they were the first well-analyzed cases of sex-linked inheritance. The fact that the genetic results exactly paralleled the behavior of the **X** chromosome was strong evidence that the gene responsible for *white* eyes is part of the **X** chromosome. At least many biologists believed the data to be highly suggestive. Now if it is established that one gene is part of a chromosome, it is a good working hypothesis that other genes are parts of chromosomes. One could even hold to the hypothesis that most or all genes are parts of chromosomes.

Scientific Methods. This early genetic work of Morgan is valuable in still another way. The experiments and the way in which they were reported are excellent examples of one of the most important procedures in experimental science, namely, the manner in which 'cause-effect' relations are discovered. A scientist is interested in the reason why things behave as they do. In this case, Morgan wondered what was the cause of the peculiar genetics of the *white* eye gene. He observed the effects and attempted to reconstruct the cause. This reconstruction, according to the philosophers who study scientific methods, takes place in well defined if not always explicitly stated steps, which are:

1. *Recognition of the problem.* In this instance the problem was to interpret the *white* eye case in genetic and cytological terms.
2. *Collection of facts pertaining to the problem.* The facts consisted of the data of the first cross together with all that Morgan knew of cytology and genetics.
3. *Formulation of a hypothesis.* From a consideration of all the particular facts, Morgan formulated a general statement, or *hypothesis,* that would explain the facts. This logical step from the particular to the general is known as *induction*. The hypothesis in this case was the symbolic scheme that explained the results of the cross in terms of chromosome behavior.
4. *Testing the hypothesis*. The correctness of a hypothesis is tested in this manner: First, we assume that the hypothesis is correct and then make certain deductions. These deductions can be tested to see if they are true or false. Morgan made four such deductions and found that the predicted results were always obtained. The more deductions that are verified, the more likely it is that the hypothesis is true.

The fate of Morgan's first hypothesis, which symbolized the *white* female as **wwXX** and the *white* male as **wwX**, should be a sobering example. It was tested by four deductions and found to be 'true.' For most scientists, this might be convincing. It did not, however, offer a convincing explanation of all the data. One had to assume that meiosis in the **WwX** males was unusual and that all *red*-eyed males are heterozygous. Morgan found, however, that a second hypothesis would explain the same data and in this case it was not necessary to introduce any qualifications, such as a special type of meiosis in males heterozygous for the eye-color gene or that all *red*-eyed males are heterozygous **(WwX)**. The second hypothesis, which symbolized the *white* female as **ww** and the *white* male as **wY,** was simpler. When one has the choice of two hypotheses, one simple and one complex, one generally selects the first. This is the famous *Occam's razor,* which admonishes the scientist to explain his results in the simplest manner possible, and to introduce no unnecessary complexity. It must be realized that both of Morgan's hypotheses explain the data. Subsequent events have shown the first one to be false and the second, and simpler one, to be true.

This episode is an example of the self-correcting nature of scientific procedures. If deductions are made and tested, the truth or falsity of the hypothesis can be established. If the hypothesis fails to account for all the data, then it must be modified or abandoned. Morgan's first hypothesis accounted for most but not all of the experimental results. It was not necessary for him to abandon the hypothesis entirely, merely to modify it.

New mutants were discovered rapidly in Morgan's laboratory and very soon there were more of them than there were pairs of homologous chromosomes. This was the difficult moment for genetics and cytology that Sutton had predicted (page 78).

The Prediction of Linkage. At the time Sutton proposed his hypothesis, he pointed out one situation in which the Mendelian laws could not apply, namely, those cases where two genes are carried on the same chromosome. Clearly, they would not obey Mendel's law of independent assortment. He foresaw that this problem would arise when more pairs of alleles had been discovered than there are pairs of chromosomes in the species being studied.

Let us consider the problem as it applies to *Drosophila melanogaster.* The diploid cells of this species have four pairs of chromosomes. Let us assume that each of the first four mutant genes discovered are located on a different chromosome pair. If this is the case, each of these four genes will show independent assortment. What will happen when we discover the fifth pair of genes? Since there is no fifth pair of chromosomes, the fifth pair of alleles must be located on a chromosome that already has one of the first four pairs of alleles. When this situation arises, obviously the two pairs of alleles cannot act in an independent way during meiosis. They would be linked in inheritance. This deduction is inevitable, if genes are parts of chromosomes.

The Discovery of Linked Genes. In 1906 Bateson and R.C.Punnett (1875–1967) reported a cross involving two pairs of genes that did not show independent assortment. Their cross was with sweet peas, where *blue* flower color **(B)** is dominant over *red* **(b)** and *long* pollen grain **(L)** is dominant over *round* pollen **(l)**. The scheme of the cross was this:

P	phenotype	*blue-long*	×	*red-round*
	genotype	**BB LL**		**bb ll**
	gametes	**B L**		**b l**

F_1	phenotype	*blue-long*
	genotype	**Bb Ll**
	expected gametes	**BL Bl bL bl**
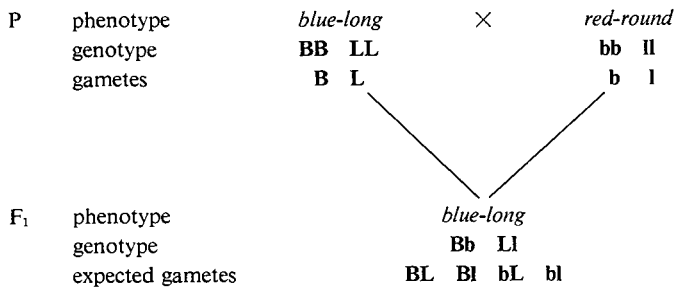
If there is independent assortment, we would expect the four classes of F_1 gametes shown in the diagram to be produced in equal numbers. Each type would account for 25 per cent of the total. The standard genetic way for finding gamete percentages is to cross the organism being tested with the pure recessive. This is called the *test cross*. For the F_1 heterozygous plant it would be this:

phenotype	*blue-long*	×	*red-round*
genotype	Bb Ll		bb ll
gametes	BL Bl bL bl		bl

phenotype	*blue-long*	*blue-round*	*red-long*	*red-round*
genotype	Bb Ll	Bb ll	bb Ll	bb ll
	25%	25%	25%	25%
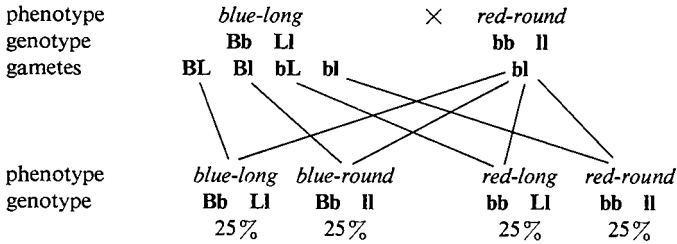
In this test cross, the phenotype of the offspring would be a measure of the gamete frequency of the plant being tested. Thus, 25 per cent of the gametes would be **BL** and 25 per cent of the offspring would be *blue-long*. This would be true for all classes of gametes, since they would be combining with a gamete having both recessive genes. (These **bl** gametes, having only recessive genes, cannot alter the expression of genes in the gametes with which they combine.)

When Bateson and Punnett made the cross, these were the results:

	EXPECTED	ACTUAL
blue-long	25%	43.7%
blue-round	25%	6.3%
red-long	25%	6.3%
red-round	25%	43.7%

Clearly these results do not conform to those expected from the Mendelian theory. Two points should be noticed.

1. The two most frequent phenotypes are those of the original parents (*blue-long* and *red-round*).
2. The percentages of the original parental types, *blue-long* and *red-round,* are the same. The percentages of the two recombination classes, *blue-round* and *red-long,* are also the same.

An even more disturbing finding was that the F_2 ratios depended largely on the genotype of the P generation. On strict Mendelian principles a cross of *blue-long×red-round* should give the same F_2 as *blue-round×red-long*. This was not so with Bateson's sweet peas. The *blue-round×red-long* cross gave the following results (once again the results are compared with what would have been expected if Mendel's rules applied):

phenotype	blue-round	×	red-long
genotype	BB ll		bb LL
gametes	B l		b L

phenotype	blue-long		×	red-round
genotype	Bb Ll			bb ll
gametes	BL Bl bL bl			bl

phenotype	blue-long	blue-round	red-long	red-round
genotype	Bb Ll	Bb ll	bb Ll	bb ll
expected	25%	25%	25%	25%
actual	6.3%	43.7%	43.7%	6.3%
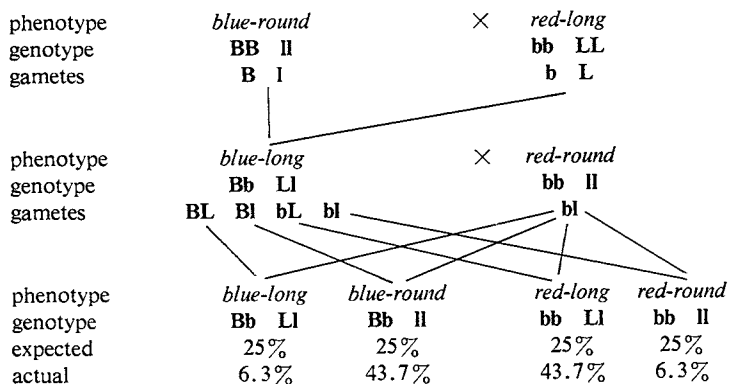
When these results are compared with those of the first cross, the percentages for each phenotype are found to be different, but again we notice a preponderance of the parental types. Both crosses suggest an orderly, though non-Mendelian, mechanism of inheritance. Some new principles must be involved.

Let us try to explain the results on the basis of Sutton's hypothesis. If we assume that the two genes, **B** and **L,** are parts of the same chromosome, this will be the schematic representation of the cross:

expected	50%	50%
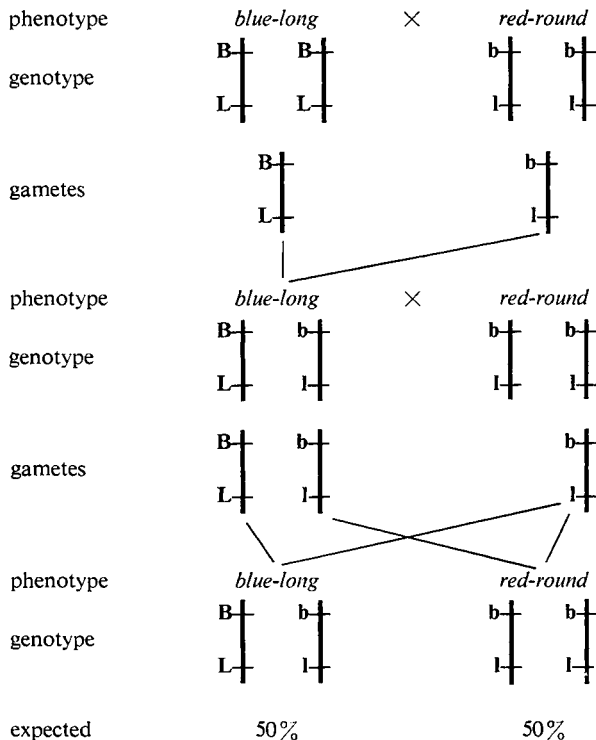
The percentages actually obtained can be compared with the ratios expected on the basis of Mendel's hypothesis and Sutton's hypothesis:

	blue-long	*blue-round*	*red-long*	*red-round*
OBSERVED	43.7	6.3	6.3	43.7
MENDEL'S	25	25	25	25
SUTTON'S	50	0	0	50

At first sight it may seem as though Sutton's explanation is the better since the *blue-long* and *red-round* classes are nearer the observed percentages. One difficulty, however, is fatal to Sutton's hypothesis. Both *blue-round* and *red-long* plants were obtained in the cross. Neither of these classes would be possible if both the **B** and **L** genes were part of the same chromosome and were inherited in the manner outlined. We may conclude that the results obtained in the cross cannot be understood on the basis of either Mendel's or Sutton's hypotheses, as originally stated.

Linkage and Crossing Over. Morgan and his associates discovered many new mutants and used them in crosses. Some of the crosses involving two pairs of genes gave the independent assortment expected in the Mendelian scheme. In other crosses, deviations of the sort found by Bateson and Punnett were encountered. Morgan was convinced of the correctness of Sutton's hypothesis that genes are parts of the chromosomes and assumed that the exceptions to independent assortment were due to the two different genes being on the same chromosome. But that simple assumption could not explain the results satisfactorily; it was necessary to assume that under some circumstances genes could be transferred from one chromosome to another. Was there any cytological evidence for this?

A possible cytological mechanism for an exchange of genes had been suggested in 1909 by F.A.Janssens (1863–1924). He described a type of behavior of chromosomes in meiosis that is now known to be nearly universal in both animals and plants. It is called *crossing over* and it occurs during the tetrad stage (Fig. 5–2). During synapsis the homologous chromosomes come close together with their long axes parallel. Both chromosomes duplicate and a tetrad of four chromatids is formed. According to Janssens, there is considerable coiling of chromatids around one another at this time and in some cases two of the chromatids break at the corresponding place on each. The broken chomatids rejoin in such a way that a section of one chromatid is now joined with a section of the other. As a result, 'new' chromatids are produced that are mosaics of segments of the original ones.

Janssens' hypothesis of crossing over could provide the basis of gene
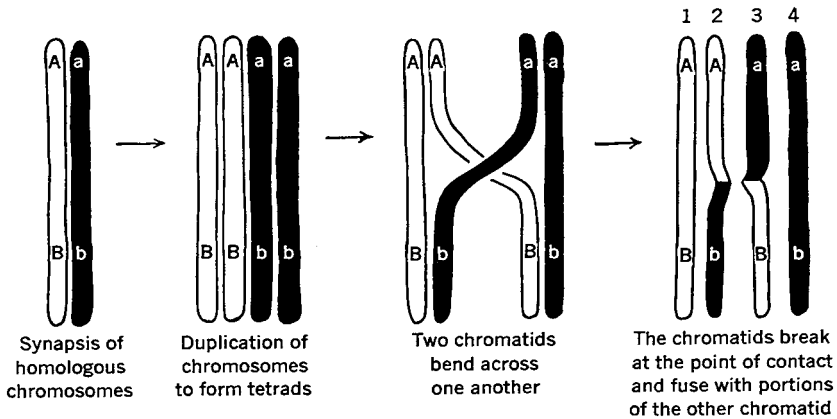
5–2 Janssens' hypothesis of crossing over.

transfer from one chromosome to another. Morgan suspected that it did and wrote in 1911:

> In consequence, the original materials will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find [linkage] in certain characters, and little or no evidence at all of [linkage] in other characters; the difference depending on the linear distance apart of the chromosomal materials that represent the factors. Such an explanation will account for all of the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes, and of the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system as of the relative location of the factors in the chromosomes. *Instead of random segregation in Mendel's sense we find 'associations of factors' that are located near together in the chromosomes. Cytology furnishes the mechanism that the experimental evidence demands.*

The term *linkage* was introduced to refer to cases where different genes are located on the same chromosome. *Crossing over* was the term applying to the coiling, breaking, and rejoining of homologous chromosomes during meiosis.

The following is an example of inheritance of two linked genes: In Drosophila *gray* body color **(B)** is dominant to *black* body color **(b)**. *Long* wing **(V)** is dominant to *vestigial* wing **(v)**. The two pairs of genes are located on the same pair of autosomes.

P   phenotype   *black-vestigial*   ✕   *gray-long*

genotype

gametes

F₁   phenotype   *gray-long*
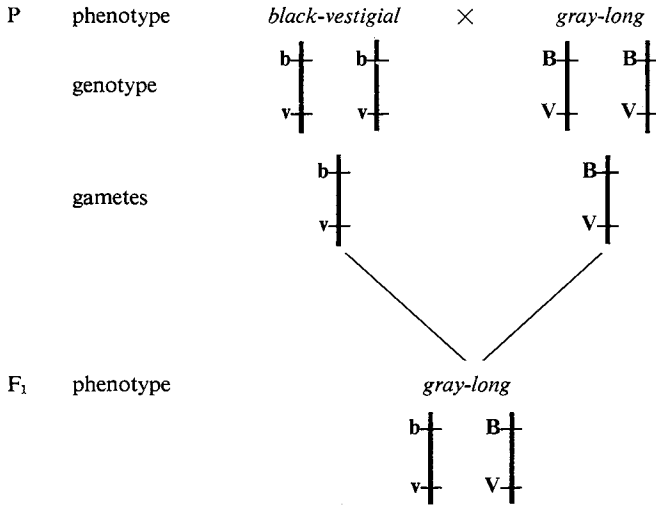
This F₁ individual will produce four types of gametes, two the result of crossing over and two non-crossovers. Since crossing over occurs in the tetrad stage we could diagram gamete formation as follows:
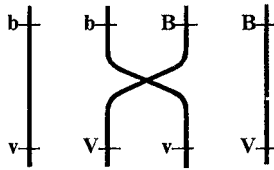
The middle pair of chromatids will break and recombine. The two meiotic divisions then occur and each resulting gamete will receive one chromosome. The four types of gametes will be as follows:

no crossing over   crossing over
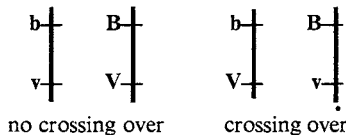
These four gametes are not produced in equal frequency. Morgan suggested that *the chance of a crossover occurring between two genes is a junction of the distance between them*. This suggestion was based on a simple argument. Let us assume that crossing over can occur at any point along a chromosome. Let us assume further that there are three genes, **A, B,** and **C,** on the chromosome. **A** and **B** are close to one another; **B** and **C** are far apart. If this is so, crossing over is more likely to occur between **B** and **C** than **A** and **B** merely because there is a longer stretch of chromosome where it might occur.

Possibly this analogy will help: We will imagine that we have a string three feet in length and of uniform strength. We will stretch this string until it breaks. The site of breakage is more likely to be at some point in the first 30 inches of the string than in the last six inches.

Continuing the experiment, we mate an F_1 female of the above cross with a *black-vestigial* male, the results are as follows:

gray-long	black-vestigial	gray-vestigial	black-long
41.5%	41.5%	8.5%	8.5%
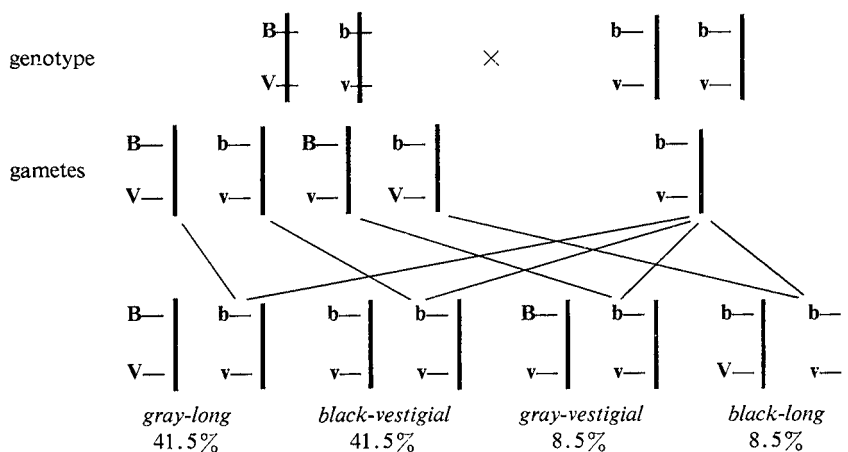
Among the offspring, 17 per cent are derived from gametes that carry chromosomes that had a crossover between the two genes being studied. The remainder, 83 per cent, are from non-crossover gametes.

The frequency of crossing over between any two genes is nearly constant. If we cross a *black-long×gray-vestigial* fly the F_1 would be *gray-long* and heterozygous for both genes. In these two respects the F_1 of this cross will be identical with that in the one previously described. In this second cross, however, one of the P generation chromosomes will be **bV** and the other **Bv**. If a female of this constitution is crossed with a *black-vestigial* male the offspring will be as follows:

41.5% *black-long*
41.5% *gray-vestigial*
8.5% *gray-long*
8.5% *black-vestigial*

Compare these percentages with the previous cross and be sure you understand the reason for the difference.

Subsequent events have shown that Morgan's explanation satisfactorily accounts for the inheritance of genes located on the same chromosome. Many details were added, such as the occurrence of double or triple crossovers, and, for reasons still not understood, the absence of crossing over in Drosophila males.

GROUP I

Name	Region Affected
Abnormal	Abdomen
Bar	Eye
Bifid	Venation
Bow	Wing
Cherry	Eye color
Chrome	Body color
Cleft	Venation
Club	Wing
Depressed	Wing
Dotted	Thorax
Eosin	Eye color
Facet	Ommatidia
Forked	Spines
Furrowed	Eye
Fused	Venation
Green	Body color
Jaunty	Wing
Lemon	Body color
Lethals, 13	Die
Miniature	Wing
Notch	Venation
Reduplicated	Eye color
Ruby	Legs
Rudimentary	Wings
Sable	Body color
Shifted	Venation
Short	Wing
Skee	Wing
Spoon	Wing
Spot	Body color
Tan	Antenna
Truncate	Wing
Vermilion	Eye color
White	Eye color
Yellow	Body color

GROUP IV

Name	Region Affected
Bent	Wing
Eyeless	Eye
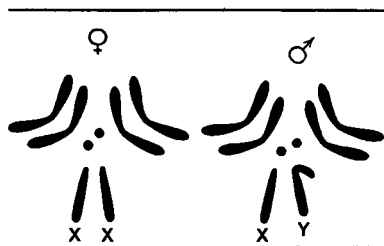
GROUP II

Name	Region Affected
Antlered	Wing
Apterous	Wing
Arc	Wing
Balloon	Venation
Black	Body color
Blistered	Wing
Comma	Thorax mark
Confluent	Venation
Cream II	Eye color
Curved	Wing
Dachs	Legs
Extra vein	Venation
Fringed	Wing
Jaunty	Wing
Limited	Abdominal band
Little crossover	II chromosome
Morula	Ommatidia
Olive	Body color
Plexus	Venation
Purple	Eye color
Speck	Thorax mark
Strap	Wing
Streak	Pattern
Trefoil	Pattern
Truncate	Wing
Vestigial	Wing

GROUP III

Name	Region Affected
Band	Pattern
Beaded	Wing
Cream III	Eye color
Deformed	Eye
Dwarf	Size of body
Ebony	Body color
Giant	Size of body
Kidney	Eye
Low crossing over	III chromosome
Maroon	Eye color
Peach	Eye color
Pink	Eye color
Rough	Eye
Safranin	Eye color
Sepia	Eye color
Sooty	Body color
Spineless	Spines
Spread	Wing
Trident	Pattern
Truncate intensf.	Wing
Whitehead	Pattern
White ocelli	Simple eye

These experiments are an example of the mutual checking that the combined genetic and cytological approach permits. As we have mentioned before, inheritance must be explained in both fields. If two genes are in the same chromosome they will be linked in inheritance. If they are not completely linked there must be a chromosomal basis for the recombination. A chromosomal basis is to be found in the phenomenon of crossing over.

Linkage Groups in Drosophila. Another interesting parallel between genetics and cytology was soon apparent. One deduction we could make from Sutton's hypothesis is this: If genes are on chromosomes and all chromosomes have genes, then the number of groups of linked genes would correspond to the number of pairs of homologous chromosomes. This deduction was verified. By 1915 Morgan and his associates had studied more than 100 mutant genes. When these were tested, *they were found to comprise four linkage groups. The number of chromosome pairs in Drosophila is also four.* The partial list that Morgan published at that time is given in Fig. 5–3.

The evidence was becoming almost overwhelming that Sutton's hypothesis was correct, though it was necessary to modify it to take crossing over into account.

In Figure 5–3, two sets of data are given: the genetic list of linked genes and a drawing of the chromosomes. As we have seen, there is good reason to believe that a relation exists between the two sets of data.

If we accept the hypothesis that the linkage groups correspond to the pairs of homologous chromosomes, how could we determine which linkage group corresponds to each of the four pairs of chromosomes? It will be worthwhile for you to consider this problem.

Another matter should be mentioned. Mendel believed that in crosses involving two pairs of alleles there is always independent assortment. This is to be expected if each pair of alleles is situated on a different pair of homologous chromosomes. Mendel worked with seven pairs of alleles and there are seven pairs of homologous chromosomes in the garden pea. Each pair of alleles could have been on different homologues but later work has shown this not so. In some cases two pairs of alleles are on the same chromosome and might be expected to show linkage,

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5–3 A total of 85 genes of *Drosophila melanogaster* were reported in 1915. These fell into 4 linkage groups. Cytological investigations showed that this species has 4 pairs of chromosomes. This parallelism between the number of chromosomes and the number of linkage groups suggested that the genes were situated on the chromosomes (T.H.Morgan, 'The Constitution of the Hereditary Material,' *Proc. Amer. Phil. Soc.* 54:143–53. 1915).

yet Mendel reports only data that suggest independent assortment. The answer to this paradox lies in the relative positions of the alleles on the chromosomes. If they are far apart, the amount of crossing over may be so great that the different alleles appear to be inherited independently.

Relation of Genes to Characteristics. The data in Figure 5–3 showing the linkage groups of Drosophila are instructive in another connection. Notice that many different genes affect the same character: 13 influence eye color and 33 modify the wings in some manner. The question arises, what determines the normal red eye color? The answer is that the wild type alleles of all of these 13 eye color genes, together with many undiscovered in 1915 when Morgan published his list, act together to produce the wild type red eye color. If an individual is homozygous for the mutant allele of any one of these genes, then the eye is not red but some other color such as white, sepia, or peach. We should think of the normal red eye color as the end product of a series of gene actions. If any of these actions is altered, the eye color will be different.

Figure 5–3 is misleading in one respect. Each mutant gene appears to have a single effect. It usually does have a single *main* effect, but most of the genes that have been studied intensively are found to have many different effects. Thus the *white* eye color gene in Drosophila is responsible not only for the absence of color in the compound eyes but also for the absence of color in the simple eyes and in some of the internal organs as well. It is called an eye color gene simply because the most obvious effect of the gene is on the color of the compound eyes. Genes that affect more than one structure are said to be *pleiotropic*.

THE CYTOLOGICAL PROOF OF CROSSING OVER

With the data so far given, the concept of crossing over as a mechanism for genetic recombination might be regarded as a good working hypothesis and nothing more. The hypothesis was invented to account for the results of genetic crosses. Thus, the absence of recombination of genes in some crosses suggested that these genes were parts of the same chromosome. This explanation did not account for all the data, however, since in a definite percentage of the individuals recombination did occur. Now if the genes in question are parts of chromosomes, these recombinations among genes of the same linkage group could only be explained on the basis of some exchange of genes between homologous chromosomes.

After the Morgan group had analyzed the situation to this extent they sought some possible cytological basis for the postulated interchange of genes between chromosomes. It was then that they came across the work

of Janssens, who had described a phenomenon that *might be interpreted* in terms of the breaking and rejoining of chromatids during the tetrad stage of meiosis. It should be emphasized that Janssens did not actually observe the breaking and rejoining of chromosomes and no one has to this day. The difficulty is this. Crossing over is assumed to occur between homologous chromatids. Since they are homologous, they are of identical appearance when viewed under the microscope. Furthermore, the act of crossing over is assumed to occur when the four chromatids of the tetrad are tightly coiled around one another. Crossing over cannot be seen in living cells, and in fixed and stained cells there is no direct way of telling whether a chromatid has exchanged portions with another chromatid or not.

Figure 5–2 is a diagram of crossing over. The two homologous chromosomes undergoing synapsis are drawn differently, but it must be remembered that in living or in fixed and stained material they would be of identical appearance. In the four chromatids shown after crossing over there is no difficulty in distinguishing the chromatids that have crossed over and those which have not, since the strands have been shaded differently by the artist. Once again this is impossible to observe in the actual material.

One could obtain critical cytological evidence for crossing over if there was some visible or detectable difference between the members of a homologous pair of chromosomes. Such evidence was not available for animals until the work of Curt Stern (born 1902) in 1931. This was nearly 20 years later than the time Morgan's group postulated the existence of crossing over. We shall consider Stern's work out of turn, so to speak, but by so doing we can complete the analysis.

By the time Stern began his work, Drosophila geneticists had a large variety of strains with different types of chromosome abnormalities. He was able to use a female that had the necessary chromosomal and genetic characteristics to demonstrate convincingly whether or not crossing over involves a transfer of material from one chromosome to another.

The female used had two structurally and genetically different **X** chromosomes (Fig. 5–4). One of the **X** chromosomes was in two portions: one portion behaved as an independent chromosome and the other was attached to one of the tiny fourth chromosomes (Fig. 5–3 shows the chromosomes of a normal ♀; the fourth chromosomes are the pair of dot-shaped structures). The other **X** of this female was unusual in that a piece of a **Y** chromosome was attached to it. These structural differences were so great that they could be seen easily in fixed and stained nuclei.
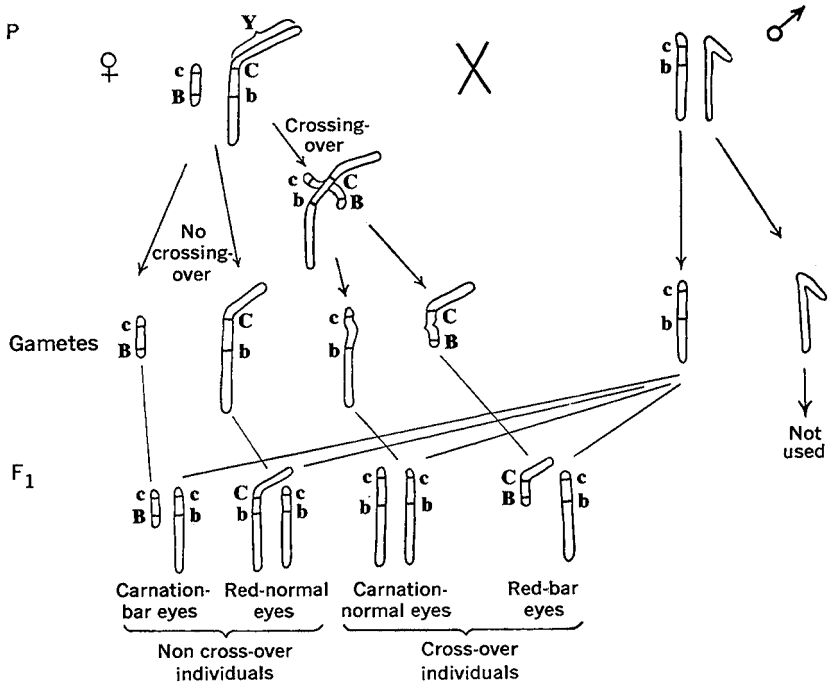
5–4 Stern's Experiment

The two **X** chromosomes, in addition to being structurally different, were also genetically different. The divided **X** had in one portion of it the recessive gene *carnation* **(c),** which when homozygous produces a dark ruby eye color, and the dominant gene *bar* **(B),** which reduces the eye to a narrow band. The other **X**, which had the piece of the **Y** attached to it, contained the wild type alleles, **C** and **b**, which when homozygous result in *red* eyes of *normal* shape.

The essential point about this female is that she had two **X** chromosomes that could be distinguished from one another on both cytological and genetic grounds.

She was crossed to a male fly carrying the genes for *carnation* color **(c)** and *normal* eye shape **(b)**. The ova of the female would be of two types if no crossing over occurred: one type of ova would contain the short **X** chromosome with the genes **c** and **B;** the other type would have the **C** and **b** genes on the **X** that had the piece of **Y** chromosome attached to it. If crossing over occurred between the two marker genes, two other types of gametes would be produced. One of these crossover

types would have the **c** and **b** genes on an **X** chromosome of normal size; the other crossover type would have the **C** and **B** genes on a short **X** chromosome to which the piece of **Y** chromosome was joined.

Stern studied only the female offspring of the cross. One can determine from Fig. 5–4 that four types of daughters are to be expected, showing all combinations of the phenotypic characters. These flies should also have four different chromosome configurations, and if the theory is correct it should be possible to predict the chromosome configuration for each phenotypic class. Thus, the flies that give genetic evidence of coming from crossover gametes will be either *carnation-normal* or *red-bar*. The *carnation-normal* flies, alone among the offspring, should have two normal-shaped **X** chromosomes. The *red-bar* flies, again alone among the offspring, should have one short **X** with a piece of **Y** chromosome attached and an **X** of normal proportions.

Stern studied the cytology of his flies and saw that the phenotype corresponded to the expected chromosomal configuration. This was a brilliant demonstration of the hypothesis that chromosomal material can be interchanged between homologous chromosomes.

MAPPING THE GENES

Morgan and his fellow workers made numerous crosses involving linked genes. It was found that, in successive experiments, the amount of crossing over between two particular genes was always the same. Depending on the genes used, this might be less than 1 per cent or nearly 50 per cent. Morgan suggested that the different values were the result of the relative positions of the genes on the chromosome: If the amount of crossing over between hypothetical genes **A** and **B** was small and between genes **C** and **D** large, one would predict that **A** would be closer to **B** than **C** would be to **D**.

The development of this concept, that linkage data could be used to map the relative positions of the genes on the chromosomes, was attempted in 1913 by Alfred H.Sturtevant when he was a graduate student of Morgan. He made crosses involving five genes carried on the **X** chromosome: *yellow* body **(y),** *white* eyes **(w),** *vermilion* eyes **(v),** *miniature* wings **(m),** and *rudimentary* wings **(r)**. From the data obtained, he constructed a genetic map showing the 'positions' of these genes on the **X** chromosome. This was his basic assumption: 'It would seem…that the proportion of "crossovers" could be used as an index of the distance between any two factors. Then by determining the distances (in the above sense) between A and B and between B and C, one should be able to predict [the amount of crossing over in the interval] AC. For, if proportion of crossovers really represents distance,

AC must be approximately, either AB plus BC, or AB minus BC, and not any intermediate value.'

The percentage of crossovers between **y** and **v** was found to be 32.2 and between **y** and **m** 35.5 On the basis of the hypothesis we would expect **v** to be closer to **y** than in to **y**. What can we conclude about the relative positions of **m** and **v**? According to Sturtevant this should be 67.7 (35.5+32.2) or 3.2 (35.5 −32.2). The reason for this is as follows: The chromosome is a long and very thin structure so we can represent it as a line. On this line we shall put **y** and **v**, as follows:

```
                            y              v
  _____ ─┐
                         ------32.2------                    │
```
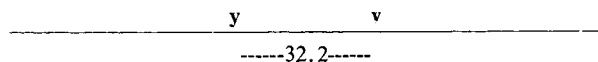
Now **m** can be either to the right or to the left of **y**, as shown here:

```
        ?                                        ?
        m                  y               v   m
  _____
           --------35.5--------   ------32.2------
                                  --------35.5--------
```
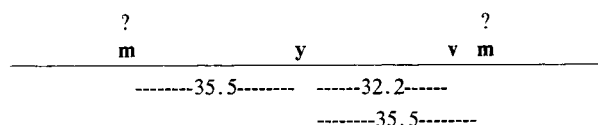
If **v** and **m** are on the same side of **y**, we would expect the amount of crossing over between **v** and **m** to be 3.2 per cent. If they are on opposite sides of **y** the value should be 67.7 per cent. When Sturtevant measured the amount it was found to be 3 per cent, which indicated that **v** and **m** were on the same side of **y**. This close correspondence between the actual and expected result was strong support for his hypothesis.

In this manner, the relative positions of the five genes were determined and a genetic map constructed. The **y** gene was taken as the reference point and the distances measured from it. This was the result.

y ·	w		v	m		r
0.0	1.0		30.7	33.7		57.6

It was found that the most reliable values were obtained when crossover values for adjacent genes were used. This was due to the occurrence of double crossover, which introduced an error into the results. Let us consider the three genes **y**, **w**, and **m** (Fig. 5–5). Sturtevant raised 10,495 flies to test the linkage relations. He found that in 6,972 flies there was no crossing over between the three genes. In 3,454, crossovers occurred between **w** and **m**; in 60, crossovers between **y** and **w** were detected; and in nine a double crossover occurred. That is, there was one crossover between **y** and **w** and another between w and m. This would result in **y** and **m** being on the same chromosome as they were before the two crossover occurred.

| No crossovers 6,972 flies | Crossovers between **w** and **m** 3,454 flies | Crossovers between **y** and **w** 60 flies | Double crossovers 9 flies |
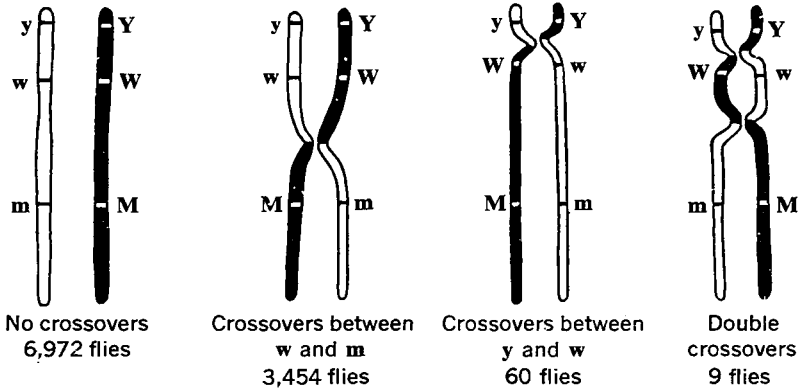
5–5 Sturtevant's Experiment

In the case of the double crossovers it should be noted that three genes are always necessary to detect the event. Thus, if only the genes **y** and **m** were used, any double crossover between them would be undetected since **y** and **m** would still be together after the chromatids had broken and rejoined. When genes are far apart, double crossovers are likely. If they are not detected they will introduce an error in the positions assigned to the genes, for the data would suggest that the genes are closer to one another than they really are. For this reason Sturtevant suggested that chromosome maps be based on crossover values of genes close to one another and not those far apart.

Now the question arises, What is the relation of the chromosome map to the position of these genes on the chromosome? Sturtevant has this to say:

> Of course, there is no knowing whether or not these distances as drawn represent the actual relative spatial distances apart of the factors. Thus, the distance **wv** may in reality be shorter than the distance **yw**, but what we do know is that a break is far more likely to come between **w** and **v** than between **y** and **w**. Hence, either **wv** is a long space, or else it is for some reason a weak one. The point I wish to make here is that we have no means of knowing that the chromosomes are of uniform strength, and if there are strong or weak places, then that will prevent our diagram from representing actual relative distances—but, I think, will not detract from its value as a diagram.

Sturtevant's chromosome map was a graphic way of expressing linkage data. Once constructed, these maps proved useful in predicting the results of untried crosses. The most important induction from the data

is that the genes are arranged in a linear order on the chromosomes, analogous to the sequence of beads on a string. The position occupied by a gene is its *locus*.

THE 'FINAL PROOF' THAT GENES ARE PARTS OF CHROMOSOMES

Beginning in 1884 with Hertwig and others, we have seen that *some* biologists thought the evidence indicated that the hereditary factors were parts of the chromosomes. It is probable that a minority held this view prior to 1910. After 1910 the Drosophila data collected by Morgan and his associates made it increasingly probable that genes are parts of chromosomes, and more and more biologists came to accept this view. Geneticists generally credit the work of Calvin Bridges, another of Morgan's students, published in 1914 and 1916 as being the 'final proof' that the genes are parts of chromosomes. The material that will now be covered should be studied more to learn the type of evidence constituting a 'final proof,' and less for the genetic details.

Bridges' experiments dealt with the inheritance of sex-linked genes in Drosophila. The hypothesis that he sought to prove was that 'sex-linked genes are located on the sex chromosomes.'

Normal Inheritance of Sex Chromosomes. In order to understand Bridges' experiments, it is necessary to have clearly in mind the normal inheritance of sex chromosomes in Drosophila. The **X** chromosome of a male is transmitted only to his daughters and his **Y** only to his sons. The **X** chromosomes of a female are transmitted to both sons and daughters. Looked at from the point of view of the offspring, a daughter receives one **X** from her father and one from her mother. The sons receive an **X** from the mother and a **Y** from the father. The following diagram depicts this. In it, the sex chromosomes of the female are indicated in large letters and those of the male in small letters.

$$♀\,\mathbf{XX} \qquad \times \qquad ♂\,\mathbf{xy}$$

gametes	**X**	**X**	x	y

F₁	**Xx**	**Xx**	**Xy**	**Xy**
	♀	♀	♂	♂
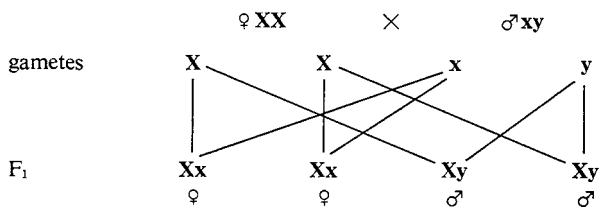
Inheritance in Non-disjunction Females. Bridges noticed that in some strains of Drosophila the inheritance of sex-linked genes was most unusual. Thus, in a cross between a *white*-eyed ♀ and a *red*-eyed ♂, some *white*-eyed daughters and *red*-eyed sons were obtained. These daughters

had inherited their sex-linked genes solely from the mother, and the sons had inherited their sex-linked genes solely from the father. This would be impossible if (a) the sex-linked genes were located on the **X** chromosome, and (b) the sex chromosomes were inherited as shown in the diagram.

Bridges realized that the unexpected breeding results could be explained on the assumption that the female parent giving the unexpected offspring had two **X** chromosomes plus one **Y**. We could designate her **XXY**, in contrast with a normal female, which is **XX**. During meiosis a normal female produces only one class of ova so far as the sex chromosomes are concerned: those with a single **X**. An **XXY** female would produce four types of ova during meiosis. These would be **XY**, **X**, **XX**, and **Y**. There was no way of predicting the frequency of each type of gamete, but we will anticipate the breeding results where Bridges found that the proportions were: 46 per cent **XY**; 46 per cent **X**; 4 per cent **XX**; 4 per cent **Y**.

Females of the **XXY** type were called *non-disjunction* females. The term refers to the fact that in some of the ova produced by these females there is no separation, or disjunction, of the two **X** chromosomes. In a normal female there is regularly a disjunction of the two **X** chromosomes with the result that a single **X** is present in each ovum.

The cross of a *white*-eyed non-disjunction female to a normal *red*-eyed male according to Bridges' hypothesis would be as shown in Figure 5–6.

It must have taken considerable courage to postulate such a seemingly preposterous hypothesis, although some such hypothesis was necessary to explain the results, if one were to continue to hold the belief that genes are located on chromosomes. The hypothesis could be verified, however, since deductions could be made and tested by observation and experiment. These were the main deductions:

1. If the hypothesis is true, we would expect 50 per cent of the daughters (classes 1 and 7 of Fig. 5–6) to be non-disjunction females. Breeding experiments showed this to be the case.

2. If the hypothesis is true, we would expect the exceptional ♂ (class 4) not to transmit the power of producing exceptions in later generations. It should behave like a normal male. Breeding experiments showed this to be true.

3. If the hypothesis is true, we would expect 46 per cent of the males to be **XYY**. These would produce sperm of four genotypes, namely, **X, YY, XY,** and **Y**. If a male of this type were crossed to a normal female, there should be no exceptional offspring (i.e. males inheriting

P   Non-disjunctional white eye ♀   Normal red eye ♂
                    𝕏𝕏Y   ×   **XY**

Gametes   𝕏Y (46%); 𝕏 (46%)   **X** (50%)
            𝕏𝕏 (4%) ; Y (4%)   **Y** (50%)

F₁   𝕏Y (46%)   𝕏 (46%)   𝕏𝕏 (4%)   Y (4%)

	1 **𝕏𝐗Y** 23%	2 **𝕏𝐗** 23%	3 **𝕏𝕏𝐗** 2%	4 **XY** 2%
X 50%	Red eye ♀ Would show non-disjunctional behavior if crossed.	Red eye ♀ Normal chromosome behavior.	Triploid X. ♀ Usually dies.	Red eye ♂ The X has come from the father and the Y from the mother. This is the reverse of the normal situation.
	5 **𝕏Y𝐘** 23%	6 **𝕏Y** 23%	7 **𝕏𝕏𝐘** 2%	8 **YY** 2%
Y 50%	White eye ♂ With extra Y chromosome.	White eye ♂ With normal chromosome behavior.	White eye ♀ Would show non-disjunctional behavior if crossed.	Dies

5–6 Bridges' Experiment

their sex-linked characteristics only from the father, and females inherit-
ing theirs only from the mother). However, every **XY** sperm that entered
a normal **X**-containing egg would produce an **XXY** daughter, which
should be a non-disjunctional female. Breeding experiments confirmed
all these predictions.

4. If the hypothesis is true, we would expect that 50 per cent of the daugh-
   ters (classes 1 and 7) would have two **X** chromosomes and one **Y**. It
   should be possible to verify this deduction by cytological examination
   of the F1 females. Bridges did this and found that approximately half of
   the daughters that he examined had two **X** chromosomes plus one **Y**
   (Fig. 5–7). The other half had two **X** chromosomes only. This was the
   crucial test of the hypothesis, since the test was of a very different sort,
   namely, cytological. (We can ignore the rare **XXX** females.)

Bridges' conclusion was as follows: '…there can be no doubt that the com-
plete parallelism between the unique behavior of the chromosomes and the
behavior of the sex-linked genes and sex in this case

means that the sex-linked genes are located in and borne by the X-chromosomes.' It seemed equally probable that the autosomal genes were likewise parts of chromosomes, though this was not proved by his experiments. A further extension was made to other species, and the hypothesis advanced that the genes of all organisms are parts of chromosomes. This extrapolation from the data was done because it appeared that inheritance was the same in all organisms being studied.

It might be of interest to inquire about the nature of this 'final proof,' in 1914, that genes are located on chromosomes. It must be apparent that it is the same type of evidence that had been offered ever since 1902. Sutton pointed out the parallel behavior of chromosomes in meiosis and fertilization with the behavior of Mendelian factors. This evidence probably convinced a few that Mendelian factors were on the chromosomes. Morgan's analysis of the *white* eye case in Drosophila showed that inheritance of the gene was an exact parallel to the inheritance of the **X** chromosome. This study convinced more biologists. The discovery that the number of linkage groups is the same as the number of chromosome pairs was further support for the theory. These and many other experiments showed that either the genes were parts of chromosomes or the genes were parts of structures that behaved precisely like the chromosomes.

Bridges' evidence was of the same type, though it differed in degree. The inheritance of eye color in his non-disjunction experiments was completely different from any other type of inheritance. If he assumed genes were carried on chromosomes, then he had to postulate some most unusual chromosome phenomena. Cytological studies verified the predictions made from the genetic data. There could no longer be a
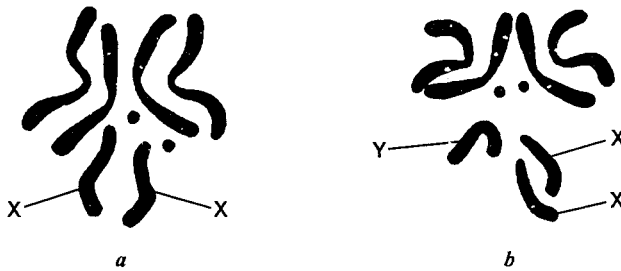
5–7 Bridges' drawings of the chromosomes of the females in his non-disjunction experiment. Approximately half of the females (class 2) had the normal chromosome complement as shown in *a*. The remaining females (classes 1 and 7) have two **X** chromosomes and a **Y** as shown in *b* (C. Bridges, 'Non-disjunction as proof of the chromosome theory of heredity,' *Genetics* 1:1–51; 107–63. 1916).

'reasonable doubt' that the genes were on chromosomes. More elaborate evidence was still to come, but for many biologists this evidence of Bridges was sufficient.

There are few cases of inheritance that seemed unrelated to chromosomes. There were grouped under the term *cytoplasmic inheritance,* since it seemed that some non-nuclear factor was responsible. Over the course of the years, many cases first thought to be due to cytoplasmic inheritance were found to be misinterpretations of the data. A few instances of cytoplasmic inheritance are well established. For the other thousands of analyzed cases, there is no doubt that the genes are parts of chromosomes. The chromosomes form the physical basis for 99.9+ per cent of inheritance.

THE CHROMOSOME BALANCE THEORY OF SEX DETERMINATION

The problem of sex determination as it was understood in the first decade of the twentieth century was discussed in Chapter 4 where it was shown that there is a constant relation between the sex of an organism and its chromosomes. The cells of *Drosophila melanogaster* females contained three pairs of autosomes and two **X** chromosomes. In the cells of males of this species there were three pairs of autosomes plus one **X** and one **Y** chromosome.

Genetic work with *Drosophila melanogaster* revealed that the **Y** chromosome contained very few genes, although it is essential for fertility in males.

The **Y** came to be looked upon as a nearly inert chromosome genetically in all organisms; a view strengthened by the discovery that in many animal species the males have a single **X** and no other sex chromosome. These data led to the concept that in species with a **XX** ♀ -**XY** ♂ sex chromosome constitution, the presence of a single **X** determined that the individual be a male and a pair of **X** chromosomes determined that the individual be a female. (Exceptions to this rule were eventually discovered—man being one of them.)

This hypothesis was strengthened further by some remarkable observations on gynandromorphs in Drosophila. Gynandromorphs are individuals in which part of the body has the morphological features of a male and the other part has the morphological features of a female. Morgan and Bridges discovered some gynandromorphs that were female on one side and male on the other. Analysis showed that these individuals began development as females. Due to some developmental accident in the early embryo, one **X** chromosome was lost from the cells that were to form one-half of the body. As a result the cells of one side

of the body remained normal and contained the three pairs of autosomes and two **X** chromosomes. This side had the external structure typical of females and internally an ovary might be present. The cells of the other side of the body where one **X** was lost contained three pairs of autosomes and a single **X** chromosome. This side had the external structure typical for males and internally a testis might be present.

These observations made more probable the hypothesis that a fly was a-male or female depending on the number of **X** chromosomes in its cells. This concept seemed to be an adequate explanation of the data and geneticists were willing to accept it until further tests were possible.

The work of Bridges on non-disjunction showed that a remarkable amount of chromosome juggling was possible in Drosophila. As a consequence it became feasible to further test the hypothesis that one dose of **X** resulted in a male and two doses of **X** resulted in a female. This concept was found to be inadequate to explain all of the data. Some Drosophila were obtained that were triploid, that is, there were three **X** chromosomes and three members of each autosome type in every cell. These individuals were females.

Bridges obtained flies with various combinations of autosomes and sex chromosomes (Fig. 5–8) and proposed the hypothesis that sex is due to a balance between the number of **X** chromosomes and the number of autosomes. In a normal ♀ there are two **X** chromosomes and
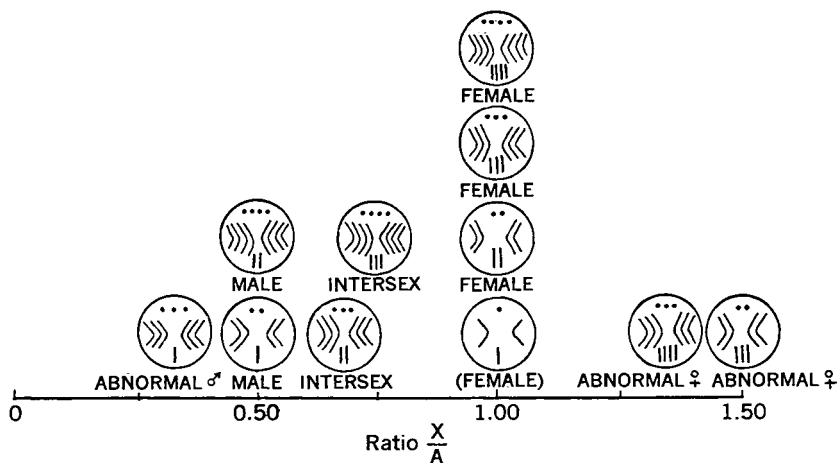
5–8 The various combinations of **X** chromosomes and autosomes obtained by Bridges and others. (The lowest circle at ratio 1.00 is a haploid female. No such fly has been obtained but some diploid flies have been observed that have haploid areas in their bodies. If these areas include sex structures, they are of the female type.)

two haploid sets of autosomes (a haploid set of autosomes consists of one of each of the three different kinds of autosomes). We could express this as $2X/2A=1.0=♀$. A normal male would be $1X/2A=0.5=♂$. The triploid female would be $3X/3A=1.0=♀$.

When a triploid female is crossed with a diploid male, some of the off-spring have two **X** chromosomes and three members of each autosome. We could write these as $2X/3A=0.67$. Now this ratio is intermediate between the value for normal males (0.5) and that for normal females (1.0) and it was observed that these individuals were intermediate in appearance between males and females. They are described by Bridges as follows:

> The 'intersexes,' which were easily distinguished from males and from females, were large-bodied, coarse-bristled flies with large roughish eyes and scolloped wing-margins. Sex-combs (a male character) were present on the tarsi of the fore-legs. The abdomen was intermediate between male and female in most characteristics. The external genitalia were preponderantly female. The gonads were typically rudimentary ovaries; and spermathecae were present. Not infrequently one gonad was an ovary and the other a testis; or the same gonad might be mainly ovary with a testis budding from its side.

The intersexes were sterile.

It was possible to obtain individuals with ratios of **X** chromosomes to autosomes that were below the normal male value or above the normal female value. Some individuals had three **X** chromosomes and two sets of autosomes. The ratio for these would be $3X/2A=1.5$. This ratio is higher than that of a normal female, and the resulting imbalance in **X** chromosomes and autosomes produces a sterile and somewhat abnormal female. Bridges was able to obtain a value below the 0.5 ratio of normal males in individuals with a single **X** and three autosome sets. These would be $X/3A=0.33$. Such flies were structurally abnormal and sterile males.

These and many other combinations of chromosomes were obtained by Bridges and others. The results formed a consistent pattern, there being a relation between the ratio of **X** chromosomes to autosome sets and the sex characteristics of the flies.

RATIO X/A	MORPHOLOGICAL TYPE
0.33	abnormal male
0.50	male
0.67	intersex
0.75	intersex
1.00	female
1.33	abnormal female
1.50	abnormal female

The significance of these ratios is to be found in the differential effectiveness of genes on the autosomes and on the **X** chromosomes. The net effect of the autosomal genes is a male-forming tendency. The net effect of the **X** chromosome genes is a female-forming tendency. In a normal male the genes of the two autosome sets overbalance the genes of the single **X** to produce the male. In the normal female the **X** chromosome genes are in a double dose and this is sufficient to produce the female body type.

MULTIPLE ALLELES AND HUMAN BLOOD TYPE GENES

In all the cases considered so far, a gene has existed in only two states. It might be involved in the production of *red* or *white* eyes, *long* or *vestigial* wings, *round* or *wrinkled* peas. The *white* eye allele appeared in a stock of *red*-eyed flies. Since the stock had been under observation for many generations, it is reasonable to assume that in one **X** chromosome the gene, which in the normal condition is involved in the production of *red* eyes, changed in such a way as to produce *white* eyes. One question this suggests is: If the original *red* eye gene can change to *white,* might it not change in another way to produce a still different result? Continued observations answered this question in the affirmative. A mutant known as *eosin* was discovered. Its phenotypic expression was a diluted red eye color. In its linkage relations and crossover behavior, it was found to occupy the same place on the **X** chromosome as the gene for *white*. In crosses involving the *red, white,* and *eosin* alleles, it was never possible to have more than two of the three in the same female. All of the data were consistent with the hypothesis that *red, white,* and *eosin* were different states of the same gene. Phenomena of this sort are known as *multiple alleles*. At the present several dozen alleles have been discovered at the *white* locus.

The A, B, O Blood Type Alleles. A well-known case of multiple alleles in man is the *ABO* blood type series. The blood types, *A, B, AB,* and *O* are determined by the interaction of three autosomal alleles, **A, B,** and **o**. (Other designations frequently used are I^A, I^B, and I^o; furthermore, additional alleles are now known for this locus.) **A** and **B** are dominant over **o**. When **A** and **B** are present in the same individual neither gene is dominant and the individual is type *AB*. The phenotypes and possible genotypes are as follows:

PHENOTYPE	GENOTYPE
Type *A*	**AA** or **Ao**
Type *B*	**BB** or **Bo**
Type *AB*	**AB**
Type *O*	**oo**

The inheritance of these alleles follows the usual Mendelian scheme.

A cross of a heterozygous type *A* with a heterozygous type *B* would be as follows:

P   phenotype   *type A*   *type B*
     genotype   Ao   Bo
     gametes   A   o   B   o

F$_1$ phenotype   *type AB*   *type A*   *type B*   *type O*
     genotype   AB   Ao   Bo   oo
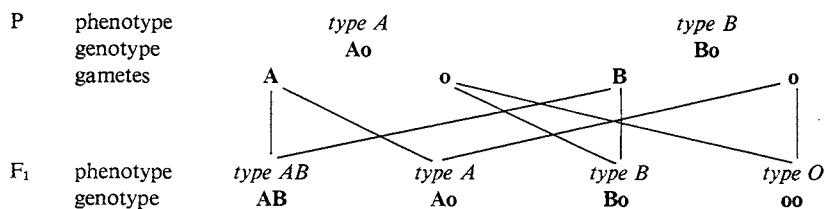
Digression on the Importance of Blood Types in Transfusions. Some years before the genetic basis of blood groups had been worked out, Karl Landsteiner found that human blood could be classified into the four types just described. The types proved to be of great importance in connection with blood transfusions. In some cases death resulted when the donor and recipient were of different types. Experimentation and observation revealed that this incompatibility was due to the interaction of *antigens* on the surface of the red blood corpuscles with *antibodies* in the plasma. There are two types of antigens, **A** and **B,** and two types of antibodies, α and β. The distribution of these substances is as follows:

BLOOD TYPE	ANTIGEN IN CORPUS-CLE	ANTIBODY IN PLASMA
A	A	β
B	B	α
AB	A and B	none
O	none	α and β

The corpuscles are agglutinated (clumped) if those containing **A** antigen come in contact with α antibody, or if those containing **B** antigen come in contact with β antibody. The important factor is the type of corpuscle introduced in a transfusion; the introduced plasma has little or no effect on the recipient's corpuscles. Any interaction, therefore, will be between the donor's antigens on the corpuscles and the recipient's antibodies in the plasma, resulting in clumping of the introduced corpuscles. The possible combinations are as follows:

RECIPIENT'S BLOOD TYPE
(Antibodies in parentheses)

	AB (none)	*A* (β)	*B* (α)	*O* (αβ)
AB (AB)	O	+	+	+
A (A)	O	O	+	+
B (B)	O	+	O	+
O (none)	O	O	O	O

Donor's blood type. (Antigens in parentheses)

A 'o' in the table signifies no reaction while a '+' indicates agglutination of corpuscles. It can be seen from the chart that the blood of an *O* type person can be used in any transfusion. For this reason type *O* is spoken of as a *universal donor*. A type *AB* individual can receive blood of any of the four types. For this reason type *AB* is spoken of as a *universal recipient*.

These interrelations are no longer quite so simple as described; it is now realized that more alleles are involved than the original three.

INDUCED MUTATIONS

The origin of mutants had been a mystery since the early days of genetics. In the initial work of Morgan and his associates, stocks of wild-type Drosophila might be kept for generations, and thousands of individuals examined, before a new mutant was discovered. The occurrence of mutations was a spontaneous event that could neither be predicted nor controlled.

Attempts to induce hereditary changes in the chromosomes were made from the very beginnings of Drosophila genetics. Various agents were tried, such as exposure of the flies to radium, X rays, and many different chemical agents. In one of Morgan's first papers he reported the appearance of several new mutants in the offspring of flies that had been exposed to radium. Several other investigators reported similar results.

Difficulties in Studying Induced Mutation. None of these early experiments was conclusive because of the difficulty of distinguishing induced from spontaneous mutations. This was the problem. Mutants were appearing in stocks not exposed to unusual radiations or to special chemical treatment. Their appearance could not be correlated with any known 'cause,' so they were termed 'spontaneous.' Spontaneous mutations were of very rare occurrence. In the experiments attempting to produce mutations by physical or chemical means, mutations occurred but they also were very rare. Thus, if we expose flies to radium in an effort to produce mutations, and if a mutant form appears among the offspring or later descendants of the irradiated flies, we could not be sure whether radium was the cause or whether it 'just happened.'

Since new mutant genes appear infrequently and most of them are recessive, the mere detecting of them becomes a problem. Assume, for example, that one autosomal gene in a sperm nucleus mutates. If this sperm then enters an egg the new individual will have one mutated allele from the father and one unmutated allele at the same locus from the mother. The mutant will, consequently, be masked by its dominant allele and the observer will see no evidence that a mutation has occurred.

Appropriate crosses could be made to produce an individual homozygous for the new mutation if there was some way of knowing which individuals to cross. Since there is no way of knowing this, the alternative would be to make innumerable crosses in the hope of having at least one fly heterozygous for the new mutant allele.

An appreciation of this problem will be gained if you determine the number of crosses that would have to be made to secure homozygous flies starting with a single adult heterozygous for a new mutant gene. If you then wished to measure the mutation rate per one million flies, what would the total number of necessary crosses be?

Muller's ClB Method. H.J.Muller (1927) was the first person to give a practical solution to the problem. He was able to do so because he devised an ingenious experiment that gave an easy and accurate measure of mutation rate. His experiments were designed to test the effects of X rays on the induction of mutations. As a control, it was necessary for him to know the spontaneous mutation rate as well.

After considerable experimental manipulation, Muller developed the **ClB** strain of Drosophila. A **ClB** ♀ contains the **C** inversion on one of her **X** chromosomes, a recessive lethal gene **l** and a dominant mutant *bar-eye* **B**, both of the genes being within the inverted section of the chromosome.

An *inversion* is a region of the chromosome that has been reversed. If the normal order of genes is **a b c d e f g,** a chromosome with genes in the order **a b e d c f g** would contain an inversion. An inversion is caused by a double break of the chromosome, in this case between **b** and **c** and between **e** and **f**. Following this there is a rotation of the central section, **c d e,** through 180° and a subsequent fusion with the two ends of the chromosome. Inversions were discovered by Morgan's group, and it was found that they have the important effect of reducing or even preventing crossing over between genes in the inverted section of one chromosome and in the corresponding normal sequence of its homologue. In the **C** inversion, crossing over is entirely prevented. This means that the **C** inversion with the **l** and **B** genes will be inherited as a unit. The **B** gene has the sole purpose, in this experiment, of serving as a ready means of recognizing a fly heterozygous for the **ClB** chromosome, since every fly that has *bar* eyes must have one **ClB** chromosome. It could not have two **ClB** chromosomes since the **l** gene, if homozygous, would result in the death of the fly.

If a female heterozygous for a **ClB** chromosome is crossed with a normal male the results are as follows:
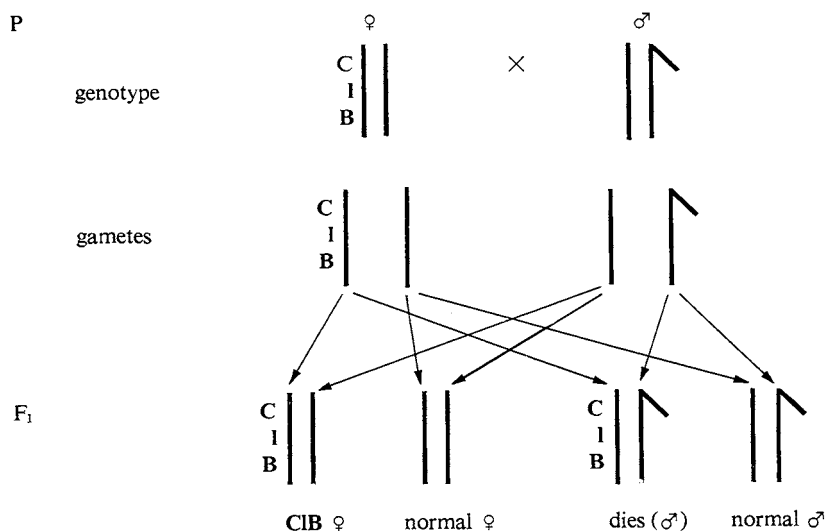
Those males that inherit the **ClB** chromosome from their mothers will have the lethal gene, **l**. Since there is no normal gene on the **Y** to counteract the effects of this lethal, these males will die. Therefore, the sex ratio will be 2 ♀:1 ♂.

It was well known at the time Muller performed his experiment that many separate gene loci can mutate in such a way as to lead to death. These lethal genes were usually recessive. Since many genes can do this, the chance of getting some lethal mutation is greater than the chance of observing mutations at a specific locus. Thus, if we studied the rate of mutations to the lethal condition on the **X** chromosome we would be measuring the sum of the rates for *all* of its genes that can form lethals by mutation. The rate for a specific locus would be much smaller.

With **ClB** flies it is possible to measure the mutation rate for lethal genes on the **X** of sperm. Once again it must be emphasized that this will not be a measure of rate for one locus, but of all the loci on the **X** that can mutate to a lethal condition. Should a lethal mutation occur on the **X** in one of the sperm, it could be detected by the cross shown at the top of page 124. The * will represent the new lethal.

If the F_1 **ClB** ♀ is then mated with a normal ♂, the marked chromosome that we are searching for will pass to the ♂ offspring and be revealed as shown in the cross at the bottom of page 124. One class of the daughters will be normal in appearance and heterozygous for the new lethal mutant gene. Another class will be **ClB** females. One of the males will carry the new lethal mutant gene and die as a result. Another class of males will inherit the **ClB** chromosome and die because of the lethal

P   genotype

♀   ×   ♂

C
l
B

gametes

C
l
B

mutation
in sperm
→ *

F₁

C
l
B *

♀   ♀

C
l
B

ClB ♀   dies (♂)   normal ♂
heterozygous heterozygous
for new   for new
lethal*   lethal*
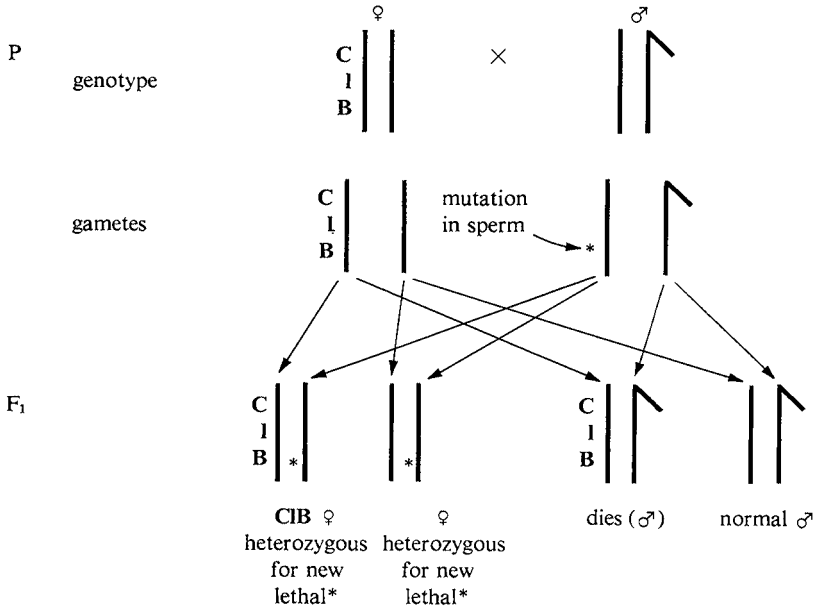
gene in the **C** inversion. Therefore, only females will appear. *Thus, if a lethal gene was present on the* **X** *of the original sperm, there will be no sons in the* F_2. This fact can be ascertained by a quick examination of the F_2 flies. This point is of considerable significance, since it makes it feasible to check many crosses in a short period of time.

genotype

♀   ×   ♂

C
l
B *

gametes

C
l
B

*

F₂

C
l
B

ClB ♀   normal ♀

C
l
B

*

dies (♂)   dies (♂)
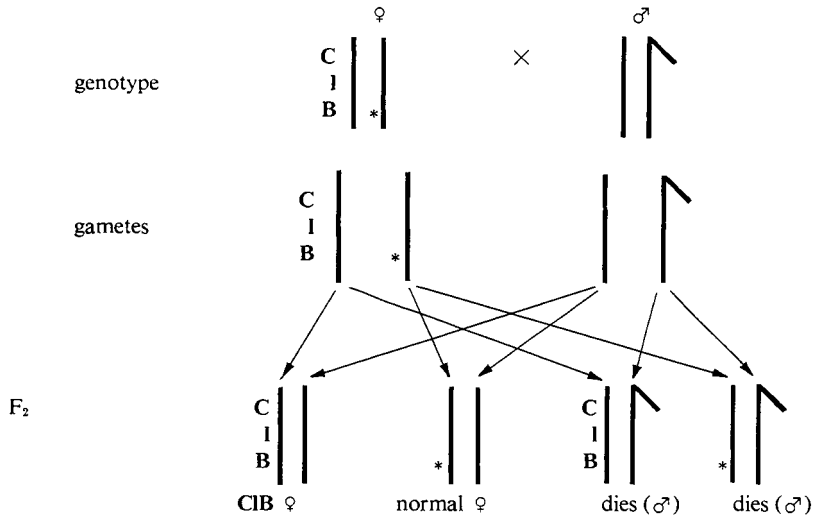
When Muller used normal untreated males in crosses of this type, he found that approximately one cross in a thousand gave solely females in the F_2. This means that the chance of a lethal mutation occurring at some locus on the **X** is 1 in 1,000 or 0.1 per cent. This is the natural, or spontaneous, mutation rate. If the males are first exposed to about 4,000 r-units of **X** rays the results are strikingly different: approximately 100 crosses in every 1,000, or 10 per cent, have only females in the F_2. This amount of radiation, therefore, increased the mutation rate 100 times.

Muller's results were not only of great theoretical importance in showing that mutations could be experimentally produced, but they gave geneticists a practical means of securing new mutants for their work. Later it was found that radiations would induce not only gene mutations but also cause inversions, translocations (the attachment of a piece of one chromosome to another), or deficiencies (elimination of a section of a chromosome).

SALIVARY GLAND CHROMOSOMES

The concepts of genetics were developed mainly on data derived from breeding experiments. That is, the localization and behavior of genes were studied without the gene ever being seen. With standard cytological techniques, the chromosomes appear as uniformly-staining structures with no differentiations recognizable as genes. By 1930 geneticists felt that genes were probably some type of protein. If this were so, it would be impossible to see them even under the most powerful compound microscopes available since protein molecules are too small to be observed with these instruments. Geneticists became resigned to investigating their invisible genes just as the chemist studies his invisible molecules and the physicist his invisible sub-atomic particles.

The Discovery of Salivary Gland Chromosomes. It was against this background that Theophilus S.Painter (1889–1969), in 1933, and somewhat later Calvin Bridges, made discoveries of the first importance concerning the finer structure of chromosomes. It was found that the chromosomes in the salivary glands of larvae of Drosophila were of enormous size, being about 100 times longer than those of ordinary body cells. Of even greater interest and importance was the presence of cross bands on the chromosomes. Figure 5–9, from Painter's first paper on the subject, shows the appearance of the salivary chromosomes.

The salivary glands are diploid, but instead of the expected eight chromosomes, Painter found only four. This is due to the fact that homologous chromosomes have fused together. They are so close to one
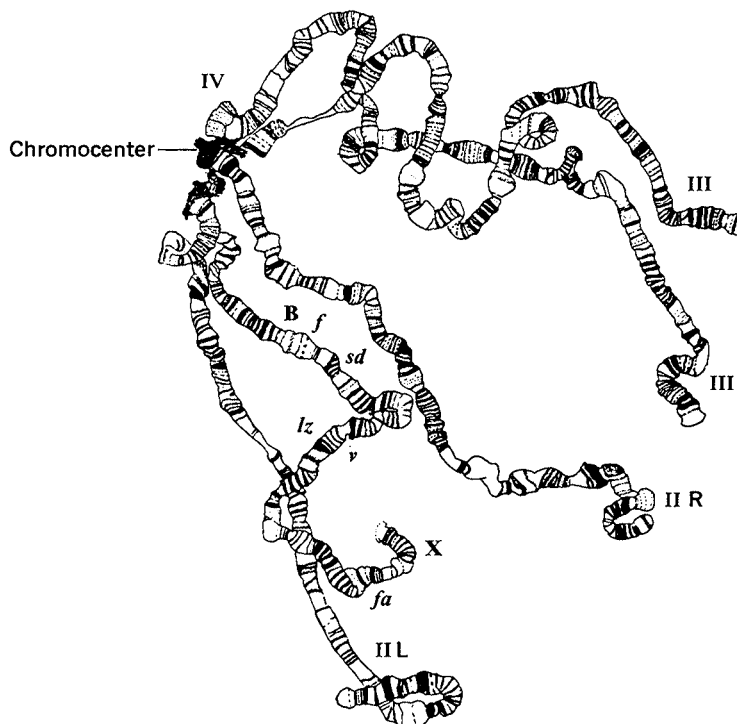
5–9 Painter's first drawing of the salivary gland chromosomes of *Drosophila melanogaster.* The chromosomes radiate out from the chromocenter. The **X** is attached to the chromocenter by one end so it appears as a single long structure. Both the II and III chromosomes are attached by their middle portions. Consequently both of these chromosomes have two arms extending from the chromocenter. The tiny IV chromosome is attached by its end to the chromocenter. The approximate location of several **X** chromosome genes (*B, f, sd,* etc.) is shown. (T. S.Painter, 'A New Method for the Study of Chromosome Aberrations and the Plotting of Chromosome Maps in *Drosophila melanogaster,' Genetics* 19:175–88. 1934.)

another that the line of separation cannot be seen in the illustration. The line of separation can be seen in some microscopic preparations, however. The pairing of the two chromosomes is so exact that the cross bands extend across both chromosomes as though they were a single structure.

In different sections of the chromosomes the bands were found to vary in size, distinctness, shape, and distance from adjacent bands. These regional differences were found to be constant for the chromo-

somes in different cells of the same larva and in different larvae. For the first time it became possible to recognize regional differences in chromosomes. The concepts of chromosome structure previously based solely on the genetic data could be confirmed.

Was there a close relation between the genetic map of a chromosome, which was nothing more than a way of describing linkage groups and crossover percentages, and the real chromosome? There were many other special situations that needed to be checked. For example, geneticists sometimes resorted to seemingly preposterous hypotheses to explain the outcome of their crosses: genes jumping from one sort of chromosome to another (a translocation); the loss of an entire gene (a deletion); a chromosome might seem to have two loci for the same gene (a duplication); and still other results were explained on the basis of a reversed order of genes (an inversion).

Critics of the Morgan school were delighted to point out that one can manufacture hypotheses to explain anything. What is needed is a means of testing hypotheses. If one postulated a genetic inversion, it was up to him to show that a section of the chromosome was, indeed, reversed. But that was impossible so long as one could not distinguish the sections of chromosomes: a chromosome with an inversion would look exactly like one without the inversion. The discovery of salivary chromosomes made it possible to test these hypotheses.

Inversions and Translocations. In the early days of Drosophila genetics, Morgan's group encountered some instances where the amount of crossing over was less than expected. For example, on the basis of previous work it might have been observed that the amount of crossing over between genes **A** and **B** was 10 per cent. Such data would have been used to fix the position of these genes on the chromosome map. But what should one conclude if, in still other experiments, the amount of crossing over between **A** and **B** was zero? This would suggest that the hypothesis that the positions of genes was constant and could be determined from the amounts of crossing over is invalid.

A new hypothesis was developed to account for the exceptional results. If crossing over was reduced or prevented, possibly this was a consequence of something interfering with synapsis. If the homologous chromosomes could not synapse, there would not be an opportunity for crossing over to occur. It had always been assumed that synapsis was possible only because the homologous chromosomes were identical in the order of their genes. Could it be that in the flies giving the exceptional results the order of the genes was different? If a section of a chromosome became inverted, then normal synapsis and crossing-over would
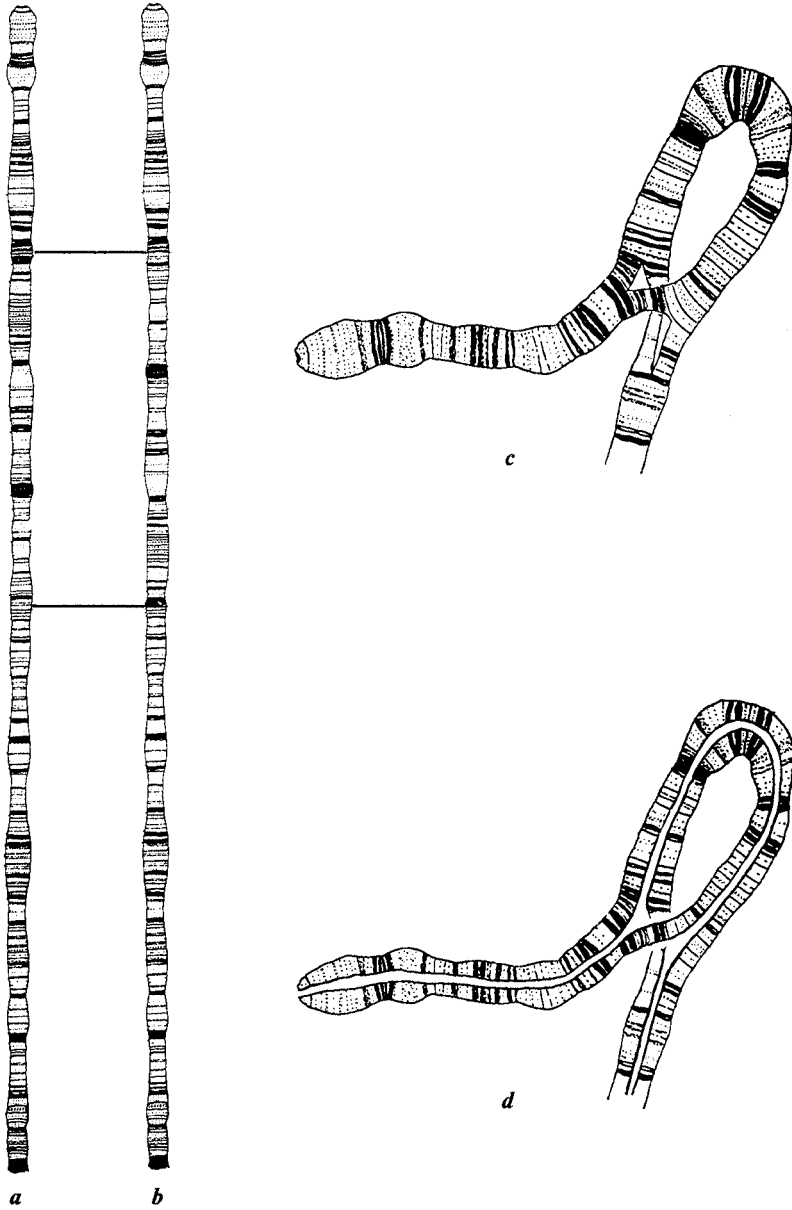
5–10 Inversions in *Drosophila pseudoobscura*. In *a* the standard band sequence in the third chromosome is shown. The chromosome shown in *b* is identical to the one in *a* except for the region between the two lines, which has the band

be prevented. This could happen if a chromosome broke in two places, to give three pieces, and the middle piece rotated 180° and then rejoined the two end sections of the chromosome. It was quite an intellectual achievement to make this hypothesis, which at first must have seemed most improbable. Direct evidence for this hypothesis could not be obtained so long as it was impossible to detect regional differences in chromosomes. When salivary chromosomes were discovered, a method of checking became possible.

Figure 5–10 gives an example. In *Drosophila pseudoobscura* the *'standard'* arrangement of bands is shown in *a*. In this species many inversions have been discovered. One of these is known as *'arrowhead.'* A chromosome with the *arrowhead* inversion is shown in *b* and it can be seen that the sequence of bands is reversed. Cytology confirmed the genetic hypothesis of chromosomal inversions.

An interesting situation arises when an individual has one *standard* and one *arrowhead* chromosome. During synapsis these two chromosomes will pair, with corresponding bands being adjacent as shown in *c*. Since a section of one chromosome has a reversed order of regions, considerable gyrations are necessary for matching of the corresponding regions to be achieved. The way it is accomplished is shown in Fig. 5–10*d*.

The occurrence of translocations, which had been predicted on the basis of genetic results, was also verified by an examination of the salivary chromosomes. In those individuals suspected of having translocations, it was found that a piece of chromosome, with its characteristic set of bands, was no longer in its customary place, but instead it was joined to another chromosome.

How did Painter decide which salivary chromosome corresponded to a particular linkage group? It was done largely by means of inversions and translocations. Painter had studied the salivary gland chromosomes of normal flies so carefully that he was able to recognize the various regions. Once he could do this, he studied flies that were known on genetic grounds to have an inversion in one chromosome—for example, the **X**. Invariably, he found that in one of their salivary

---

sequence reversed. This is a cytological demonstration of an inversion. If an individual fly has one of each of these chromosome types, the salivary gland picture will be as in *c*. Pairing is achieved by one chromosome forming an inverted U and the other a loop. As a result of these contortions, it is possible for the corresponding bands of the two chromosomes to be situated opposite to one another. In *d* the two chromosomes are separated slightly to show more clearly the manner of pairing. (Modified from Dobzhansky and Sturtevant, 'Inversions in the chromosomes of *Drosophila pseudoobscura,*' *Genetics* 23:28–64. 1938.)

chromosomes there was a region of reversed bands. This chromosome was, therefore, the **X**. He had numerous inversions and translocations for study, so it was possible to identify each salivary chromosome with the corresponding linkage group.

The Gene Locus. So much for the gross problems: Now we can ask, Where are the genes? Chromosomal structural aberrations were the basis for solving this problem, small deficiencies being especially useful. The following example, from the work of Milislav Demerec (1895–1966) and M.E. Hoover, will show how it was done (Fig. 5–11). They studied Drosophila with deficiencies near one end of the **X** chromosome. Most deficiencies, except those that are very small, are lethal when homozygous. In the heterozygous condition they do not cause death, but
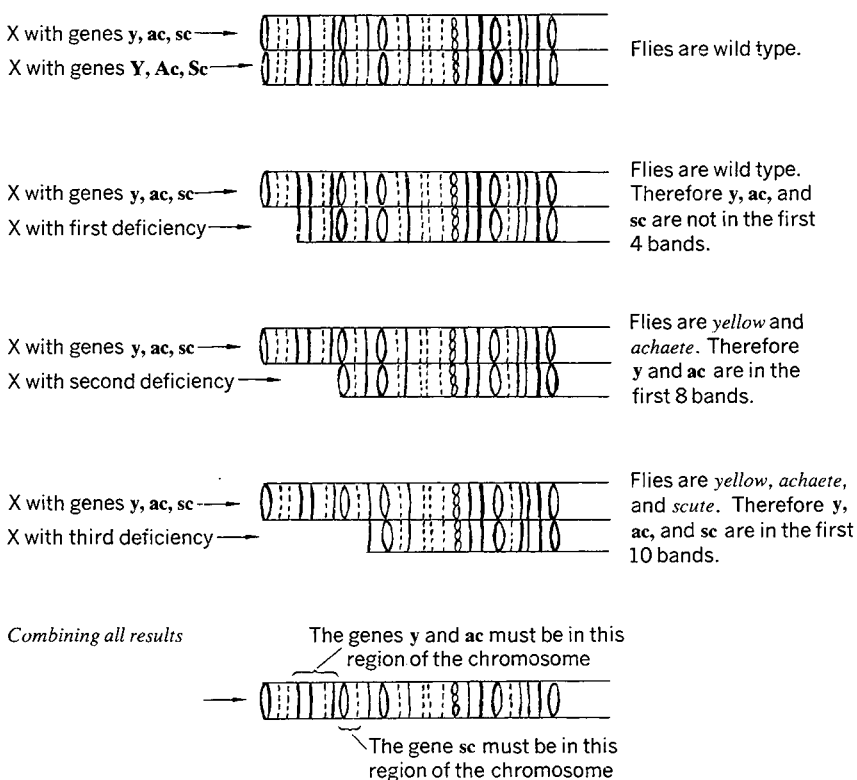
5–11 Diagrammatic representation of the experiment of Demerec and Hoover showing how the positions of genes on the salivary gland chromosomes can be determined.

they do have a characteristic genetic effect, which can be brought out by the following consideration.

Let us assume that a fly is heterozygous for a deficiency including the locus of the gene **A**. That is, there will be no **A** on the chromosome with the deficiency, but in the normal chromosome the locus will be present. The result is that the genes at the **A** locus on the normal chromosome will be the one determining the character of the individual. If the dominant **A** allele is present, it will have its usual effect. If the recessive a allele is present it will produce its effect, since there is no gene on the chromosome with the deficiency to influence its action. These results should not be too surprising. All the **X** chromosome characters of the Drosophila male that we have studied behave in a similar way. The **Y** lacks nearly all loci normally present on the **X** and, therefore, acts as one giant deficiency.

Demerec and Hoover used three stocks, each with a different small deficiency at one end of the **X** chromosome. The locations and extents of the deficiencies were determined by study of the salivary chromosomes. Genetic crosses had previously established that the genes **y** *(yellow),* **ac** *(achaete),* and **sc** *(scute)* were close to the end of the **X** chromosome. Demerec and Hoover made their crosses in such a way that a fly would receive one normal **X** chromosome carrying the recessive genes **y, ac,** and **sc** and another **X** carrying a deficiency but no mutant genes. If the deficiency included the locus of any of these genes, then the fly would exhibit the recessive character since there would be no normal allele to counteract its action.

The first deficiency tried was a small one. It removed the first 4 bands of the **X** chromosome (Fig. 5–11). When this deficient chromosome was present with the **X** carrying **y, ac,** and **sc,** the flies were normal. This means that none of these genes is in the part of the **X** having the first 4 bands.

The second deficiency removed the 8 terminal bands. When this chromosome was present with an **X** carrying **y, ac,** and **sc,** the flies were *yellow* and *achaete.* This experiment showed that **y** and **ac** were in that part of the chromosome having bands 1 through 8. The previous cross showed they were not in the region covered by bands 1 through 4. Therefore, the genes **y** and **ac** must be in the region marked by bands 5 through 8. This cross also showed that the locus for **sc** is not in the area covered by the first 8 bands.

The third deficiency removed the 10 terminal bands. When one of these chromosomes was present with an **X** carrying **y, ac,** and **sc,** the flies were *yellow, achaete,* and *scute.* This indicated that **y, ac,** and **sc** were in the area covered by the first 10 bands. In the previous experi-

ment we saw that **sc** was not in the first 8 bands. The present cross shows it is somewhere in the first 10. Combining these results, we can conclude that the **sc** gene is in that region of the chromosome containing bands 9 and 10.

In this manner it was possible to give an approximate location for many genes. In a few cases genes were localized to a portion of the chromosome having but a single band. No genes were found in areas without bands. *These observations led to the tentative hypothesis that the bands are gene loci.*

If the bands are gene loci, it should be possible to determine the number of genes in Drosophila by counting the number of bands. This attempt was made but one difficulty made an exact determination impossible. Not all of the bands are equally distinct: they vary from those that stain distinctly to those so indistinct as to be at the limit of visibility. Approximately 5,000 bands could be seen and this figure was taken as the tentative minimum number of genes in Drosophila.

Once genes were located on salivary chromosomes, a comparison could be made with the genetic maps. One example is given in Figure 5–12, which shows corresponding parts of a small section of the salivary second chromosome and the genetic map. The point of greatest importance is the close resemblance of the two. The genetic map is
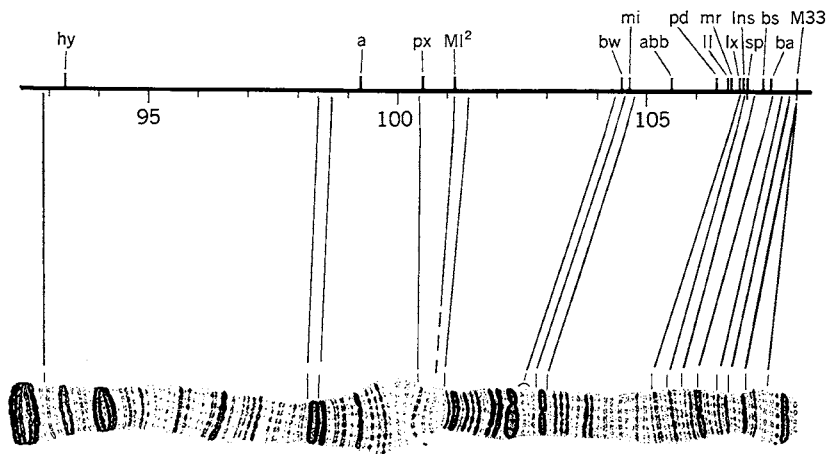
5–12 Corresponding points in the salivary chromosome and linkage map for the tip of the second chromosome of *Drosophila melanogaster*. (Modified from C. B.Bridges, 'Correspondence between linkage maps and salivary chromosome structure, as illustrated in the tip of chromosome 2R of *Drosophila melanogaster*,' *Cytologia*. Fujii Jubilee Volume, pages 745–55, 1937.)

based on breeding experiments and the arrangement of loci is based on the percentages of crossing over. These data suggest that the genes are in a linear order and in a certain sequence. When it became possible, with the salivary gland techniques, to determine the actual position of genes on the chromosomes, it was found that the order and sequence predicted by genetic means was verified by cytology. Once again, these mutually supporting fields had established a hypothesis as 'true' beyond a reasonable doubt.

Salivary gland chromosomes are of great importance in many genetic problems being studied today. One of their more spectacular applications has been in the field of evolution.

As an interesting historical footnote we might add this bit of information: The banding of salivary chromosomes in flies had been observed and recorded by cytologists in 1881, but this was not known to geneticists. If the Morgan group had been aware of this, their efforts would have been made much easier. Their prediction of chromosomal aberrations such as inversions, translocations, and deficiencies, largely on genetic data, was a tremendous intellectual achievement. All this time a simple method for demonstrating these cytological phenomena was buried and forgotten in the archives of biological literature.

Cases such as this, which are not infrequent, make all scientists wonder what important facts have been discovered, forgotten, and now await rediscovery and a realization of their worth.

Suggested Readings

Morgan's Croonian Lecture (1922) before the Royal Society of London and his Presidential Address (1932) before the Sixth International Congress of Genetics are reprinted in *Readings in Heredity and Development*. A more complete bibliography is also provided there.

The development of ideas in genetics, to produce what Morgan called 'The Theory of the Gene,' is discussed in:

CREW, F.A.E. 1966. *The Foundations of Genetics.* New York: Pergamon.

DUNN, L.C. Editor. 1951. *Genetics in the 20th Century. Essays on the Progress of Genetics During Its First 50 Years.* New York: Macmillan.

DUNN, L.C. 1965. *A Short History of Genetics.* New York: McGraw-Hill.

STURTEVANT, A.H. 1965. *A History of Genetics.* New York: Harper & Row. As recalled by one who did so much to make genetics an exact science. Chapter 7 is a fascinating account of life and science in the "Fly Room" at Columbia University, where so much of the research was done.

STURTEVANT, A.H., and G.W.BEADLE. 1939. *An Introduction to Genetics.* Philadelphia: W.B.Saunders. Reprinted by Dover Books, New York.

Much of the material presented in this chapter will be found, differently presented, in the standard books on genetics. Some of the more notable ones are:

BEADLE, GEORGE and MURIEL. 1967. *The Language of Life. An Introduction to the Science of Genetics.* Garden City, New York: Doubleday Anchor Book. Elementary. Paperback.

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STRICKBERGER, MONROE W. 1968. *Genetics.* New York: Macmillan.

SRB, ADRIAN M., RAY D.OWEN, and ROBERT S.EDGAR. 1965. *General Genetics.* San Francisco: W.H.Freeman.

WHITEHOUSE, H.L.K. 1969. *Towards an Understanding of the Mechanism of Heredity.* New York: St. Martin's Press.

WINCHESTER, A.M. 1966. *Genetics. A Survey of the Principles of Heredity.* Boston: Houghton Mifflin.

The principal research papers referred to in the chapter are:

BRIDGES, C.B. 1914. 'Direct proof through non-disjunction that the sex-linked genes of Drosophila are borne by the X-chromosome.' *Science 40:*107–9.

BRIDGES, C.B. 1916. 'Non-disjunction as proof of the chromosome theory of heredity.' *Genetics 1:*1–52, 107–63.

BRIDGES, C.B. 1921. 'Triploid intersexes in Drosophila melanogaster.' *Science 54:* 252–54.

BRIDGES, C.B. 1939. 'Cytological and genetic basis of sex.' In *Sex and Internal Secretions,* edited by E.Allen.Williams and Wilkins.

DEMEREC, M., and M.E.HOOVER. 1936. 'Three related X-chromosome deficiencies in Drosophila.' *Journal of Heredity 27:*206–12.

MORGAN, T.H. 1910. 'Sex-limited inheritance in Drosophila.' *Science 32:*120–22.

MORGAN, T.H. 1911. 'Random segregation versus coupling in Mendelian inheritance.' *Science 34:*384.

MULLER, H.J. 1927. 'Artificial transmutation of the gene.' *Science 66:*84–87.

MULLER, H.J. 1928. 'The production of mutations by X-rays.' *Proceedings of the National Academy of Sciences 14:*714–26.

PAINTER, T.S. 1934. 'A new method for the studying of chromosome abberrations

and the plotting of chromosome maps in Drosophila melanogaster.' *Genetics* *19:*175–88.

PAINTER, T.S. 1934. 'Salivary chromosomes and the attack on the gene.' *Journal of Heredity 25:*465–76.

STERN, C. 1931. 'Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktoraustauschs.' *Biologisches Zentralblatt. 51:*547–87.

STURTEVANT, A.H. 1913. 'The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association.' *Journal of Experimental Zoology 14:*43–59.

Questions

1. In the light of what you now know about genetics, try to explain the ten types of observations that Darwin attempted to account for by his Theory of Pangenesis (Chapter 1).

2. Can you suggest an explanation for Darwin's observations on color blindness (page 12)?

3. The 'porcupine man' transmitted his defect only to his male descendants (p. 9). Assume that this is the true pattern of inheritance. What genetic explanations are possible?

4. What beliefs concerning the importance of the nucleus were held by Schwann, Flemming, and Morgan?

5. Compare the evidence available in 1900 and 1920 for the participation of the nucleus in inheritance. Do you believe that the evidence of 1900 would have convinced you?

6. Discuss the experiment of Bridges that is referred to as the "final proof" that genes are parts of chromosomes. How did his proof differ from earlier proofs?

7. In Drosophila, *vestigial* **(v)** is an autosomal recessive to *long wings* **(V)**. *Sepia* **(s),** another autosomal character, is recessive to *red eyes* **(S)**. These two genes are located on different chromosomes. Describe (genotype and phenotype) the F_1 and F_2 of a cross between a *vestigial* female and a *sepia* male. Also the F_1 and F_2 of a cross between a *sepia* female and a *vestigial* male. In all these questions assume homozygosity for the normal allele unless you are told otherwise.

8. Two flies, both heterozygous for *sepia* and *vestigial,* are crossed. Describe the offspring of this mating.

9. A *normal*-appearing fly is crossed with one which is both *sepia* and *vestigial.* The offspring are:

1/4 *sepia-vestigial*	1/4 *sepia-long*
1/4 *red-vestigial*	1/4 *red-long*

What is the genotype of the *normal* parent?

Hint. When doing genetics problems of this type, it is best to solve for one character at a time. It is also necessary to keep in mind how the usual ratios

are obtained. Thus, a 3:1 ratio results from the crossing of two heterozygous individuals (**Aa×Aa**). A 1:1 ratio results from the cross of a heterozygous individual with a homozygous recessive individual (**Aa×aa**).

10. In an F_1 the following results were obtained:

3/8 *red-long*	1/8 *sepia-long*
3/8 *red-vestigial*	1/8 *sepia-vestigial*

What were the genotypes and phenotypes of the parents?

11. A female with *sepia eyes* and *long wings* was crossed with a male fly. The F_1 gave the following ratios:

3/4 *red-long*	1/4 *red-vestigial*

What were the genotypes of the parents?

12. In Drosophila the character *vestigial* wings **(v)** is an autosomal recessive to *long* wings **(V)**. *White eyes* **(w)** is a sex-linked recessive to *red eyes* **(W)**. Describe the F_1 and F_2 of a cross between a *white-eyed* female and a *red-eyed* male.

13. Describe the F_1 and F_2 of a cross of a homozygous *red-eyed* female and a *white*-eyed male.

14. A *white-eyed, long-winged* female is crossed to a *red-eyed, vestigial* male. Describe the F_1 and F_2.

15. In an F_1 the following results were obtained:

Males—1/2 *white-vestigial;* 1/2 *white-long.*
Females—1/2 *red-vestigial;* 1/2 *red-long.*
What were the genotypes of the parents?

Hint. In doing problems of this nature, involving both autosomal and sex-linked genes, solve for the autosomal gene first. When you begin with the sex-linked gene remember these points:

a. a female has two **X** chromosomes, which are distributed to both sons and daughters.

b. the male has one **X** chromosome, which goes to the daughter, and one **Y** which is transmitted to the son.

c. the son, therefore, receives his **X** only from his mother. The phenotype of the sons will, therefore, identify the genotype of the mother.

d. the daughter receives one **X** from the father and one from the mother. With the genotype of the mother already established, the genotype of the father can be determined by considering the known genotype of the mother and the phenotypes of the daughters.

16. In an F_1 the following results were obtained:

Males—1/4 *white-long;* 1/4 *white-vestigial;*
1/4 *red-long;* 1/4 *red-vestigial.*
Females—1/2 *red-long;* 1/2 *red-vestigial.*
What were the genotypes of the parents?

17. In an F_1 the following results were obtained:

Males—3/8 *white-long;* 3/8 *red-long;*
1/8 *white-vestigial;* 1/8 *red-vestigial.*

Females—3/8 *white-long;* 3/8 *red-long;*
1/8 *white-vestigial;* 1/8 *red-vestigial.*

What were the genotypes of the parents?

18. In an F_1 the following results were obtained:

Males—3/8 *white-long;* 3/8 *red-long;*
1/8 *white-vestigial;* 1/8 *red-vestigial.*
Females—3/4 *red-long;* 1/4 *red-vestigial.*
What were the genotypes of the parents?

19. In man *hemophilia* **(h)** is a sex-linked recessive to *normal* clotting blood **(H)**. Apparently hemophilia is an embryonic lethal when homozygous in females. At least no homozygous females have been recorded. *Brown eyes* **(B)** is autosomal and dominant to *blue eyes* **(b)**. Describe the offspring of a female heterozygous for *hemophilia* and a *normal* male.

20. Describe the offspring of a female heterozygous for *hemophilia* and a *hemophiliac* male.

21. A *blue-eyed* female heterozygous for *hemophilia* marries a male heterozygous for *brown eyes* and with *normal* blood. Describe the possible offspring.

22. A case is recorded of a couple living in Outer Mongolia who were the parents of 193 children. Of the children, 99 were females and 94 were males. Of the 94 males, 12 were *blue-eyed* and had *hemophilia;* 10 were *blue-eyed* and *normal;* 33 were *brown-eyed* and had *hemophilia;* 39 were *brown-eyed* and with *normal* blood. Of the 99 females all had *normal* blood but 26 were *blue-eyed* and 73 had *brown eyes*. What were the genotypes of these fortunate parents?

23. In man *brown eyes* **(B)** is dominant over *blue* **(b)**. The gene is autosomal. *Colorblindness* **(c)** is a sex-linked recessive to *normal* vision **(C)**. What are the probable genotypes and phenotypes of two individuals who had the following children?

Daughters:	1 *blue-normal*
	4 *brown-normal*
Sons:	2 *blue-color blind*
	5 *brown-color blind*

24. The blood groups in man, *A, B, AB,* and *O* are determined by the interaction of three multiple alleles, **A, B,** and **o**. A and B when present together result in *type AB*. Both **A** and **B** are dominant over **o**. *Type A* is either **AA** or **Ao**. *Type B* is either **BB** or **Bo**. *Type O* is the homozygous recessive **oo**. These are autosomal genes. What types of children would be expected from the following crosses: **AB×oo; AB×AA; AB×Bo; Ao×Bo**.

25. Two mothers, one whom we shall call M (husband is N) and the other R (husband is S), delivered two children, X and Y, in an obscure British hospital during a power failure. In the confusion no one remembered which baby belonged to which mother. It was therefore necessary for all individuals concerned to submit to a blood test. Baby X was found to be of *type O* and

baby Y of *type A*. Mother M was of *type A* and her husband N was also of *type A*. Mother R was *type AB*. Her husband, S, being on a business trip to Ceylon at the time, could not be tested. With this information, was it possible for the hospital to make the proper allocation of babies?

26. In chickens a common type of comb, *single,* occurs when two different recessive genes, **p** and **r,** are homozygous. *Rose comb* is formed when one or two dominant **R** genes are present. *Pea comb* is formed when one or two of the dominant **P** genes are present. If one or two **R** genes and one or two **P** genes are present in the same individual, the result is a *walnut comb*. The loci for **P** and **R** are on different chromosomes. What will be the phenotypes and genotypes in the F_1 and F_2 of a cross of a homozygous *rose* and a homozygous *pea* chicken?

27. Let us assume that an organism has a pair of autosomes and a pair of sex chromosomes (♀**XX** and ♂**XY**) and that crossing over occurs in the female only. In the ♀, one of the autosomes has the genes **a** and **B** and the other **A** and **b;** one of the **X** chromosomes has the genes **c** and **D** and the other **C** and **d**. Assume that crossing over between the **a** and **b** loci occurs in 40 per cent of the cases and between **c** and **d** loci in 20 per cent of the cases. This ♀ is crossed with a ♂ homozygous for a and **b** and with **c** and **d** on his **X** chromosome. Assume that there are no loci of **c** and **d** on the **Y** chromosome. Diagram the chromosomal events, beginning with the diploid cells in the ovary and testis and ending with the first division of the zygote.

28. In Drosophila, *ebony* (e) and *stripe* (s) are recessive autosomal genes located on the same chromosome. They show 8 per cent crossing over. Describe the offspring of a cross of a female heterozygous for both genes (her father was *ebony-stripe* and her mother homozygous-normal) with an *ebony-stripe* male. There is no crossing over in Drosophila males.

29. In Drosophila *bar eyes* **(B)** is a sex-linked dominant to *normal* eyes **(b)**. *Red eyes* **(W)** is a sex-linked dominant to *white eyes* **(w)**. The two loci are 50 cross-over units apart. Cross a *white-normal* female with a *red-bar* male. Then cross the F_1 male with a *white-bar* (heterozygous) female. What will be the genotypic and phenotypic ratios of the resulting offspring?

30. A cross is made of a Drosophila female with *white eyes* and a *red-eyed* male. One of the eggs was fertilized with a sperm carrying an **X** chromosome. Something happened in the very young embryo and the father's **X** was eliminated from the cells that were destined to form the entire left side of the body. What would be the eye color of this fly when it reached the adult stage?

31. *Bar eye* is a dominant sex-linked gene of Drosophila that reduces the normal circular eye to a bar-shaped structure. Solely on the basis of genetic crosses, Sturtevant and others advanced the hypothesis that the *bar* gene is a duplication. That is, it differs from a normal gene in having two loci, which are adjacent, on the same chromosome. At the time it was impossible to test this hypothesis by studying the chromosomes. Years later when the salivary

gland techniques were known, Bridges examined the region of the **X** chromosome of normal flies and of *bar-eyed* flies where the *bar* locus was thought to be located. This is what he saw (modified from Bridges 1936; Science *83:* 210).

Bar

Normal
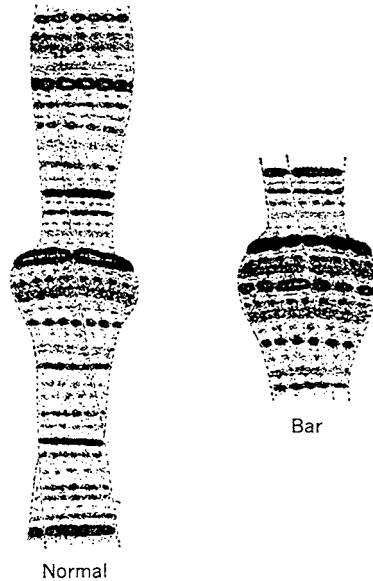
Was Sturtevant correct?

32. If volume 44 of Science (1916) is available to you, read Bateson's review (pages 536–543) of 'The Mechanism of Mendelian Inheritance' by Morgan, Sturtevant, Muller, and Bridges. Do you agree with his caution?

33. Darlington in 'Genetics and Man' (page 118), mentions the reluctance of most European biologists to accept Morgan's Theory of the Gene. Read what he has to say and see if you understand the biological and philosophical problems involved.

6 Genetics—Old and New

By the 1930's, after millions of crosses had been made and billions of off-spring classified, geneticists had the satisfying feeling that the big questions that had been asked for centuries had been answered. When studies were made of inheritance in species not previously investigated, the results confirmed the rules of Mendel and Morgan. The science of genetics had reached an acceptable level of maturity: it could predict the outcome of experiments.

If one were to ask the question, however, just what *had* been accomplished, the answer might seem unimpressive. One understood how the genes for eye color were inherited, but there was no understanding of either the molecular structure or the mode of action of the gene. What had been worked out were the rules governing the transmission of genes from parent to offspring. These rules held for plants, animals, and microorganisms: their universality was impressive.

Before we ask the new questions let us summarize the answers to the old. In the following list, the generally established concepts of classical genetics appear in *italics;* expansions or exceptions to each concept—which for the most part have not been mentioned before—are in roman type.

1. *The basic morphology, physiology, and biochemistry of an organism is determined by its inheritance, acting in a definite environment; that is, by a process of reproduction it has originated from other organisms similar to it.*
2. *Inheritance is the transmission of genes from parents to offspring.*

Some of the characteristics of the individual, especially those of the early embryo, are determined solely by the action of the maternal genes during the formation of the ovum in the ovary. Thus the sperm enters an ovum that has some of its characters, such as size, color, and rate at which mitosis will occur, already determined. The paternal genes will not affect these characters but they will affect the ova produced when the individual reaches maturity. The effect of the paternal genes on the early embryo, therefore, may be delayed for a generation.

3. *Genes are situated on chromosomes.* There are few exceptions to this generalization. A fraction, possibly very small, of inheritance is dependent upon non-chromosomal structures such as mitochondria, plastids, and some virus-like bodies. Even the genes of bacteria and viruses are parts of chromosome-like structures.

4. *Each gene occupies a particular site, or locus, on a chromosome.* In some instances the position of the locus may be changed by inversions and translocations, which shift the position of one or more genes in relation to other genes.

5. *Each chromosome has many genes, and these are arranged in a linear order.* As an exception to this we should remember that some chromosomes, such as the **Y** of Drosophila, have only a few genes.

6. *The cells of an animal, except those in the process of ova or sperm formation, contain two of each kind of chromosome (diploid condition); that is, all chromosomes are present in homologous pairs, and each gene locus will be represented twice.* There are several well-known exceptions: **(a)** In some species there are differences in chromosome number between different kinds of individuals. In bees, for example, the females (queens and workers) are diploid and the males (drones) are haploid. **(b)** It is generally believed that an individual will have the same number of chromosomes in all of its cells (except the germ cells) but some exceptions to this are known. In the liver cells of some species of vertebrates, for example, different classes of cells exist. Some have the expected diploid number but others may have twice this number, **(c)** Sex chromosomes, as in the case of **XO** males, offer still another exception to chromosomes existing in homologous pairs,

7. *For each mitotic cycle every gene is duplicated from the chemical substances in the cell. Cellular reproduction involves a concurrent genic reproduction.*

8. *Genes are capable of existing in several states (alleles), each having detectable effects. The change from one such state to another is known as mutation.* In spite of this ability to change, genes are very stable. On the average, a gene might be expected to duplicate at least a million times before a mutation occurs.

9. *Genes can be transferred from one homologous chromosome to another by crossing over.* This process is a normal part of meiosis. Crossing over seems not to occur in some instances—for example, in the male of Drosophila. Genes can also be transferred to non-homologous chromosomes by translocations.

10. *Every gamete receives one chromosome of each homologous pair. This distribution of chromosomes to the gametes is a matter of chance.* Thus, each type of chromosome in every pair of homologous chromosomes will be distributed to 50 per cent of the gametes. In the case of males with **XO** sex chromosomes, half of the gametes will receive no sex chromosome at all. This is a complication, though not an exception, to the rule as stated.

11. *The distribution to the gametes of the chromosomes of one homologous pair has no effect on the distribution of the chromosomes of the other pairs.* There are, however, a few cases known in which the chromosomes enter the gametes in specific groups.

12. *Fertilization consists of the random union of male and female gametes, each with one chromosome of every homologous pair. Therefore, the zygote receives one chromosome of each homologous pair from its father and one from its mother.* Once again, some sex chromosomes, as in **XO** males, introduce a complication to this general rule.

13. *When the cells of an organism contain two different alleles of the same gene (heterozygous condition), one allele (the dominant) has a greater phenotypic effect than the other (the recessive). In most cases of this sort the heterozygote appears to be identical with individuals homozygous for the dominant alleles.* In a few exceptional cases, the heterozygotes are intermediate in appearance between the homozygous dominant and homozygous recessive types.

14. *Genes produce their effects through the production of chemical substances, which in turn control the biochemical reactions of the cell.* A fruitful hypothesis, though unproved before 1940, was that each gene controls the production of a specific enzyme, which in turn controls a specific biochemical reaction.

From these simple propositions one can deduce most of the phenomena of classical genetics.

Once the concepts of transmission genetics had been established, geneticists turned to three other major problems. Some who wished to understand better the role of genes in evolution went on to revolutionize evolutionary biology, but their discoveries will not be discussed here. The other major problems were: What is the gene? How does it act? The 'what is it?' question will be continued in the next chapters; the

'how does it act?' question will start now and continue in the next chapter.

Inborn Errors of Metabolism. In man there is a rare disease—affecting about one in a million—known as *alkaptonuria.* It makes its presence known very early, since afflicted babies stain their diapers. The urine of these babies contains a substance known as homogentisic acid (or alkapton), which becomes dark red or black when oxidized. The disease is benign, although in later life it may be associated with arthritis. An English physician, Archibald E.Garrod (1857–1936), noticed that the parents of these children were often first cousins and he speculated about the possibility of the disease being inherited. He consulted Bateson, who in 1902 suggested that the available data could be understood if one assumed that alkaptonuria is caused by a recessive gene. This was the first Mendelian recessive discovered in man.

Garrod recognized alkaptonuria as a genetic disease and spoke of it as an 'inborn error of metabolism.' He suggested that individuals with alkaptonuria lack an enzyme. This enzyme is present in normal individuals where it converts homogentisic acid to simpler substances, which are excreted in normal urine. In the absence of the enzyme, therefore, the urine contains homogentisic acid. Normal individuals, and heterozygotes, are able to produce the enzyme. Thus Garrod hypothesized that one of the things that genes can do is to make enzymes.

Neither Garrod nor alkaptonuria is mentioned in any of the books written by the Morgan school in the years of active discovery. Morgan and many other geneticists were adamant in ignoring hypotheses they could not test. As he expressed it: 'It is the prerogative of science, in comparison with the speculative procedures of philosophy and metaphysics, to cherish those theories that can be given an experimental verification and to disregard the rest, not because they are wrong, but because they are useless.' There was very little that Morgan and his school could cherish in the hypothesis 'genes control the production of enzymes'—the techniques of biochemistry were too poorly developed.

Beadle, Tatum, and Neurospora. By the late 1930's, George W.Beadle (born 1903) with two associates, first Boris Ephrussi (born 1901) and later Edward L.Tatum (born 1909), thought the time ripe to make a vigorous attempt to discover how genes act. Somehow the genes must produce or control the production of molecules and the interactions of these molecules must result in the phenotypic expression of the genes. It was impossible to think of gene action in any other way; but how could one possibly get at the problem? Every cell has thousands of

genes: how would one investigate the molecules produced by any one of them?

First, Beadle and Ephrussi studied the production of eye color in Drosophila. The experiments seemed to show that many substances were involved and that some were probably enzymes. Enough was discovered to suggest the hypothesis: *one gene—one enzyme,* meaning that the primary function of each gene is to produce a specific enzyme. But the biochemistry of Drosophila proved to be too complex to test the hypothesis adequately and, for the first time, that noble animal let a geneticist down. Some other biological system would have to be used.

Metabolic Pathways in Cells. During the 1930's biochemists had been successful in learning about many of the molecular reactions that occur in cells. Thus, the oxidation of glucose, which is so basic for life and which for generations had been expressed:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,H_2O + 6\,CO_2$$
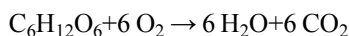
was found to consist of dozens of intermediate reactions, each controlled by a specific enzyme. The speed of these reactions is incredible: from glucose to H_2O and CO_2 requires far less than a second.

Very special methods had to be devised to discover intermediate reactions passed through so quickly. It was found, for example, that a poison may produce its effects by destroying or inhibiting a specific enzyme. When the enzyme is made ineffectual, the sequence of reactions is blocked and its substrate (the molecules it should have changed) accumulate in the cell. For example, let us suppose that this sequence of reactions occurs:

$$\longrightarrow A \xrightarrow{\text{enzyme}} B \xrightarrow{\text{enzyme}} C \xrightarrow{\text{enzyme}} D$$
$$\ 1 \qquad\quad 2 \qquad\quad 3$$
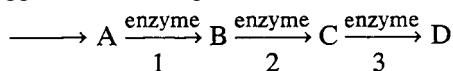
If enzyme 1 is eliminated, the reaction cannot go beyond the production of molecules of A. Thus, B, C, and D will not be formed but the amount of A may increase to the point where it can be detected. One can check the hypothesis that enzyme 1 is not working by supplying the cell with molecules of B. The cell should then be able to make C and D. Similarly, if something goes wrong with enzyme 2, molecules of B will accumulate. Thus, by throwing chemical wrenches into the biochemical gears of the cell, one can learn about the sequence of reactions in normal metabolism.

With this in mind, Beadle and Tatum sought to apply the methods of genetics and biochemistry to learn if the genes do, in fact, make enzymes. They reasoned that if gene **A** is responsible for producing enzyme 1, a mutation to an **a** allele might result in an abnormal and

ineffectual enzyme, or none at all. This was a reasonable hypothesis, and unbeknown to them, proposed long before by Garrod. But how could one test it? If enzyme 1 is necessary for life, all **aa** individuals would die or possibly never appear at all. Geneticists were rather sure that many mutants did behave this way. They were familiar with a large number of lethal genes which kill homozygous individuals in various unknown ways. Quite possibly these individuals were dying because they lacked some essential enzyme. But which one?

Try to imagine how one might test the hypothesis: each enzyme in the cell is produced under the influence of a specific gene. By noticing the stained diapers, Garrod had developed the hypothesis that individuals homozygous for the alkaptonuria gene lack the enzyme that normally degrades homogentisic acid. But if the diapers had not been stained in a specific way, how could he have discovered the nature of the disease? One cannot expect that all types of molecules produced by genetic diseases are excreted in the urine, and certainly very few are helpful enough to reveal their presence by turning black in the presence of air.

Recall that Mendel's principal innovation was remarkably simple: he counted the numbers of offspring in each phenotypic class. Beadle and Tatum were to be equally simple and elegant. The standard procedure for a geneticist had been to determine what the genes do: they produce blue eyes, round seeds, or vestigial wings. It was reasonable to assume that these phenotypic expressions of genes were, themselves, consequences of various biochemical reactions. One always started with the gene. Beadle and Tatum reversed this procedure. Instead of beginning with the genes and trying to find the biochemical reactions, they started with the biochemical reactions and searched for the genes that controlled them. Sounds good but, again, what does one do? They had tried to experiment with Drosophila but it proved to be far too complex. Instead they deliberately sought an organism that had the biochemical and genetic properties that would enable them to answer their questions. In 1932, when Morgan had been asked to predict how new discoveries were to be made in genetics he replied, 'By a search for favorable material' (*Readings,* Chapter 5). The favorable material that Beadle and Tatum found was the fungus *Neurospora crassa*.

Neurospora crassa. This is the red bread mold. It can be grown on a variety of media in the laboratory but, for reasons that will shortly become clear, it was necessary for Beadle and Tatum to know exactly what sorts of molecules are required for growth. The list was discovered to be surprisingly short: air, water, inorganic salts, sucrose, and a single vitamin—biotin. Thus, sucrose and biotin are the only organic molecules

required. From these simple foods, Neurospora is able to synthesize all of the molecules necessary for its structure and life: amino acids, proteins, vitamins, nucleic acids, carbohydrates, fats, and so on.

Neurospora has a life cycle almost as simple as its nutritional requirements. The colonies are haploid for most of their life cycle. All look more or less alike but, in reality, there are two mating types, *A* and *a,* which correspond to the sexes of other organisms. If *A* and *a* colonies are grown together, parts will fuse and *A* nuclei will unite with (fertilize) *a* nuclei to produce diploid zygotes. The zygote immediately undergoes meiosis to produce four haploid nuclei. These then divide by mitosis to produce eight haploid spores capable of growing into as many new colonies. These eight spores are encased in a spore sac, or ascus, from which they can be removed one by one and allowed to grow into a new colony. Since each colony will be haploid, every gene will be expressed—there are no dominant alleles to suppress the recessives.

The amino acid arginine is among the many molecules that Neurospora normally synthesizes. Let us suppose that we wish to learn if the synthesis of arginine is under genetic control. As a working hypothesis, Beadle, Tatum, and their associates assumed that a specific gene supervises the production of a specific enzyme that, in turn, catalyzes the reaction that leads to the formation of arginine from some unknown precursor. Presumably this gene could mutate to an allelic form that would be unable to make the enzyme. Unless such a mutation occurred, one would never suspect the presence of the original gene. A spore with the mutant allele could never produce a colony: such a mutant allele would be a 'lethal.'

Thus, to solve the problem, Beadle and Tatum had to devise a method for detecting these lethal mutations and for maintaining them in culture. This sounds impossible, but they did it. First, they used X-rays to produce mutations—as Muller had done a decade before. They assumed that the radiations would produce all sorts of mutations but, if they were lucky and looked long enough, they might discover some mutants involved in the synthesis of arginine. Spores from the irradiated colonies were placed on the culture medium that contained the minimum variety of molecules necessary for growth. Most of the spores grew, showing that if mutations had occurred they did not prevent the Neurospora from synthesizing its constituent molecules from the few simple chemicals of the basic medium. Some of the spores did not germinate. Presumably these had genes that had mutated to allelic states than made the production of some essential enzyme impossible. But what enzyme or enzymes?

Beadle and Tatum reasoned that if one of the genes necessary for the synthesis of arginine had mutated, the defect might be overcome by add-

ing arginine to the medium. So the spores that had not germinated on the minimal medium were transferred to a minimal medium supplemented with arginine. Most of these spores still did not germinate, but others did and from them strains were established that could grow on the minimal medium enriched with arginine.

The next step in the analysis was to determine if the inability to grow without arginine has a genetic basis. This can be done by crossing the presumed mutant strains to normal Neurospora of the opposite mating type. Spores obtained from such a cross were grown in separate tubes. Half were able to grow on the minimal medium but half required arginine. These results were consistent with the hypothesis that in the wild type Neurospora there is a gene, **A,** which somehow is necessary for the synthesis of arginine. The X-rays used in the experiment had caused **A** to mutate to **a,** which was unable to play some essential role in arginine synthesis.

Numerous strains that required arginine were found in Beadle and Tatum's laboratory. Were all of them the same genetically? There were two main possibilities:

1. All could have originated from mutations at the same locus: $A \rightarrow a$.
2. Many different genes might be involved in arginine synthesis: A_1, A_2, A_3, etc. Any one of these might mutate to an allelic state that could no longer function. Thus A_1 could mutate to a_1, A_2, to a_2, and so on.

Genetic crosses could be made to test the alternatives. Thus, if both strains are **a,** all of the offspring will be **a**. Alternatively, if different gene loci are involved, some wild type colonies will appear among the offspring. For example, consider a cross of $a_1 \times a_2$. If a mutation had occurred at only one locus, which is overwhelmingly probable, and not at both A_1 and A_2, the a_1 strain would also have the A_2 allele. Similarity, a_2 would have A_1. The diploid zygotes formed in this cross will be A_1 a_1 A_2 a_2. These will produce spores. If the A_1 and A_2 loci are on different chromosomes, the haploid colonies formed from the diploid zygotes should be:

1/4 A_1A_2.	These are normal and will be able to grow on minimal media.
1/4 A_1a_2.	These will require arginine since the a_2 allele will not function.
1/4 a_1A.	These will require arginine since a_1 is not functioning.
1/4 a_1a_2.	These will require arginine since neither a_1 nor a_2 is able to function.

If the loci are on the same chromosome, the frequency of each class of recombinants will depend on the amount of crossing over.

By making these crosses, Beadle and Tatum found seven genetically different mutants, each requiring arginine for growth. One can conclude, therefore, that a minimum of seven genes are required by Neurospora to make arginine. This is interesting but our primary concern is to learn what these genes do. If we assume that they are involved in the formation of enzymes, there are two main possibilities. First, all seven genes could be involved in the formation of a single enzyme that has the function of converting some unknown precursors into arginine. Second, each of the seven genes could be involved in the formation of a different enzyme, each catalyzing a different reaction in the metabolic pathway to arginine (the one gene-one enzyme hypothesis). We could think in terms of this abbreviated model:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} F \xrightarrow{E_6} G \xrightarrow{E_7} \text{Arginine}$$
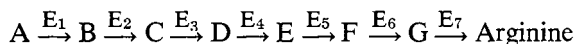
A is an unknown starting compound that finally becomes arginine after passing through intermediate stages B…G, each step being catalyzed by one of the gene-controlled enzymes $E_1…E_7$. The failure of any enzyme would have the same consequence so far as arginine is concerned: there would be none.

Beadle and Tatum favored the hypothesis that each of the seven mutants that required arginine for growth had something wrong with a different enzyme, rather than the hypothesis that all the mutants were involved with the same enzyme. But preference hardly makes a hypothesis probable. They would have to learn more about how arginine is made in the cells.

Recall that Beadle and Tatum were using biochemical reactions to discover genes rather than genes to discover the reactions. They had chosen to work with arginine because a good deal was already known about its metabolism. In 1932 Hans A.Krebs had discovered that in some vertebrate cells arginine is formed from citrulline, citrulline from ornithine, and ornithine from an unknown precursor. A specific enzyme is required for each transformation. Thus the reaction can be abbreviated:

$$\text{precursor X} \xrightarrow{E_x} \text{ornithine} \xrightarrow{E_o} \text{citrulline} \xrightarrow{E_c} \text{arginine}$$
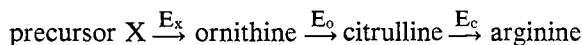
If Neurospora has a similar metabolic pathway, one should be able to see how the seven mutants are involved, since the failure of each specific enzyme would have a different consequence. If something was wrong with enzyme E_x, the reaction would end with precursor X;

arginine would not be formed, but neither would ornithine or citrulline. Similarity, if enzyme E_o was absent or ineffectual, ornithine would be formed but neither citrulline nor arginine.

It is experimentally possible to test these various alternatives. For example, let us suppose that a mutant strain requires arginine for growth because enzyme E_x is absent or ineffectual. If this is so, then growth should not depend only on the addition of arginine; either ornithine or citrulline should be equally effective. Similarity, if enzyme E_o is absent, either citrulline or arginine should make growth possible. In this case, the addition of ornithine would not help. If enzyme E_c is absent or defective, only arginine can cure the deficiency.

Close study of the seven mutants gave the answer. In the case of four, growth was possible if ornithine, citrulline, or arginine was added. This suggested that the sequence of reactions was blocked in the unknown pre-ornithine part of the metabolic pathway. Two of the strains would grow if either citrulline or arginine was added to the basal medium, but ornithine did not help. This suggested that the block was between ornithine and citrulline. The data suggested something else of great interest: since two genetically different mutants both blocked the reaction, it is reasonable to think that ornithine does not change directly into citrulline. Instead ornithine probably changes to some unknown intermediate, in a reaction catalyzed by one enzyme, and then from the unknown intermediate to citrulline under the influence of a second enzyme. Finally, one strain was found that would grow only on arginine: neither citrulline nor ornithine would permit growth. This suggested that enzyme E_c was absent or inactive.

We can tentatively conclude that the metabolic pathway ending in arginine has at least seven gene-controlled enzymes and at least seven products of the reactions catalyzed by these enzymes. Three of the products are known. We can, therefore, modify the original model to read as follows:

$$ A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} \text{ornithine} \xrightarrow{E_o} F \xrightarrow{E_6} \text{citrulline} \xrightarrow{E_c} \text{arginine} $$
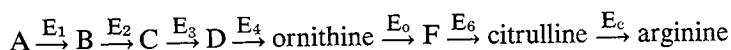
In the experiments described so far, the presence or absence of enzymes had been suggested, not demonstrated. Additional data, obtained after new laboratory techniques had been perfected, proved that the mutants either lack enzymes or have ones so abnormal that they cannot function.

Thus Beadle and Tatum had been able to show that one way genes act is to produce enzymes that control specific biochemical reactions. This hypothesis was established as true beyond reasonable doubt. At the time two additional extensions of the hypothesis seemed probable.

1. Every enzyme is dependent on a specific gene for its formation.
2. The primary effect of all genes is the formation of enzymes and other molecules.

Much as Sutton had linked cytology and genetics in the early 1900's, Beadle and Tatum linked genetics and biochemistry in the early 1940's. The type of experimentation introduced by Beadle and Tatum was vigorously pursued by numerous investigators, using not only Neurospora, but other molds, yeasts, and bacteria as well. While this was going on still another approach to the study of genetics at the molecular level was being made. This was a line of investigation that began in the late 1920's and eventually led to the identification of the gene as DNA—the topic of the next chapter.

Suggested Readings

H.J.Muller's Pilgrim Trust Lecture of 1945 to the Royal Society of London, entitled 'The Gene,' is reprinted in *Readings in Heredity and Development*. It is a masterful synthesis of what could be induced about the nature of the gene from the data of classical genetics. A more complete bibliography on the nature of the gene and the pioneering work of Beadle and Tatum will be found in the *Readings*.

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Questions

1. Considering the data available at the time, what could one have concluded about the nature of the gene in 1900, 1905, 1915, 1930, and 1945?
2. Why was it necessary for Beadle and Tatum to know what substances are required for growth by Neurospora?
3. If you wished to use Drosophila to answer the sorts of questions that

Beadle and Tatum asked of Neurospora, outline how you would plan your work.

4. Assume that Neurospora synthesizes molecules of substance A from substrate B. Four genetically different strains are discovered that cannot grow on the minimal medium but can grow if A is added. What can you conclude about the synthesis of A?

5. Two strains of Neurospora are discovered that cannot grow unless the amino acid tryptophan is added to the growth medium. How would you decide whether the strains are genetically same or different?

6. The meiotic and mitotic divisions that produce the eight spores of Neurospora occur in a geometrically exact manner. Thus, if the four chromatids of a tetrad on the first meiotic spindle are designated, left to right, $A\ A^1\ B\ B^1$, they will occupy predictable positions in the four cells formed by the two meiotic divisions. Thus, A will be in the left-most cell, A^1 next, then B, and finally B^1 on the extreme right. The mitotic division that follows produces the eight spores, which are arranged in a linear order: $A\ A\ A^1\ A^1\ B\ B\ B^1\ B^1$. This regular arrangement will occur if there is no crossing over.

A strain of Neurospora that can synthesize arginine is crossed with one that cannot. A spore case formed in this cross is opened and the eight spores removed in order and each is placed in a tube with minimal medium. Describe what will happen in each tube.

7. A cross of the above type is made and the spores are removed in order and placed in separate tubes. Growth occurs in tubes 1, 2, 5, and 6 but not in 3, 4, 7, or 8. How would you explain these results?

7 The Substance of Inheritance

Pneumonia in man is commonly caused by the bacterium *Diplococcus pneumoniae,* known also by an older name—pneumococcus. Before the days of sulfa drugs and antibiotics, it was one of our most serious diseases. Diplococcus also produces disease in monkeys, rabbits, and mice. Horses, swine, sheep, dogs, cats, guinea pigs, chickens, and pigeons are resistant.

Diplococcus pneumoniae shows a large amount of variability, much of which is now known to be genetic. For example, there are many dozen different types, usually designated by roman numerals. In the United States Type I and Type II are the most common. Types III and IV, as well as all the others, are less common. The cells of the various types seem to be identical so far as their general structure is concerned. Their specificity is due to the chemical composition of the capsule that surrounds them. The capsule is a thick, slimy, polysaccharide (a complex carbohydrate resembling the starch of plants).

The types are identified by their reaction to antibodies. Thus, if Type II cells are injected into a rabbit, the rabbit will form Type II antibodies. These antibodies will react with the polysaccharide capsules of Type II cells but not with those of the other types.

When capsulated cells are grown on culture plates they form colonies that are *smooth* and shiny in appearance. Mutations that give rise to cells lacking capsules are infrequent but, since the bacteria are in such tremendous numbers, even rare mutations can easily be observed. Cells lacking the polysaccharide capsules form colonies that are *rough* in

appearance. The reverse mutation, of cells lacking capsules giving rise to cells that possess them, occurs rarely.

There is an important biological property based on the presence or absence of the capsule. When capsules are present, the cells produce disease; when capsules are lacking the cells are harmless.

Transformation. An experiment on these bacteria that was eventually to usher in a new era in genetics was reported by F.Griffith in 1928. Griffith was a Medical Officer working for the British Ministry of Health. His interests in Diplococcus, as judged by his publications, were entirely medical. He gave no suggestion of the tremendous implications that his work was to have for genetics. This is easy to understand, since in 1928 it was not believed that the variations observed in bacteria were comparable to the genetically controlled variations of higher organisms. Furthermore, the medical profession was nearly wholly ignorant of genetics, and geneticists were yet to begin a study of inheritance in bacteria.

Griffith began one of his experiments with a culture of Type II bacteria that possessed capsules. Mice died if injected with these cells. As was usual, when these virulent bacteria were grown on culture plates, they produced the smooth colonies characteristic of capsulated cells. After repeated culturing, a few rough colonies appeared, which lacked the ability to synthesize the polysaccharide capsule. Thirty mice were injected with these capsuleless bacteria but no bacteremia occurred. The bacteria were harmless.

Griffith observed, as had others before him, that only living capsulated cells would produce bacteremia in mice. If, for example, he killed the capsulated bacteria with heat, they could be injected into mice and no disease would result. It was established, therefore, that the capsular material itself would not produce disease.

Next Griffith made a double injection: his mice received living capsuleless Type II bacteria plus heat-killed capsulated Type II. On the basis of the data given so far, we might predict that the mice would remain healthy. After all, they were receiving the harmless variant of living bacteria and dead cells of the virulent strain. Yet all of the four mice injected died after five days. Upon examination, their blood was found to be rich with Type II capsulated bacteria! So far as could be determined, they were the same as other strains of capsulated Type II cells.

This was an almost unbelievable result. It appeared that the ability to synthesize a capsule had been transferred from the dead cells to the living cells. Any geneticist of 1928, who might have known of these

experiments, would have shuddered and rededicated himself to *Drosophila melanogaster*. Whatever the nature of the change, it seemed to have nothing to do with the rules that governed inheritance in peas, fruit flies, man, and every other species that had been studied.

How could the results be explained? Possibly the first explanation that comes to mind would be the occurrence of a mutation of a gene lacking the ability to synthesize a capsule to an allele which could. Though possible, this seems unlikely. There had been thirty control mice, which were injected with capsuleless cells. They did not die. Yet all four mice receiving living capsuleless cells plus heat-killed caspulated cells had died.

Another experiment showed that mutation was not the explanation, and also shed new light on the problem. Once again the experiment consisted of giving the mice a double injection: living capsuleless bacteria plus dead capsulated bacteria. This time, however, *the cells were of different types:* the living capsuleless cells were of Type II, but the dead capsulated cells were of Type I. Eight mice were injected. Two died, one on the third and one on the fifth day. Numerous bacteria were in the blood of these two famous mice and, when cultured and tested, they were found to be Type I capsulated cells. Somehow the Type II capsuleless cells had been converted into Type I capsulated cells. This was not a temporary change: the cells were cultured generation after generation, and they remained Type I. The change was permanent and hence, in the broad sense, genetic. Griffith did not use such terms, but we would say today that one specific genetic type had been converted into another specific genetic type. The change was not a mutation but was apparently due to some influence of the dead cells—strange genetics indeed.

Variations of the experiment gave similar results. Thus, Type II non-capsulated cells were converted to Type III capsulated cells, and Type I non-capsulated cells were converted to Type III capsulated cells. The changes occurred only when living non-capsulated cells plus dead capsulated cells were injected into mice. Griffith was unable to observe the same change when the experiments were carried out in test tubes. The transformation occurred in a living mouse but not *in vitro*.

If we consider the tremendous medical problems caused by *Diplococcus pneumoniae,* it is not surprising that bacteriologists throughout the world were studying its biology. M.H.Dawson, of The Rockefeller Institute for Medical Research in New York, was one of these. In fact, he was doing experiments similar to those of Griffith in England. In 1927 Dawson, together with Oswald T.Avery (1877–1955), confirmed the even earlier observations that, when non-capsulated cells were in-

jected into mice, capsulated cells would usually be produced. One of their observations was: 'In all cases in which transformation has been effected, reversion has invariably been toward the specific form from which the [capsuleless] form was originally derived.' This was 1927, a year before Griffith had published the results of his experiments using living and dead cells of different Types.

In 1930, Dawson reported that he had confirmed Griffith's experiments of 1928. He refined the experiments in important ways in order to ensure that the strange observations, though unexplainable, were true. One of his improvements was to begin the strains of bacteria from single cells, rather than use many cells as Griffith had done. A single cell was allowed to reproduce and form a large population. Since this entire population had a single ancestor, and reproduction was by asexual means, it should have a high degree of genetic uniformity. With this precaution, one could rule out the possibility that the change from one type to another was not real but due to the use of mixed cultures. Using the strains obtained from single cells, Dawson did the following:

Non-capsulated cells derived from a Type II strain were injected into mice together with heat-killed capsulated cells of Type I, Type III, or Group IV. In each case the non-capsulated cells were transformed into capsulated cells of the type represented by the heat-killed cells.

In later experiments, Dawson and his associates were able to produce the transformations *in vitro.* This was a most important discovery, making it far easier to find out what it was in the preparation containing the heat-killed cells that led to the transformation. In 1932 and 1933 J.L.Alloway reported that a crude extract of the capsulated cells would cause the transformation.

The Chemistry of Transformation. The evidence was becoming quite convincing that a chemical substance was responsible for the transformations. It is probable that most workers expected the polysaccharide of the capsule to be the active agent. After all, the polysaccharide was responsible for the Type specificity but, wrote Alloway,* the polysaccharide 'when added in chemically purified form, has not been found effective in causing transformation of non-capsulated organisms derived from Diplococcus of one Type into capsulated forms of the other Type. When non-capsulated cells change into the capsulated form they always acquire the property of producing the specific capsular substance. The immunological specificity of the encapsulated cell depends upon the

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* What follows is not a direct quotation; I have modernized some of the technical terms.

chemical constitution of the particular polysaccharide in the capsule. The synthesis of this specific polysaccharide is a function peculiar to cells with capsules. However, since the non-capsulated cells under suitable conditions have been found to develop again the capacity of elaborating the specific material, it appears in them this function is potentially present, but that it remains latent until activated by special environmental conditions. The fact that a non-capsulated strain derived from one Type of Diplococcus, under the conditions defined in this paper, may be caused to acquire the specific charac- ters of the capsulated forms of a Type other than that from which it was origi- nally derived implies that the activating stimulus is of a specific nature.'

There is nothing in this long quotation, or in any other writing of this period, to suggest that transformation might be a genetic phenomenon. Alloway and others seemed to regard the phenomenon as some sort of a phys- iological modification—a perfectly reasonable hypothesis on the basis of the available data.

Dawson's discovery that transformation could occur *in vitro* and Alloway's discovery that a substance causing transformation could be extracted from the bacterial cells, suggested additional experiments. What was the chemical nature of the transforming substance? Alloway had demon- strated that it was not the polysaccharide of the capsule surrounding the cells. A likely guess was that it was a protein, for, in the 1930s, it seemed that nearly every important event that occurred in a living system involved or was controlled by proteins. The answer, however, lay elsewhere.

DNA and Transformation. Work on the problem continued slowly at The Rockefeller Institute and, in 1944, a most important announcement was made. Avery, MacLeod, and McCarty reported that they had obtained the transforming substance in a highly purified state and had established its chem- ical nature beyond a reasonable doubt. They began with huge amounts of Type III cells, using in some experiments the cells from as many as 75 liters of culture medium. The cells passed through a procedure that involved extrac- tion, washing, precipitation, dissolving, and so on. In the end they had no more than 10 to 25 mg. of the active transforming substance. At frequent steps in the procedure they tested the preparation by seeing if Type II non- capsulated cells could be transformed into Type III capsulated cells. Their final extract, though small in amount, was highly active. In fact it was far more active, per unit of weight, than the original mass of cells. What was its chemical nature?

The methods used in purifying the extract should have removed all

protein and all fat. As a check, however, the extract was tested for protein and none was found. Numerous other tests were made, including one for the presence of deoxyribonucleic acid (DNA). The extract was found to be exceedingly rich in this substance.

Tests for the closely similar compound, ribonucleic acid (RNA), gave only weakly positive results. In an effort to check on the reliability of the method, some purified DNA from animal cells was tested for RNA. This animal DNA gave the same weak test for RNA as did the purified transforming substance.

These results suggested that the extract contained a large amount of DNA and possibly some RNA. One could not conclude, definitely, however, that the transforming substance was either compound. After all, other substances might be present and one or several of these be the active principle. Nevertheless a good working hypothesis was: the transforming substance is DNA. The next step was to test the hypothesis.

First a comparison was made of the elemental composition of the purified extract and the elemental composition of DNA. The percentage composition for the extract was: 34.88 carbon; 3.82 hydrogen; 14.72 nitrogen; and 8.79 phosphorus. In 1944 the structure of DNA was not accurately known, but the theoretical percentages of these elements were thought to be: 34.20 carbon; 3.21 hydrogen; 15.32 nitrogen; and 9.05 phosphorus. The parallel was striking. Furthermore, the ratio of nitrogen to phosphorus, which was theoretically 1.69 in DNA, was found to be 1.67 in the extract. If we assume that the extract consisted largely of the transforming substance, then the results suggested that the transforming substance *could be* DNA and that it *could not be* protein, nor fat, nor carbohydrate.

Further tests substantiated this view. If we assume that the transforming substance is protein, then its activity should be destroyed by the enzymes that digest protein. Two protein-digesting enzymes of pancreatic juice, trypsin and chymotrypsin, were added to an extract containing the transforming substance. There was no loss of activity.

Another experiment indicated that the transforming substance was not RNA. Other workers had discovered an enzyme, ribonuclease, which destroys RNA. When this enzyme was added to the extract, there was no loss of activity. Avery, MacLeod, and McCarty concluded, The fact that trypsin, chymotrypsin, and ribonuclease had no effect on the transforming principle is further evidence that the substance is not ribonucleic acid or a protein susceptible to the action of tryptic enzymes.'

Thus they were reasonably sure of some of the substances that the active principle could not be, but how could they prove the hypothesis

that it was DNA? Convincing evidence would be obtained if they could use some agent that specifically destroyed DNA. If such an agent destroyed the ability of the extract to transform cells, one could conclude that the transforming substance was DNA.

Four years earlier, two investigators had reported that tissue extracts and blood serum contain an enzyme that breaks down the large molecules of DNA. The enzyme, which was obtained in a crude form, is known today as deoxyribonuclease (DNase). Avery, MacLeod, and McCarty prepared some of this enzyme and added it to their active extract of transforming substance. There was a complete loss of ability to transform cells. This was most convincing. In addition, many other experiments were carried out and the results were all explainable on the basis of the hypothesis that the transforming substance was DNA.

Preliminary observations suggested that the molecular weight of this DNA was very large—about 500,000. Its biological activity was also quite impressive: transformation could be induced when the DNA was present in a concentration of one part in 600 million.

The following quotation shows how the authors explained their observations that an extract of the DNA of Type III capsulated cells could cause Type II non-capsulated cells to start producing capsules that were specific to Type III.

> In the present state of knowledge any interpretation of the mechanism involved in transformation must of necessity be purely theoretical. The biochemical events underlying the phenomenon suggest that the transforming principle interacts with the [non-capsulated]* cell giving rise to a coordinated series of enzymatic reactions that culminate in the synthesis of the Type III capsular antigen. The experimental findings have clearly demonstrated that the induced alterations are not random changes but are predictable, always corresponding in type specificity to that of the encapsulated cells from which the transforming substance was isolated. Once transformation has occurred, the newly acquired characteristics are thereafter transmitted in series through innumerable transfers in artificial media without any further addition of the transforming agent. Moreover, from the transformed cells themselves, a substance of identical activity can again be recovered in amounts far in excess of that originally added to induce the change. It is evident, therefore, that not only is the capsular material reproduced in successive generations but that the primary factor, which controls the occurrence and specificity of capsular development, is also reduplicated in the daughter cells. The induced changes are not temporary modifications but are permanent alterations which persist provided

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* The terms in brackets are substitutions for older terms.

the cultural conditions are favorable for the maintenance of capsule forma-tion. The transformed cells can be readily distinguished from the parent [non-capsulated] forms not alone by serological reactions but by the presence of a newly formed and visible capsule which is the immunological unit of type specificity and the accessory structure essential in determining the infective capacity of the microorganism in the animal body.

> It is particularly significant in the case of [the bacterial cells] that the experi-mentally induced alterations are definitely correlated with the development of a new morphological structure and the consequent acquisition of new antigenic and invasive properties. Equally if not more significant is the fact that these changes are predictable, type-specific, and heritable.

> Various hypotheses have been advanced in explanation of the nature of the changes induced. In his original description of the phenomenon Griffith sug-gested that the dead bacteria in the inoculum might furnish some specific pro-tein that serves as a 'pabulum' and enables the [non-capsulated] form to manu-facture a capsular carbohydrate.

> More recently the phenomenon has been interpreted from a genetic point of view. The inducing substance has been likened to a gene, and the capsular anti-gen which is produced in response to it has been regarded as a gene product. In discussing the phenomenon of transformation Dobzhansky has stated that "If this transformation is described as a genetic mutation—and it is difficult to avoid so describing it—we are dealing with authentic cases of induction of spe-cific mutations by specific treatments…."

> It is, of course, possible that the biological activity of the substance described is not an inherent property of the nucleic acid but is due to minute amounts of some other substance adsorbed to it or so intimately associated with it as to escape detection. If, however, the biologically active substance isolated in highly purified form as the sodium salt of deoxyribonucleic acid actually proves to be the transforming principle, as the available evidence strongly sug-gests, then nucleic acids of this type must be regarded not merely as structurally important but as functionally active in determining the biochemical activities and specific characteristics of [the bacterial] cells. Assuming that the sodium deoxyribonucleate and the active principle are one and the same substance, then the transformation described represents a change that is chemically induced and specifically directed by a known chemical compound. If the results of the present study on the chemical nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.

Dobzhansky's belief that the DNA was inducing mutations was probably shared by most geneticists. After all, it was clearly established that mutations could be produced experimentally. Muller had demonstrated this, in 1927, by using X rays (page 125). Other investigators sub-

sequently discovered that ultraviolet light and some chemical substances would cause mutations.

Stimulus or Substance. There is a difference, of fundamental importance, between the effects of X rays and the phenomenon of transformation in bacteria. X rays do not produce *specific* mutations. One could not use X rays to produce mutations only at the white-eye locus in Drosophila. Instead, the X rays would produce many kinds of mutations. Possibly one would be at the white-eye locus, possibly not. The DNA extracted from capsulated bacteria, in contrast, could produce specific changes in non-capsulated cells. Since these specific changes were inheritable, there were grounds for calling them mutations. One could imagine the mechanism to work somewhat as follows: the bacteria contain a gene, which can be either in the capsule-producing or non-capsule-producing state. We can call the alleles c^+ and c^-, respectively. We would assume that DNA extracted from c^+ cells has the ability to cause the mutation $c^- \rightarrow c^+$.

The implications of this hypothesis, if true, were enormous. Man could control inheritance to a degree never before possible. He might mold his own species and others of importance to him. To be sure, he could do this for only a single gene and in only one species of bacteria, but this limitation might be overcome with further research.

Of course, there was no theoretical reason to suppose that the mutation process could not be controlled. Mutation, whatever it was, could only be a physical or chemical event—a scientist can conceive of no other possibility. Therefore any mutation, such as $c^- \rightarrow c^+$, would become controllable once the necessary information about the biology of cells was at hand. Prior to 1940 a geneticist might have predicted that the cell biologist should be able to supply him with the necessary information about the year 2000. It was all the more remarkable, therefore, that the feat was accomplished in 1944.

But the true explanation of transformation in bacteria by DNA lay elsewhere.

Bacteriophage. Bacteria have their health problems too. Microorganisms known variously as the bacterial viruses, bacteriophages, or phages can attack bacterial cells and so disrupt the cell's metabolism that death results. In recent years, one bacterium and its many phage parasites have given important new insights into genetic mechanisms.

The bacterium, *Escherichia coli,* is a harmless inhabitant of the large intestine of man. It can be grown readily in the laboratory. Large populations are easy to obtain since cell division occurs about once every 20 minutes. Thus a geneticist, who would have to wait about 75 years

for three generations in man, or six weeks in Drosophila, could observe three generations in *E. coli* in one hour. If *E. coli* is infected with a phage, such as one called T_2, the bacterial cell is killed in about 20 minutes. The main steps are as follows. The phage attacks the cell and produces a profound change in the cell's metabolism. Before the phage appeared, the cell was synthesizing its own specific molecules: bacterial proteins, bacterial nucleic acids, and on on. The phage changes all this. In some manner it assumes control of the cell's synthetic machinery and directs it to produce phage molecules instead of *E. coli* molecules. In about 20 minutes, the bacterial cell will have been forced to make about 100 phage particles. It is at this point that the bacterial cell ruptures and liberates the newly formed phage. Each new phage can infect another *E. coli* cell and repeat the cycle.

Phage particles have a specific identity that is shown in many ways: the T_2 phages can grow only in living *E. coli* cells; they have a characteristic structure which is revealed when they are photographed with an electron microscope; chemically they are comparatively simple, being composed of an outer coat of protein and a core of DNA.

The phage particles that are released by the bursting bacterial cell are, in the vast majority of cases, the same as the particle that first entered. Thus the phages exhibit genetic continuity. It follows, therefore, that a biological system consisting of no more than a protein coat and a DNA core contains all the genetic information needed to direct a bacterial cell to make more T_2 phage. These phage particles are only about one-fifth of a micron in length. They are, therefore, far smaller than the familiar carriers of genetic information— the chromosomes. In this genetic system, so simple that it consists of only two parts, it might be possible to determine which part is the molecular basis of inheritance. Does inheritance in the T_2 phage depend on the protein coat, on the DNA core, or on the interaction of both? A partial answer to this question was provided in 1952 by A.D.Hershey and Martha Chase.

Core or Coat. Hershey and Chase used radioactive substances to tag separately the protein coat and the DNA core of the phage. This is possible because of a fundamental chemical difference between the protein and DNA. DNA is rich in phosphorus, but it contains no sulfur. On the other hand, the protein of the phage coat contains sulfur, but little or no phosphorus. In 1952 radioactive isotopes of both phosphorus, such as P^{32}, and sulfur, such as S^{35}, were available. It should be possible, therefore, to obtain phage with its protein containing radioactive sulfur and its DNA containing radioactive phosphorus.

Since the phage reproduced only in the living cells of *E. coli,* ma-

terials in the cell of *coli* must be the source of the newly synthesized phage. It was necessary, therefore, to introduce the radioactive markers into the T_2 phage by way of the bacterial cell.

The procedure was as follows. One group of bacteria was grown in a medium that contained small amounts of P^{32}. The phosphorus entered the bacterial cells and became part of the cell's molecules. Another group of bacteria was grown in a medium containing S^{35}. Each group of bacteria was allowed to grow for about four hours and was then infected with phage. The phage entered the bacterial cells and reproduced. The new phage was produced, of course, from the materials in the bacterial cells, which contained the radioactive markers. Thus in one group of phage the protein coats became marked with S^{35} and in the other group the DNA was marked with P^{32}. It would then be possible to trace the movements of the radioactively labelled phage protein in one case and the DNA in the other during the infection of bacteria.

At this point we must digress to mention an important observation

7–1 The T_2 phage as photographed with the electron microscope at a magnification of 37,000× (photo by J.S.Murphy. *Journal of General Physiology* 36:28).

concerning the mechanics of infection. Other investigators had found that the T_2 phages were elongate structures with a wide cylindrical 'head' and a narrow cylindrical 'tail' (Fig. 7–1). Photos taken with an electron microscope showed the tail of the phage attached to the cell wall of the bacterium. To these observations, Hershey and Chase added others that seemed to indicate that the phage became attached to the bacterium and then injected its DNA into the cell. If only the DNA of the phage entered the cell, one could conclude that the DNA alone carried genetic information. Having tagged either the protein or the DNA of the phage, Hershey and Chase were in a position to test this hypothesis.

Bacteria were infected with phage that had their protein coats labelled with S^{35}. A few minutes later the bacteria were put in a Waring blendor. The cells are too small to be injured by the whirling blades. But the solution was agitated so violently that the phage particles were torn loose from the bacterial cell walls. The cells were then separated from the fluid medium. Both fractions were tested for S^{35}. It was found that 80 per cent of the S^{35} was in the fluid and only 20 per cent was associated with the cells. Nevertheless, the cells were infected: in 20 minutes they burst and liberated a new crop of phage.

In a parallel experiment, other bacteria were infected with phage that had their DNA labelled with P^{32}. In a few minutes the bacteria, plus phage, were put in a Waring blendor, the phage ripped from the bacterial cells, and then the cells and phage separated. Analysis of the cells and fraction containing the phage gave results precisely opposite to those observed with S^{35}. This time it was found that about 70 per cent of the P^{32} was associated with the cells and only 30 per cent with the detached phage particles. Once again, the phage reproduced in the cells, in spite of the drastic treatment.

Thus infection and phage reproduction occurred when most of the DNA entered the cell and most of the protein coat stayed on the outside. The results were somewhat equivocal, for all of the S^{35}, which was the marker for protein, had not remained on the outside. Nevertheless it was not unreasonable to advance this working hypothesis: the phage DNA carries all the genetic information needed for phage replication.

Another way to test this hypothesis is based on the following argument. The hereditary substance is undoubtedly more stable than other substances in the organism. We should expect, therefore, that it would persist intact generation after generation, while any non-hereditary materials would not. If the P^{32} is associated with the hereditary material but the S^{35} is not, one would predict different behaviors for them.

Consider first the case of a phage particle with its DNA marked with P^{32} infecting a cell. If Hershey and Chase were correct, the DNA alone would enter the cell. Reproduction would begin, and the original DNA would be divided among the daughter phage particles and become diluted, so to speak, but not diminished in total amount. After the infection cycle was completed, and the cell ruptured, the 100 liberated phages should have among them the original DNA of the entering phage. Any hereditary substance should be expected to behave in this way. If the protein were a hereditary material, it should behave in the same way; if not, we might expect that only a small portion of the S^{35} that entered the cell would appear in the progeny.

Hershey and Chase put these ideas to experimental test. They found less than one per cent of the S^{35} of the initial phage was recovered in the daughter phages. Other investigators (including James D.Watson of whom we shall hear more shortly) had just reported that about 50 per cent of the P^{32} that first entered the bacterial cells was recovered in the daughter phage particles.

Hershey and Chase concluded, 'Our experiments show clearly that a physical separation of the phage T_2 into genetic and non-genetic parts is possible…. The chemical identification of the genetic part must wait, however, until some of the questions asked above have been answered.' These questions were: '(1) Does any sulfur-free phage material other than DNA enter the cell? (2) If so, is it transferred to the phage progeny? (3) Is the transfer of phosphorus (or hypothetical other substance) to progeny direct—that is, does it remain at all times in a form specifically identifiable as phage substance—or indirect?'

Hershey and Chase were showing necessary and commendable caution in interpreting their remarkable experiments. The implication of their experiments was clear, however: DNA *is* the substance of inheritance in T_2 phage. When one remembers the near universality of genetic phenomena, it is not too difficult to extend the hypothesis to cover all organisms: DNA is the substance of inheritance; the chemical compound of which the genes are composed.

With this hypothesis in mind, we can reinterpret the experiments on transformation in Diplococcus. Avery and his co-workers had shown that DNA extracted from capsulated cells could cause non-capsulated cells to form capsules. They suggested that the DNA stimulates a specific gene mutation in the non-capsulated cells. In the light of the work of Hershey and Chase, an entirely different mechanism for transformation might be proposed: The extracted DNA consists, in part, of genes that have the ability to cause the cell to synthesize capsules; these genes enter the non-capsulated cells and become part of the genetic machinery

of the invaded cells; in their new environment they initiate the synthesis of capsules. Avery's extract, therefore, could be thought of as containing functional genes. These genes could 'infect' bacterial cells in much the same way as phage infect *E. coli.*

The experiments of Avery, MacLeod, and McCarty, and of Hershey and Chase, were outstanding examples in a large body of data that made it increasingly probable that the substance of inheritance is DNA. In a few viruses, such as the tobacco mosaic virus, the closely similar ribonucleic acid (RNA) appeared to be the hereditary material. Apart from these few exceptions, all of which are viruses, the most likely candidate for the hereditary molecule was DNA.

Many important observations were made possible by a staining procedure known as the Feulgen reaction. This procedure, first developed in 1924, proved to be a specific stain for DNA. That is, when properly used, only the DNA of cells is stained. It is possible, therefore, to use this technique to localize the DNA in cells. Numerous observations revealed that the nuclei of animal and plant cells are rich in DNA. The cytoplasm is never stained, or is at most, stained very feebly. During mitosis the chromosomes stain deeply whereas in non-dividing cells the nuclei are nearly uniformly stained. Thus the DNA was localized in precisely that part of the cell where the geneticists had unequivocally located the genes.

Late in the 1940s, A.W.Pollister, of Columbia University, and others perfected a photometric method for measuring the amount of DNA in a single nucleus. The Feulgen reaction can be used as a quantitative method for measuring DNA, that is, the amount of dye bound is proportional to the amount of DNA. Cells are stained and put under a microscope. Exceedingly sensitive photo-cells (working on the same principle as the familiar exposure meters of the photographer) are used to measure the amount of light that passes through the nucleus. The amount of light that passes depends on the amount of stain in the nucleus. If the nucleus contains much DNA, the stain is heavy, and little light will pass. This method allows one to measure quite accurately relative amounts of DNA in different nuclei.

Many investigators used this technique to measure the DNA in a wide variety of tissues of animals and plants. The basic findings were these: in any species the diploid nuclei of the somatic cells appear to have the same amount of DNA; after meiosis, however, the nuclei of sperm and ova have only half as much DNA. An exact parallel is found, therefore, between the amounts of DNA and the number of chromosomes.

The work surveyed in this chapter leads to this tremendous thought: the once mysterious gene, which could be mapped but not known, was

revealed as an identifiable molecule—deoxyribonucleic acid. Clearly we must learn more about the nature of this DNA, and we shall do so in the next chapter.

Suggested Readings

A few general references are given here but, since the questions raised in this chapter find their answers in the next, one should also refer to the general references listed there. The *Readings* include a fascinating letter written by Avery to his brother describing his experiments on transformation.

DUNN, L.C. 1969. 'Genetics in historical perspective.' In *Genetic Organization.* Volume 1. Edited by Ernst W.Caspari and Arnold W.Ravin. New York: Academic Press.

RAVIN, ARNOLD W. 1965. *The Evolution of Genetics.* New York: Academic Press.

WHITEHOUSE, H.L.K. 1969. *Towards an Understanding of the Mechanism of Heredity.* New York: St. Martin's Press.

The original papers referred to most frequently in this chapter are:

ALLOWAY, J.L. 1932. 'The transformation in vitro of **R** pneumococci into S forms of different specific types by the use of filtered pneumococcus extracts.' *Journal of Experimental Medicine 55:*91–9.

AVERY, O.T., C.M.MACLEOD, and M.MCCARTY. 1944. 'Studies on the chemical nature of the substance inducing transformation of pneumococcal types.' *Journal of Experimental Medicine 79:*137–58.

DAWSON, M.H. 1930. 'The transformation of pneumococcal types.' *Journal of Experimental Medicine 51:*99–147.

GRIFFITH, FRED. 1928. 'The significance of pneumococcal types.' *Journal of Hygiene 27:*113–59.

HERSHEY, A.D. and MARTHA CHASE. 1952. 'Independent functions of viral protein and nucleic acid in growth of bacteriophage.' *Journal of General Physiology 36:*39–56.

8 DNA—Structure and Function

Few chemistry textbooks written before 1950 had much to say about nucleic acids. Little was known of their chemical nature and nothing of their function. Apparently they were always associated with proteins, forming a class of compounds known as nucleoproteins. Their history began in 1868 when Friedrich Miescher obtained the first crude preparation by extracting used surgical bandages, which were permeated with pus cells. Later Miescher extracted nucleic acid itself from fish sperm. Sperm might be expected to be a rich source of nuclear substances because the nucleus occupies such a large part of the cell. In fact the ratio of nucleus to cell is higher for sperm than for any other cell. Later it was discovered that the thymus gland is also a rich source of nucleic acid. Many chemical studies were made on calf thymus glands, which were obtained from local slaughter houses.

When nucleic acid of the thymus gland was hydrolyzed, it was found to consist of only a few components: adenine, guanine, cytosine, thymine, deoxyribose (a sugar), and phosphoric acid (Fig. 8–1).

Yeast cells were also extensively studied. Their nucleic acid was found to be much like that of the thymus but it differed in having uracil instead of thymine and ribose instead of deoxyribose. Gradually the belief arose, now known to be incorrect, that animal cells have one type of nucleic acid (with thymine and deoxyribose) and plants have another (with uracil and ribose).

The nucleic acids and nucleoproteins remained the orphans of the chemist for so long largely because they had no obvious importance either inside or outside the cell. Other proteins were clearly of enormous

importance: some were the enzymes that controlled the reactions of the living cells; others were the hemoglobins that carried oxygen; still others were hormones, with their dramatic effects on a variety of life processes. In the first third of the twentieth century nucleoproteins were not extensively studied because there was no urgent reason for doing so. The number of scientific problems that *might* be studied is always far greater than the number that *can* be studied—scientific manpower is always insufficient.

The biochemists of this period concentrated largely on problems associated with the release and utilization of energy within the cell. Here was a problem of clear and obvious importance. It was vigorously investi-
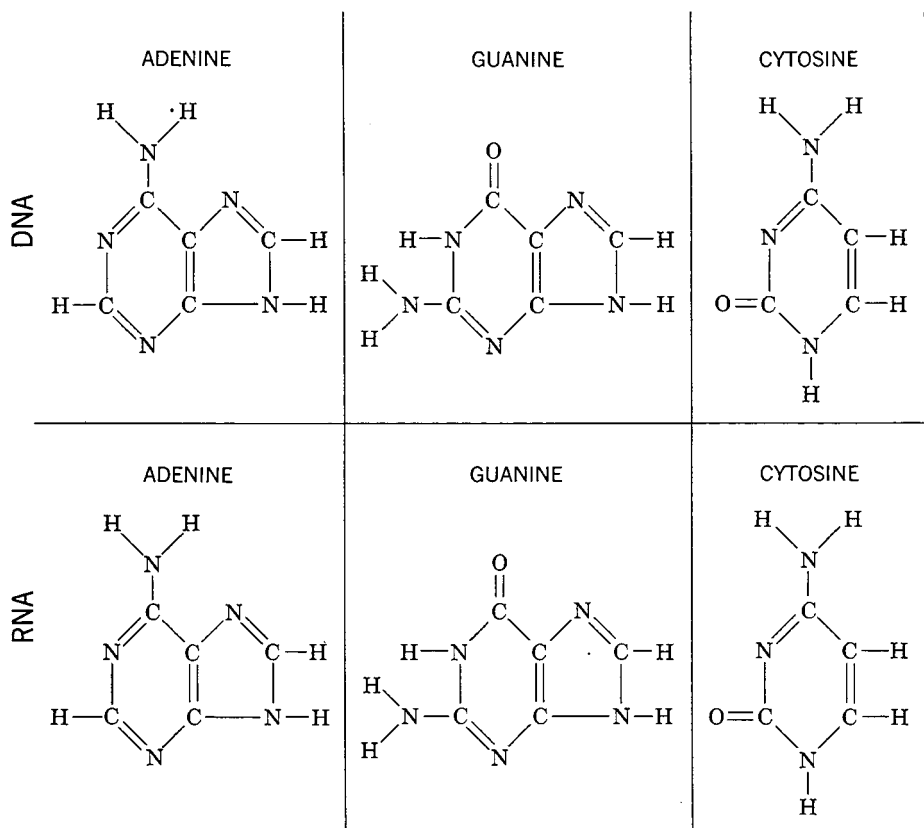
8–1 The hydrolysis products of DNA and RNA.

gated and, at one level of analysis, essentially answered. By 1950 the numerous reactions, each with a specific enzyme, in the pathway from glucose to the end products, carbon dioxide and water, were thought to be known. Adenosine triphosphate (ATP) had been identified as a key substance in the storage and transfer of energy within the cell. These biochemical pathways were adorned with many Nobel Prizes.

The experiments described in Chapter 7, however, suggested that DNA is vitally involved in inheritance. In 1944 it was established that DNA is the transforming substance in Diplococcus; evidence obtained in 1952 suggested strongly that the entire genetic information of the T_2 phage is DNA. With leads of this sort, it is not surprising that many

THYMINE	DEOXYRIBOSE	PHOSPHORIC ACID	
			DNA
URACIL	RIBOSE	PHOSPHORIC ACID	
			RNA
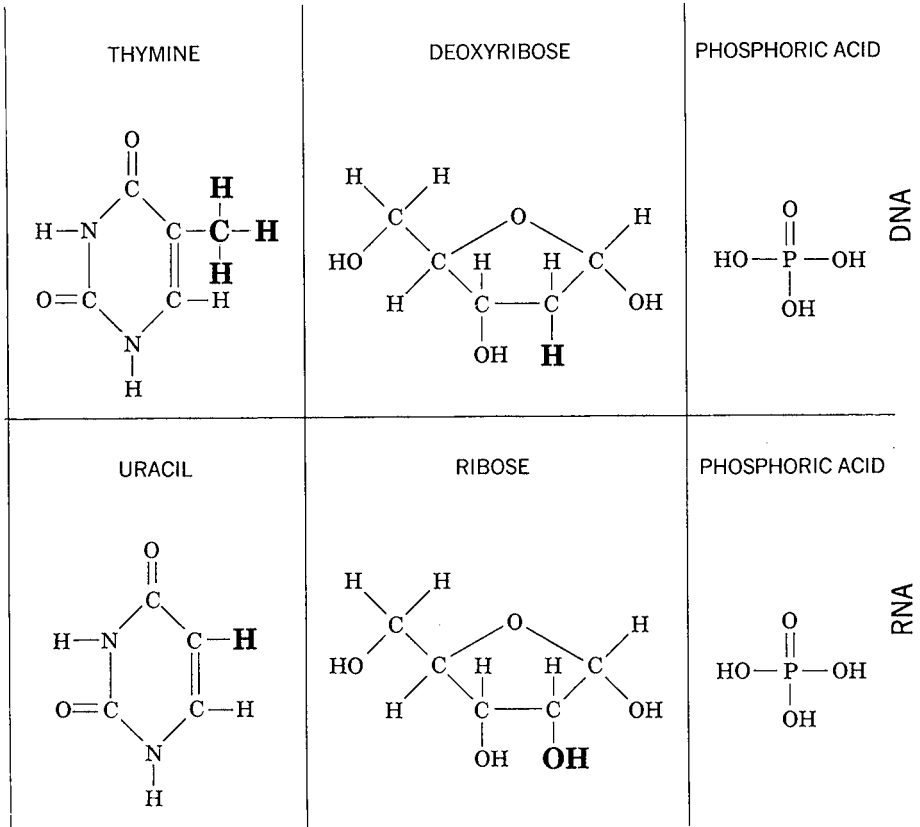
biologists turned their attention to DNA. There working hypothesis was: DNA is the hereditary material.

The possibilities for gaining new insights into genetic mechanisms were enormous with a hypothesis so specific. The hypothesis linked two fields, chemistry and genetics, creating the possibility of testing deductions of a chemical nature with genetic data. Conversely, genetic deductions could be tested with data on the chemical nature of DNA.

It is worth a brief digression to emphasize the tremendous utility of hypotheses of this type. They have been characteristic of genetics since the early days, and one might even say that they were largely responsible for the rapid progress in the field. Recall that Sutton's basic hypothesis was that genes are parts of chromosomes (Chapter 4). If this is so, then one should observe a parallel between the behavior of chromosomes in meiosis and fertilization and the behavior of the Mendelian factors in inheritance. Sutton found such a parallel and speeded genetics on its road to becoming a science. In later years geneticists and cytologists constantly checked the discoveries of one field against those of the other. Genetic data first suggested the hypothesis of crossing over. A basis for the event was then found in careful studies of the chromosomes during meiosis. Bridges advanced the hypothesis of non-disjunction on the basis of genetic data and tested his hypothesis by a study of the chromosomes of his experimental material. The hypothesis that pieces of chromosomes may become inverted was suggested by genetic data and confirmed by a study of the salivary gland chromosomes. This type of rigorous checking of the hypotheses of one field by the data of another has not been generally possible in biology.

To return to the main argument: we can test our hypothesis about the DNA molecule with the well-established principles of genetics. Similarly we can anticipate that, as knowledge of the chemistry of DNA becomes available, new insights into genetic mechanisms will be obtained. In order to proceed, we shall accept as true the hypothesis that the gene is DNA. The following deduction follows logically: DNA must have a structure and a composition that will account for the known properties of genes. Let us recall some of these basic properties.

Linkage data localized the gene as part of a chromosome. Experiments on crossing over showed that the genes are in a linear order and in a definable site on the chromosome. Genes were found to be exceedingly stable. Barring mutation, which is a rare phenomenon, the gene maintained its integrity generation after generation. This stability continued even with frequent replication. At each mitotic division every gene becomes two, and at anaphase one gene goes into each daughter

cell. A gene might replicate a hundred thousand times or more without making a mistake. Yet from time to time mistakes—or mutations—occur. Such mutations are essential for the welfare of the species, for they are the raw materials of evolutionary change.

But genes do more than merely maintain themselves. They have specific effects that the geneticist observes as the phenotype of the cell or individual.

These more important properties of genes can be summarized as follows:
1. Genes have the ability to make copies of themselves.
2. Genes carry hereditary information.
3. Genes are able to transfer this information to the rest of the cell.

On the basis of our hypothesis, therefore, the DNA molecule must have a structure that can replicate, carry information, and translate this information into the phenotype of the cell.

THE WATSON-CRICK MODEL

Did DNA have the necessary properties to be the gene? No one knew in 1950, but an American biologist, James D.Watson (born 1928, now of Harvard University), and his English associate, Francis Crick (born 1916, of Cambridge University), addressed themselves to the problem. They sought to devise a model of the DNA molecule that would satisfy the requirements imposed by the genetic data.

In two papers, one published in April and the other in May of 1953, they showed how the few facts known about DNA could be used to construct a model of its structure. This model was, in terms of scientific methodology, a hypothesis. These were the facts:
1. DNA is composed of six kinds of molecules; adenine, guanine, thymine, cytosine, deoxyribose, and phosphoric acid. Each adenine, guanine, thymine, and cytosine combines with a molecule of deoxyribose and phosphoric acid. The four combinations are known as *nucleotides*.
2. Many of these nucleotides combine to form the huge DNA molecule. (Watson and Crick were attempting to determine the precise nature of their combination.)
3. The available data on the X-ray diffraction patterns suggested to Watson and Crick that the DNA molecule consists of two long fibers twisted around one another to form a double helix (like double spiral staircases, one for ascent and one for descent).

4. X-ray data indicated that the diameter of the double helix is about 20 Ångstrom units.
5. Each fiber of the double helix consists of phosphate and deoxyribose units alternating with one another: phosphate-deoxyribose-phosphate-deoxyribose, and so on.
6. The adenine, guanine, cytosine, and thymine units (collectively known as nitrogenous bases) are attached to the phosphate-deoxyribose chain.
7. In different cells of the same species, the relative amounts of adenine, guanine, thymine, and cytosine are the same.
8. In different species, the relative amounts of adenine, guanine, thymine, and cytosine vary greatly.
9. In all cells that had been studied, the amount of adenine was found to equal the amount of thymine and the amount of guanine was found to equal the amount of cytosine. This was discovered by Erwin Chargaff of Columbia University.

Neither Watson nor Crick discovered even one of these facts about DNA, which had been slowly accumulating over the years and were available to all interested in DNA. It was Watson and Crick who first saw how the data could be unified into a model that would satisfy both the genetic and the chemical requirements for the molecular structure of DNA. Their triumph was of the mind, not of the laboratory. Half a century earlier, Sutton had made a similar contribution. Although he did study the chromosomes of grasshoppers, he merely confirmed what others had already established. He saw the relation of the data of Mendel and of the cytologists and combined them to arrive at the hypothesis: genes are parts of chromosomes. Sutton's feat was an exercise of pure intellect, as was that of Watson and Crick. But what *was* their model?

The critical aspect of the Watson-Crick model is the positioning of bases on the two entwined strands. Since the relative amounts of the different bases vary from species to species, there can be no single structure for all DNA. Yet a striking regularity exists: the amount of adenine always equals that of thymine and the amount of guanine always equals that of cytosine.

This led Watson and Crick to predict that, wherever there is an adenine on one strand, there is a thymine opposite it on the other strand; and similarly, that guanine and cytosine are also opposite one another. Thus if we unwind the double helix, the arrangement of the bases will be as shown in Figure 8–3. The adenine and thymine, as well as the guanine and cytosine, were assumed to be held loosely to one another
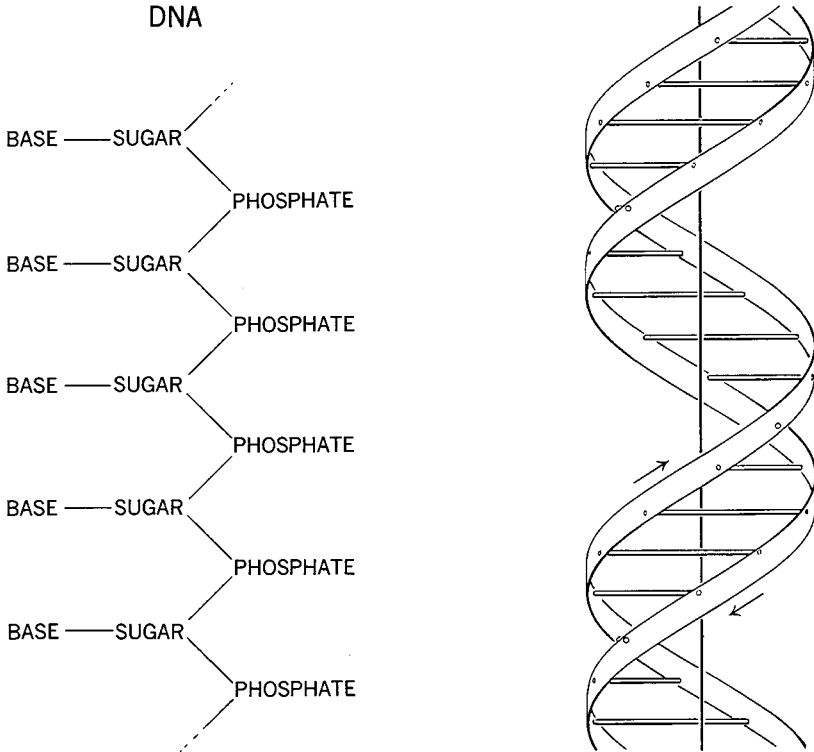
8–2 Figures 1 and 2 (redrawn) from the original paper by Watson and Crick (*Nature 171*:965).

by hydrogen bonds. These bonds would form if the bases were opposite one another in the positions suggested by the model.

Further evidence for this specific pairing came from data on the relative sizes of the bases and of the diameter of the DNA molecule. Two of the bases, thymine and cytosine, are relatively small. Adenine and guanine are larger, as can be seen from the diagrams of the molecules in Figure 8–1. Thus the pairing that Watston and Crick assumed is always of one large and one small base. Such pairing fits well with the apparently uniform diameter of the DNA, which X-ray data revealed to be 20 Ångstroms. If the pairing were between a cytosine on one strand and a thymine on the other, the diameter would be less than 20 Ångstroms. Similarly if adenine and guanine were to pair, the diameter of the double helix would be more than 20 Ångstroms. The data, there-
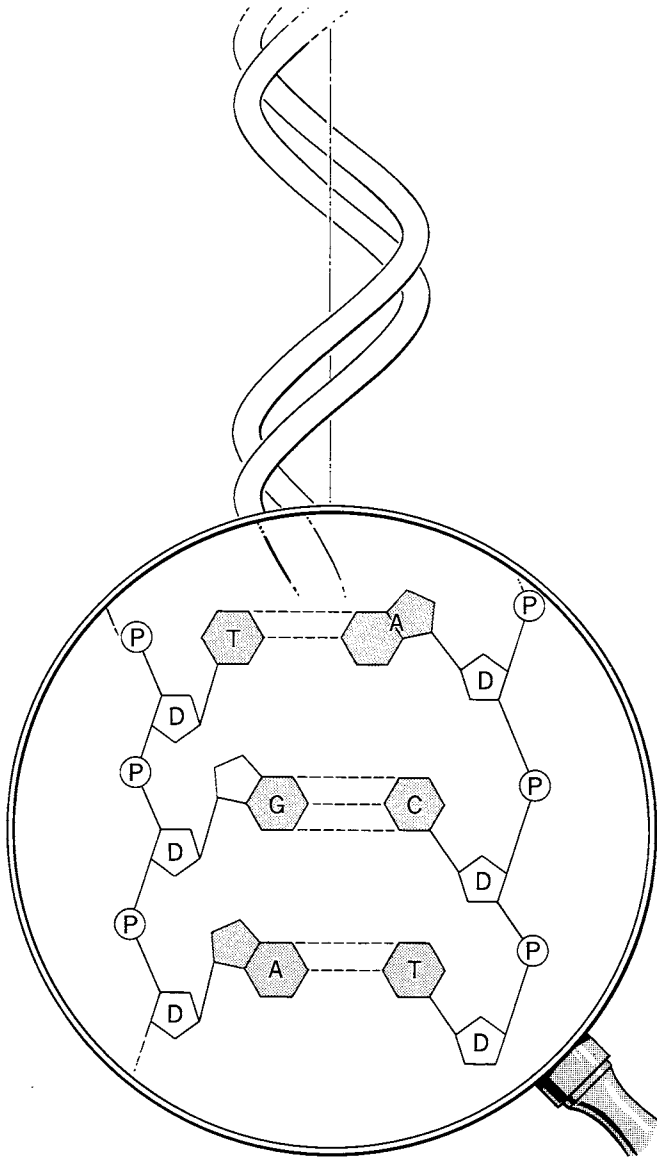
8–3 A highly schematic reconstruction of the double helix formed by the DNA molecule. The lower part of the helix is enlarged to show the bases adenine (A), thymine (T), guanine (G), and cytosine (C) and how these bases are linked with deoxyribose (D), and phosphoric acid (P). Refer to Figure 8–1 for more details of the molecular structures.

fore, were best explained on the assumption that adenine always pairs with thymine and guanine always pairs with cytosine. So far as size relations are concerned, adenine could pair with either of the smaller molecules—cytosine or thymine. Similarly, guanine could pair with either cytosine or thymine. One would then expect the amount of adenine+guanine to equal the amount of cytosine+thymine. Chargaff's measurements showed this to be true but they showed more than that: he found that the amount of adenine is equal to the amount of thymine and the amount of guanine equals that of cytosine. Had each base also accounted for 25 per cent of the total, one could still maintain that either large base could pair with either small base at random. But the amounts are not equal: the amount of adenine+thymine may differ considerably from the amount of guanine+cytosine. The data were best explained, therefore, by assuming a specific pairing of adenine with thymine and guanine with cytosine.

The Watson-Crick model for DNA, then, consists of two long and closely associated strands wound around one another. The strands are complementary to one another, in the sense that what is present on one strand automatically specifies what is on the other. Thus if the sequence of bases on one strand is adenine-adenine-cytosine-thymine-guanine-thymine, that of the other would have to be thymine-thymine-guanine-adenine-cytosine-adenine. Since the sugar-phosphate parts of the molecule are always the same and only the sequence of the bases can vary, Watson and Crick hypothesized 'it therefore seems likely that the precise sequence of bases is the code which carries the genetical information.'

Accounting for Replication. The gene can make an exact copy of itself; if DNA is the gene it must have the same ability. This was possible with the Watson-Crick model and the argument was developed as follows: 'Previous discussions of self-duplication have usually involved the concept of a template, or mould. Either the template was supposed to copy itself directly or it was to produce a "negative," which in its turn was to act as a template and produce the original "positive" once again. In no case has it been explained in detail how it would do this in terms of atoms and molecules. Now our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds [between the bases opposite to one another in the two strands] are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have *two* pairs of chains, where we only had one before. More-

Replication
not begun

Replication
in process

Replication
completed
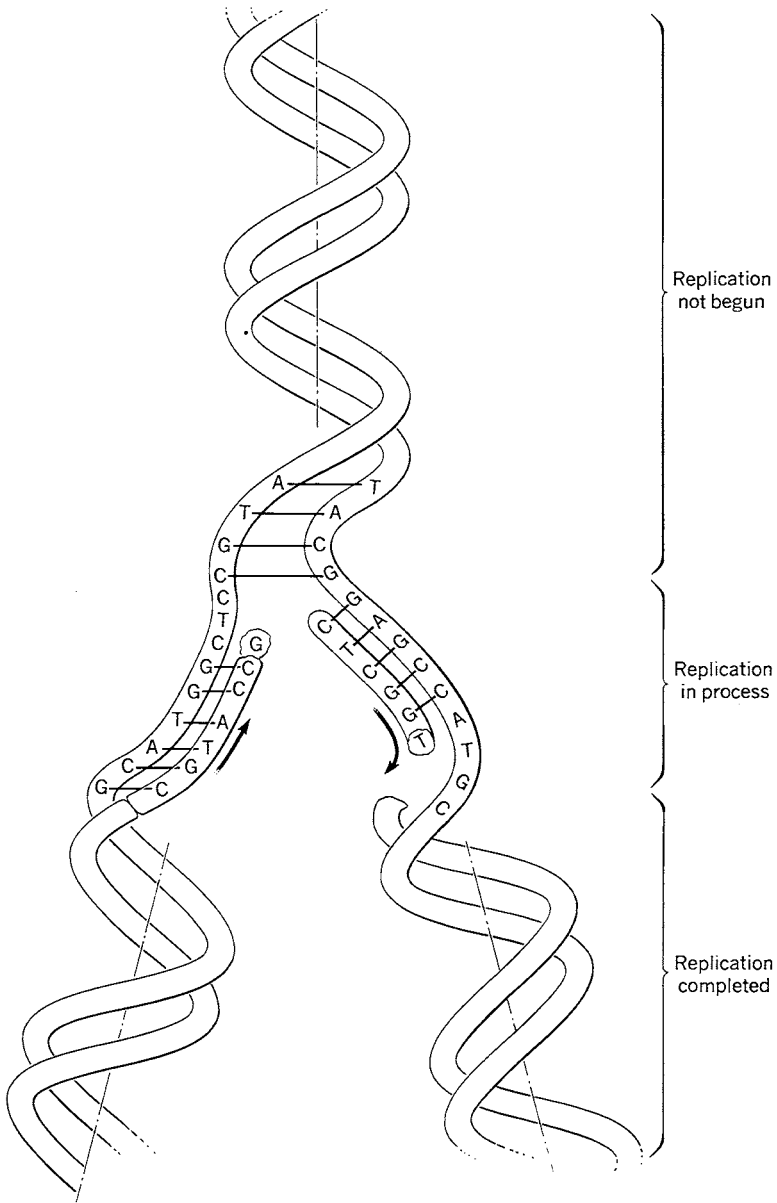
8–4 The replication of DNA. During replication the two strands of DNA separate from one another. Each strand then serves as the template for the synthesis of a complementary strand. The available data indicate that replication occurs over short stretches at a time. In this diagram the upper portion of the DNA double helix has not started to replicate. In the central section the strands have separated

over, the sequence of the pairs of bases will have been duplicated exactly…. Despite [some] uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems —the molecular basis of the template needed for genetic replication. The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.' Figure 8–4 shows in diagrammatic form how the replication is thought to occur.

In this way the Watson-Crick model accounts for the important genetic fact that genes can make exact copies of themselves. The model was a hypothesis, which opposed no known chemical or genetic facts. During the next decade an ever-increasing amount of chemical and genetic data suggested that the hypothesis was indeed correct. In 1962 Watson and Crick shared the Nobel Prize with Maurice Wilkins, the physicist from Cambridge University who had supplied much of the X-ray data indicating that DNA is a double helix with a uniform diameter of 20 Ångstrom units.

The other primary attribute of genes, their specific function in the cell, was not discussed by Watson and Crick in their first papers. This part of the problem was studied subsequently and now a probable hypothesis is at hand.

Genes and Protein Structure. The hypothesis that the chief function of genes is the control of protein synthesis became increasingly probable during the 1940s and early 1950s. A large body of work, much of it on the mold Neurospora, was best interpreted as indicating that genes produce or control the production of enzymes, which in turn control the numerous biochemical events that occur in all cells (Chapter 6). There was no clear understanding of how genes exert their control; do

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and nucleotides are combining to form the new strands. It is probable that the direction of synthesis, as indicated by the arrows, is anti-parallel. Replication produces two identical daughter strands. For example, note the site on the double helix just above the area of replication: there is a C-G unit. When these separate, the G will be united with a C in its new complementary strand and the C with a G in its new complementary strand. The two new double helices, therefore will be C-G and G-C. The lower section shows a section where replication has been completed. You will notice a few loose ends of DNA strands; these will be joined once replication has been completed in the central section. This diagram is based on DNA replication in bacteria; in higher organisms the process is similar but not identical.

they produce enzymes and other proteins directly or indirectly? Considerable light was shed on this question by the intensive study of a disease of man—sickle cell anemia.

Throughout much of central Africa, sickle cell anemia is common among the natives. The primary effect of the disease is on the hemoglobin of the red blood cells. When these cells are in capillaries where the oxygen concentration is low, they may change from a round to an elongate or even to a sickle shape. These abnormally shaped cells may clog the capillaries and the smallest arteries. Many are destroyed, which causes the anemia. The number of red blood cells may be as few as two million per cubic millimeter, in contrast to the normal number of five million. Infant mortality is high and few individuals with the disease live beyond 40 years.

Genetic analysis has shown that the disease is caused by an autosomal gene, which is symbolized Hb_1^S (the normal allele is Hb_1^A). Homozygous individuals, $Hb_1^S Hb_1^S$, have the severe anemia already described. Heterozygous individuals, $Hb_1^S Hb_1^A$, are nearly normal, however their red blood cells do show abnormal shapes when subjected to very low oxygen concentrations.

Hemoglobin is obviously an important protein and a great deal is known about its chemistry. Hemoglobin A is the common type in man. There are several other kinds, all differing only slightly from one another in the sequence of amino acids of which they are composed. Each molecule of hemoglobin A consists of about 600 amino acids, of 19 different kinds. These amino acids are arranged in four polypeptides—long chains of amino acids. Each molecule of hemoglobin is composed of two α polypeptides and two β polypeptides. The α and β chains differ from each other in length and in the sequence of their amino acids. The four polypeptides of the molecule are linked together and folded in a compact and specific manner to give the hemoglobin molecule a globular shape.

Since the most obvious feature of sickle cell anemia is the abnormality of the red blood cells, it is reasonable to suppose that the hemoglobin of these cells might also be abnormal. Linus Pauling (born 1901) and his associates at the California Institute of Technology began an investigation to see if this was so.

Their material was blood from three types of individuals: normal, $Hb_1^A Hb_1^A$; sufferers from sickle cell anemia, $Hb_1^S Hb_1^S$; and the heterozygotes, $Hb_1^A Hb_1^S$. The blood was fractionated and the hemoglobin obtained in a nearly pure form. In most features the three hemoglobins were identical. When they were compared in an electrophoresis apparatus, however, striking differences were observed.

An electrophoresis apparatus consists basically of a long tube containing a liquid or semi-solid gel. An electric current is passed through the tube. Any charged substances placed in the gel will move, with the rate of movement depending on the size of the particle and its charge. Using this apparatus, it is frequently possible to separate different kinds of molecules in a mixture. Thus, if the three types of hemoglobin differed in their charges, they could be separated, and thereby shown to be different.

This analytical device shows that normal hemoglobin, which we can call hemoglobin A, differs from sickle cell hemoglobin, which we can call hemoglobin S (Fig. 8–5). Furthermore, heterozygous individuals produce both kinds of hemoglobin.

Pauling's findings are striking evidence that genes can affect the structure of proteins. In the presence of the Hb_1^A gene, the protein hemoglobin A is synthesized in the red blood cells; in the presence of the Hb_1^S allele, hemoglobin S is synthesized.

The next step is to compare the structures of hemoglobin A and hemoglobin S. This is an obvious step, perhaps, but one beset with tremendous difficulties. The problem was to determine the exact posi-
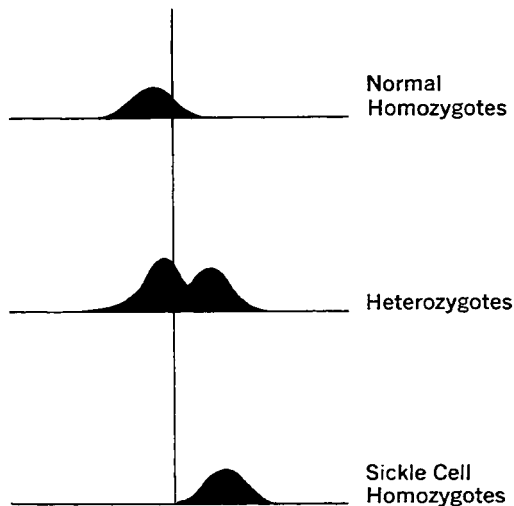
8–5 Electrophoretic patterns of hemoglobin from normal individuals, from heterozygotes, and from individuals homozygous for the sickle cell gene (modified from Pauling, Itano, Singer, and Wells, 1949).

tion of each amino acid in the total of 600. When Pauling carried out his experiments, the structure of not a single protein was known. It was not until 1954 that Frederick Sanger (born 1918), and his coworkers at Cambridge University, finally succeeded in determining the complete amino acid sequence for a protein. After ten years of intensive work they knew the position of each of the 51 amino acids in the insulin molecule.

If it took ten years to determine the structure of insulin with its 51 amino acids, how long might it take to determine the structure for hemoglobin with its 600 amino acids? The task was begun by Vernon Ingram (born 1924), then also at Cambridge University and now at the Massachusetts Institute of Technology, and his associates. They were able to learn the answer by an analytical short cut—they did not have to determine the complete structure of hemoglobin. This, however, they have since accomplished.

It was nearly impossible to study the huge hemoglobin molecule intact. A more practicable method of investigation was to break down the large molecule into smaller molecules and then to study the smaller molecules. If the structure of each of the smaller molecules could be determined, and if it could then be determined how these smaller molecules are combined, one would know the structure of hemoglobin. The older methods of the analytical chemist were not of much help. Typically, he hydrolyzes the proteins with acid and gradually breaks down the large molecules until only amino acids remain. The intermediate breakdown products are not uniform, however. A confusing mixture of large and small molecules is formed and this is not suitable for the careful analysis that Ingram was attempting. He required a precise method for breaking down the hemoglobin molecule into smaller molecules. The method he chose was to treat the protein with the enzyme trypsin. Trypsin is highly specific in action, hydrolyzing the protein only at the carboxyl side of arginine and lysine. Since these amino acids are always in the same places in the hemoglobin molecule, the hemoglobin will always be broken down in the same way. When the hemoglobin molecule is broken with trypsin, the result is 28 kinds of smaller molecules, averaging about ten amino acids each. In this manner, Ingram hydrolyzed both hemoglobin A from normal individuals and hemoglobin S from sufferers of sickle cell anemia.

Next he separated the 28 smaller molecules by a process combining-electrophoresis and paper chromatography. He put a drop of the hydrolyzed hemoglobin mixture on a piece of paper and an electric cur-

rent was passed through the paper. This method is essentially the one used by Pauling to separate the entire molecules of hemoglobin A and hemoglobin S. In this case, Ingram was attempting to separate the 28 hydrolysis products on the basis of their electrical charges. He achieved considerable separation, but not enough for analytical purposes. Then he tried another method. The edge of the same piece of paper was put in a liquid that would dissolve the 28 hydrolysis products. As the liquid moved through the paper it carried the 28 kinds of molecules with it. The different kinds were carried at different rates. At the end of the experiment, the 28 types of molecules occupied different positions on the sheet of paper—each in a specific place (Fig. 8–6). This type of separation, using both electrophoresis and paper chromatography, gave consistent results. That is, in repeated experiments with hemoglobin A, the 28 spots always occupied the same positions relative to one another.

When hemoglobin S was analyzed in a similar way, again there were 28 spots but when these spots were compared with those from hemoglobin A, there was an important difference. Twenty-seven of the spots occupied the same relative positions on two sheets of paper. The twenty-eighth pair, however, occupied slightly different positions (Fig. 8–6).
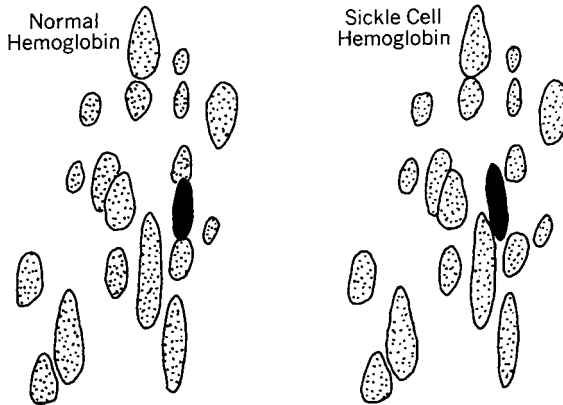
8–6 The hydrolysis products of normal hemoglobin and sickle cell hemoglobin. The products have been separated by paper chromatography and electrophoresis (not all are shown here). In most cases the hydrolysis products of normal hemoglobin and sickle cell hemoglobin occupy equivalent positions. One spot in each, shown in black, occupies a slightly different position (modified from Ingram, 1958).

The implication is that hemoglobin A and hemoglobin S are identical in all but one of the units that result from hydrolysis with trypsin. It was, therefore, important to study the one instance of difference. If the structure of this variant could be determined, one would know the difference between hemoglobin A and hemoglobin S without knowing the entire structure of either.

The variant spots were found to consist of the amino acids at the end of the β chain. Ingram was able to determine the exact sequence which, using abbreviations for the amino acids, proved to be this:

Hb A: -lys—glu—glu—pro—thr—leu—his—val
Hb S: -lys—glu—val—pro—thr—leu—his—val

The two sequences are identical except for the sixth position from the end: in hemoglobin A this is occupied by glutamic acid; in hemoglobin S by valine. This single difference in the β chains seems to be the ultimate cause of sickle cell anemia. When the hemoglobin molecule has valine instead of glutamic acid at this one site, it cannot function normally. The blood of heterozygous individuals, having both the Hb_1^A and Hb_1^S alleles, has hemoglobin of both kinds.

The conclusions that can be drawn from these observations are simple, specific, and staggering. In a normal individual, the β chain of hemoglobin A is made under the influence of one allele at the Hb_1 locus. In individuals with sickle cell anemia, an abnormal hemoglobin, hemoglobin S, is made under the influence of a mutant allele. When formed by the mutant allele, the β chain of the hemoglobin differs from the normal in a single amino acid substitution: valine rather than glutamic acid is the sixth amino acid from the end of the long chain of 146 amino acids. Thus genes can be implicated in the very basic steps in protein synthesis: the insertion of single amino acids in a polypeptide chain. Since the cell produces its proteins by combining amino acids, genes can affect the most elementary steps in the process.

The link between gene and protein was now secure. The line of investigation that began uncertainly with Garrod and was renewed by Beadle and Tatum, was revealing the molecular biology of the gene.

Having established that genes can control the synthesis of proteins, it then became essential to discover how they do it. This proved to be quite an undertaking. After all, the genes are in the nucleus and most of the cell's proteins are in the cytoplasm. How, then, could one account for the origin of the cytoplasmic proteins? One could hypothesize that they are produced in the nucleus and then pass into the cytoplasm. Alternatively, one could imagine that some influence, or information, passes from the nucleus to the cytoplasm and directs the synthesis of cyto-

plasmic proteins. The preliminary answers to these questions came from studies of the distribution of nucleic acids in the cell and on purified fractions of cells that seemed to be able to carry out *in vitro* the same reactions that occur *in vivo*.

Localizing Nucleic Acids. During the 1930s methods were perfected for detecting both DNA and RNA *in situ*. Some of the methods depended on the different staining reactions of DNA and RNA. The Feulgen method, already mentioned, was specific for DNA. Another cytological method involved the use of two dyes, methyl green and pyronin. Methyl green was found to stain DNA and pyronin to stain RNA. Spectrophotometric methods were also developed to detect the nucleic acids. The bases in these compounds (and in a few others in the cell, such as ATP) intensely absorb ultraviolet light having a wave length of 260 mμ. There are no other compounds in the cell having this specific absorption. Since the bases are situated largely in DNA or RNA, a peak absorption at 260 mμ indicates the occurrence of nucleic acids. This method will not distinguish between DNA and RNA; it measures total DNA plus RNA plus any other substances, such as ATP, that contain the bases. If one knows the total, however, and then measures the DNA by the Feulgen method, the difference will be largely the RNA.

These early methods suggested that the nucleic acids are distributed differently in the cell. The DNA appeared to be largely or entirely restricted to the nucleus. Most of the RNA was thought to be in the cytoplasm but nucleoli were rich in it and some observers thought they saw RNA closely associated with the chromosomes.

Once it was known that DNA is of great importance in the cell, it was reasonable to suppose that the closely similar RNA was also of importance. The differences between the two nucleic acids are small: RNA has uracil instead of thymine as one of its four bases and its sugar is ribose instead of deoxyribose (Fig. 8–1).

One of the first hypotheses of the role of RNA in the cell was based on the observation that cells that synthesize large amounts of protein are rich in RNA. In the liver and pancreas, where there is much protein synthesis, there is usually from two to eight times as much RNA as DNA. In the kidney, brain, spleen, and thymus, which synthesize much less protein, there are usually equal amounts, or there may even be more DNA than RNA. Possibly this correlation has a causal significance.

This was the view of Jean Brachet, of the Free University of Brussels. In the mid 1940s he observed repeatedly that cells synthesizing large amounts of protein stain heavily for RNA. The stain seemed to be taken up by tiny granules but these were too small to be studied with

the compound microscope. Later, when the electron microscope methods were perfected for studying cells, the existence of these granules was confirmed. Since they are rich in RNA, they are called ribosomes. Some appear to be free in the cytoplasm; others are situated on the walls of a network of tubes known as the endoplasmic reticulum (Fig. 8–7). Brachet and others suggested that the ribosomes must have something to do with protein synthesis. Since the ribosomes are rich in RNA, possibly it is the RNA that is concerned with protein synthesis.

If this hypothesis was to be tested, new methods had to be developed. These methods involved the isolation of different parts of the cell by such gentle procedures that they could still carry out some of their functions.

Cell-Free Systems. In the century following the formulation of the cell theory, the cell came to be regarded not only as a unit of structure but
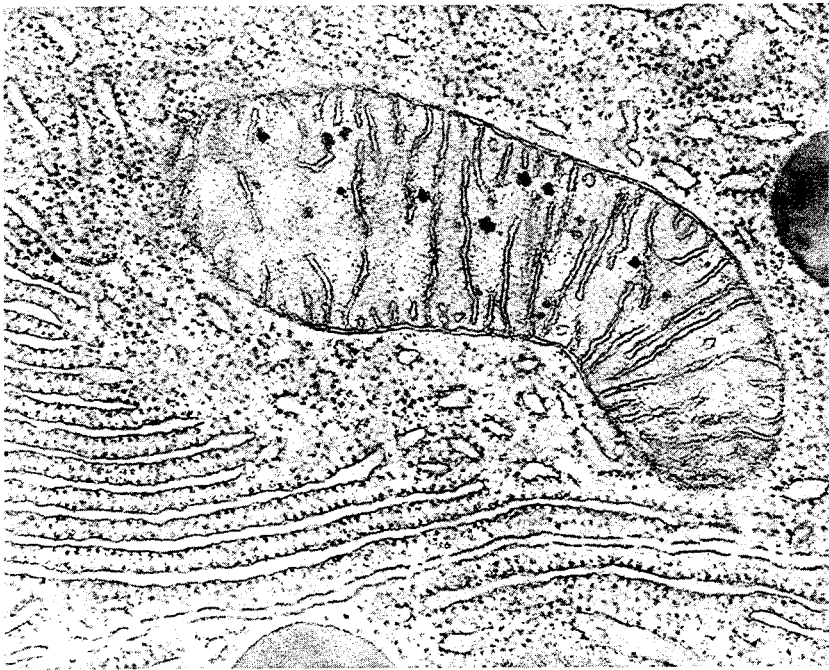
8–7 A portion of a cell from the pancreas of a bat. The large sausage-shaped structure is a mitochondrion. The tubes below it are parts of the endoplasmic reticulum. The dark granules associated with the endoplasmic reticulum, and also free in the cytoplasm, are ribosomes (photograph by Keith Porter).

also as a seemingly indivisible unit of function. Few of the complex events that occurred in cells could be duplicated apart from them. It was known that enzymes could act *in vitro* and that some small organic compounds could be synthesized from inorganic molecules. But a biochemist, who could easily synthesize fats, carbohydrates, and proteins in his body, was powerless to accomplish this feat in his laboratory. This inability to reproduce *in vitro* common events occurring *in vivo* did not suggest the need for some vitalistic principle. It was realized that the biochemical events that occur within cells frequently require dozens of different kinds of enzymes, specific sources of energy, and varied raw materials. The oxidation of glucose, for example, was found to require dozens of enzymes and many complex molecules such as the riboflavins, cytochromes, and so on. There was no reason to believe that even these intricate chains of reactions could not be eventually carried out apart from living cells. It was merely a matter of waiting for the slow accumulation of knowledge and for the perfecting of the necessary methods to reproduce the desired conditions.

Beginning in the late 1940s there was increasing success in isolating cell fractions that were functional. The general method is to grind up, or homogenize, tissues or masses of cells and then to fractionate the homogenate, usually by centrifuging. When this is done, the heavier particles, such as nuclei and unbroken cells, are thrown to the bottom of the centrifuge tube. Smaller and less dense particles form layers above the nuclear layer. For example, a distinct layer immediately above the nuclear layer contains nearly all of the mitochondria. Above this is a layer consisting almost solely of fragments of the endoplasmic reticulum with the attached ribosomes. The uppermost layer, or supernatant, is a liquid free of all but the smallest particles. The layers are far from pure, but with this analytical procedure it is possible to obtain crude preparations of mitochondria or ribosomes (methods were developed for removing the ribosomes from the fragments of the endoplasmic reticulum).

Some of these cell-free fractions are able to function in limited ways and for limited periods of time. Thus the fraction containing the mitochondria is capable of carrying out oxidative reactions and of forming adenosine triphosphate (ATP), which is the immediate source of energy for all the cell's reactions. Of greater interest to us, Paul C.Zamecnik and his associates discovered that protein synthesis could also occur in cell-free fractions of cells. In this case, the layers containing the ribosomes and the supernatant were necessary.

At last it had become possible to study directly the synthesis of proteins and to learn how this might be controlled by genes. Probably more individuals were involved in these investigations than took part in the

entire development of genetics before 1950. The critical element in this research was the generous financial support made available by the United States Government through its agencies such as the National Science Foundation, the Public Health Institutes, and the Atomic Energy Commission. Some of the key experiments involved large teams of investigators and the use of equipment costing hundreds of thousands of dollars. Without this help many of the experiments could not have been done. Interestingly, many outstanding scientists in other countries were also supported by the U.S. This was considered to be a proper use of public funds since discoveries in science would be for the benefit of all mankind.

The theory of gene action that was eventually formulated is elegant and relatively simple, but a tortuous path led to the conceptual goal. It is useful, therefore, briefly to anticipate the conclusions so that the narrative can be followed more readily.

The primordial substance is DNA. It serves as a template for the synthesis of three kinds of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Messenger RNA carries the message 'how to make a protein' from the DNA to the cytoplasm where it comes in contact with the ribosomes. Transfer RNA combines with amino acids and brings them to the mRNA bound to the ribosomes. It is here that the amino acids separate from the transfer RNA and join one another to form a polypeptide.

The direction of this genetic control is from the gene to the amino acids but the argument can be better developed by beginning with the amino acids and working back to DNA.

Amino Acid Activation. Proteins are not only huge molecules—recall that hemoglobin is composed of nearly 600 amino acids—but they are synthesized in an exact manner. Recall again that a difference in one amino acid in β chain of hemoglobin changes that molecule from the normal oxygen-carrying substance to the defective molecule associated with sickle cell anemia. The synthetic mechanisms in the cell, therefore, must be capable of producing extraordinarily precise products.

The cell makes its proteins nearly exclusively from 20 kinds of amino acids, small amounts of which are present in the fluid portions of the cell. The initial step in the union of amino acids so they can ultimately form polypeptides involves ATP and a group of enzymes known as the aminoacyl tRNA synthetases. Energy is required and, as is usual in cells, it comes from the ATP.

For purposes of description let us assume that the cell is making a polypeptide composed of only two kinds of amino acids, alanine and

glycine, and that these alternate with one another to form a chain of 100 amino acids.

The amino acids are first *activated* by joining with ATP. This reaction occurs on the surface of one of the aminoacyl tRNA synthetases. The cell contains at least 20 kinds of these synthetases, one or more for each type of amino acid. Thus, in our example, 50 alanine molecules would combine with ATP on the surface of 50 molecules of alanine aminoacyl tRNA synthetase and the 50 molecules of glycine would do the same except that the enzyme would be glycine aminoacyl tRNA synthetase.

Transfer RNA. The next step is the union of the activated amino acid, now on the surface of the aminoacyl tRNA synthetase, with a tRNA molecule.

Transfer RNA molecules are made on the DNA template by regions that can be called the tRNA genes. They have a structure complementary to the DNA that makes them. They are comparatively short chains: some of the first for which the structure was determined consist of 77, 78, or 85 nucleotides. They are composed largely of the bases typical for RNA—arginine, guanine, cytosine, and uracil—plus small amounts of others such as inosine, psue-douridine, and ribothymidine. The cell has more kinds of tRNA than there are kinds of amino acids, yet each tRNA can react with only one kind of amino acid. Consequently in some cases there may be several kinds of tRNA for a specific amino acid. All of the tRNAs are alike in having the same sequence of bases at one end of the molecule: cytosine-cytosine-adenine.

Let us return to our example. A reaction now occurs between each molecule of activiated alanine, joined to its synthetase, with a molecule of alanine tRNA. Similarly each molecule of activated glycine on the surface of its synthetase reacts with a molecule of glycine tRNA. The reaction is the same in both cases: the amino acid is transferred from the aminoacyl tRNA synthetase to the end of the tRNA molecule that has adenine (the C-C-A end). The end result will be 50 alanine+ alanine tRNA molecules and 50 glycine+glycine tRNA molecules. The synthetases, having been freed, can now combine with other amino acid molecules and repeat the process.

The extraordinary specificity of these reactions is due to the composition of the molecules involved as well as to their precise form. The combination of the amino acid and the tRNA might appear to be non-specific, since the combination is always with an adenine at the C-C-A end of the tRNA molecule. However, the fact that a specific kind of amino acid can combine first with a specific kind of synthetase and then the combination join only with a specific kind of tRNA is due to the

three-dimensional structure of the molecules involved. The shapes of the molecules must 'fit,' otherwise there will be no reaction.

The next step will involve the separation of the amino acids from tRNA and their union in precise ways to form proteins. Ribosomes, messenger RNA, and various sorts of enzymes are involved.

Ribosomes. The ribosomes, which Brachet and others suggested were involved in protein synthesis, appear as tiny granules under the electron microscope. They may be extremely abundant: in rapidly dividing *E. coli* cells about one-third of the dry mass is composed of ribosomes. They are composed of two subunits, one large and one small. Ribosomes seem to be composed almost entirely of three types of ribosomal RNA (about 60 per cent) and protein (about 40 per cent). The rRNA is made directly on DNA by the ribosomal RNA genes. The ribosomal proteins are made indirectly by DNA in the process now being outlined. Two of the rRNAs, the longest and the shortest (they are called 23S and 5S), seem to be restricted to the larger ribosomal subunit and the middle size rRNA (called 16S) seems to be in the smaller subunit. Ribosomal RNA differs in composition from both tRNA and messenger RNA in having larger amounts of guanine and cytosine.

Messenger RNA. Messenger RNA molecules are long chains of nucleotides formed on the surface of DNA. They are complementary in composition to the DNA. That is, if the sequence of bases in a short section of DNA is:

—thymine—adenine—cytosine—guanine

then the mRNA made by this section of DNA will be:

—adenine—uracil—guanine—cytosine.

A molecule of mRNA may be formed by one gene or by several sequential genes. A special enzyme, RNA polymerase, is required for the joining of individual nucleotides to produce the mRNA molecules. After being formed, the mRNA molecules move from the surface of the DNA into the cytoplasm.

When the mRNA reaches the cytoplasm, it becomes closely associated with ribosomes. The tRNA molecules with their attached amino acids then come in contact with the mRNA. Next the amino acids separate from their tRNA carriers and join one another to form a long polypeptide chain.

Proteins are formed by the reactions just outlined but the account omits any mechanism that will lead to an exact specificity of protein

structure. Recall that we are attempting to synthesize a polypeptide chain composed of 100 amino acids, half alanine and half glycine. Let us make the reasonable assumption that our molecule cannot play its normal role in the cell unless the alanine and glycine are linked in some precise manner—we could say that the order for one end of the polypeptide might have to be: A—G—G—A—G—A—A—A—A—. How could this be done?

The discovery of the manner in which the specificity of DNA becomes reflected in the specificity of proteins was one of the seminal events in modern genetics. For this reason it is worthwhile to review the experiments that led to an understanding of what it is about a gene that permits it to have a specific function. Some of the conclusions have been anticipated but they were stated as facts. Now we will learn the experimental basis of the facts. Most of the observations and hypotheses concerned bacteria and viruses but the general conclusions are now believed to apply to all organisms.

The DNA in a bacterial cell is part of a chromosome that is free in the cytoplasm; in more complex organisms the chromosomes are in the nucleus, which is separated from the cytoplasm by a nuclear membrane. One could imagine that proteins could be made in close proximity to DNA, in which case the supervision of protein synthesis by DNA could be fairly direct. It was discovered, however, that proteins are made largely in the cytoplasm, often at a relatively great distance from the DNA.

Considerations such as these led Francis Crick, in 1958, and François Jacob and Jacques Monod (of the Pasteur Institute in Paris), in 1961, to the hypothesis that a substance must carry the message from DNA in the chromosomes to the ribosomes in the cytoplasm. Theoretical considerations, as well as some data, suggested that the hypothetical substance might be a specific kind of RNA. Therefore the name *messenger* RNA seemed appropriate.

The DNA of the gene, therefore, can be thought of as doing two things. First, it can make copies of itself in the manner suggested by Watson and Crick (Fig. 8–4). Thus, if one DNA strand has the base sequence:

adenine-thymine-adenine-cytosine-guanine-thymine

it can serve as the basis for making the complementary strand consisting of:

thymine-adenine-thymine-guanine-cytosine-adenine.

Second, the same strand of DNA can also serve as a basis for making

RNA. Remembering that RNA has uracil instead of thymine, the base sequence for a messenger RNA made from the DNA of our example will be:

uracil-adenine-uracil-guanine-cytosine-adenine.

The hypothesis of Jacob and Monod was this: genetic information is carried from genes to ribosomes by mRNA. They suggested two deductions:

1. Molecules having the assumed properties of mRNA must be present in cells that are making proteins. The molecules must be polynucleotides that are produced by the chromosomes and become attached to the ribosomes. Furthermore, the base composition of the messenger RNA must be complementary to the base composition of the DNA that makes it.

2. The same ribosomal particle should be able to participate in the synthesis of different proteins at different times, depending on the type of mRNA.

Both of these deductions have been tested and found to be true. Two experiments will be chosen from the many that establish the existence of mRNA—the first deduction.

If one wishes to observe the formation of a substance in the nucleus and its movement into the cytoplasm, special techniques must be used. Presumably these events are rapid, occurring in seconds or minutes, and the amounts of materials involved are too small to be detected by the usual methods of the analytical chemist. In many instances technical problems of this sort can be surmounted by the use of radioactive isotopes. If one wishes to trace the movements of RNA it is necessary to mark the RNA in some specific way, so that it can be distinguished from all other substances in the cell.

In 1960, M.Zalokar used a substance specific to RNA, namely, uridine (uracil plus ribose) which contains tritium (a radioactive isotope of hydrogen $-H^3$). The mold Neurospora was given the radioactive isotope for short periods of time, one to four minutes. Then he examined the cells at frequent intervals. During the first few minutes the radioactivity was restricted to the nucleus, indicating that the uridine was located in this part of the cell. After eight minutes the label began to appear in association with the ribosomes. These observations were interpreted as follows: the uridine enters the cell and, in the nucleus, is incorporated into RNA; later this RNA moves into the cytoplasm and joins with the ribosomes. This movement is exactly what one would expect of the hypothetical messenger RNA. Other data obtained with the

radioactive uridine showed that at least 99 per cent of the cell's RNA is made in the nucleus and then migrates into the cytoplasm.

Another experiment had been reported by E.Volkin and L.Astrachan in 1957. Recall that part of the messenger RNA hypothesis demands that the base composition of the messenger RNA must conform to the base composition of the DNA by which it is formed. Recall also the results when the bacterium *Escherichia coli* is infected with the T$_2$ phage (page 161): almost immediately the bacterial cells stop making their own specific molecules and begin to make phage DNA and phage proteins. If the messenger RNA hypothesis is correct, the events would be as follows: mRNA would be made on the phage DNA instead of on the bacterial DNA; this new and different mRNA would move to the ribosomes where it would give the instructions for making phage proteins. If the bacterial DNA and the phage DNA differ in their base compositions, there should be a difference between the RNA produced by an uninfected bacterial cell and that produced by a cell after it has been infected by T$_2$ phage. This expectation was borne out. After the phage had entered the cell the synthesized RNA reflected the base composition of the phage DNA, not the bacterial DNA.

The second deduction of Jacob and Monod, that the ribosome is non-specific, was also shown to be true. In 1961 S.Brenner (of Cambridge University), F.Jacob (of the Pasteur Institute), and M.Meselson (then at the California Institute of Technology) worked together at the California Institute of Technology along the general lines of those of Volkin and Astrachan. They also used *E. coli* and T$_2$ phage. Bacterial cells were given various isotopes and the experiments were designed so that the ribosomes and RNA produced before and after the phage entered the cells could be distinguished. They were able to show that '(1) After phage infection no new ribosomes can be detected. (2) A new RNA with a relatively rapid turnover is synthesized after phage infection. This RNA, which has a base composition corresponding to that of the phage DNA, is added to pre-existing ribosomes…. (3) Most, and perhaps all, protein synthesized in the infected cell occurs in pre-existing ribosomes.' Thus, 'Ribosomes are non-specialized structures which synthesize, at a given time, the protein dictated by the messenger they happen to contain.'

The Message. The sender of the message (DNA), the messenger (messenger RNA), the helphers (ribosomes), and the consequence of the message (a specific protein) have all been described—but what is the message?

Part of the answer came from arm chair speculation and part from

some extraordinarily sophisticated experimentation. The speculation, which we shall consider first, has consisted, more or less, of playing the 'numbers game.'

The basis of protein specificity lies in the sequence of amino acids that comprise the protein. The data available in 1960 suggested that, beyond a reasonable doubt, the sequence of amino acids is determined by the genes.

If DNA were composed of 20 different bases, one would suspect that each base would correspond to an amino acid. Thus a thymine in a particular location in DNA might specify that leucine should occupy a specific spot in a protein molecule. Such a scheme cannot work, of course, because there are only four bases in DNA: thymine, adenine, guanine, and cytosine.

Could two bases specify a particular amino acid? Thus thymine-guanine might be thought to be the code for leucine or for some other amino acid. This is also impossible: there can be only 4^2, or 16, permutations of pairs of the four bases—and there are 20 amino acids.

With three bases, however, there are 4^3, or 64, possible permutations. Thus a code composed of triplets of bases would be the minimum number required. The total of 64 is more than three times the number required. There is the possibility that the code for some amino acids might consist of two bases and that for others of three bases. But the scientists' love of symmetry and order led most of them to adhere to the hypothesis that groups of three bases in the DNA molecule must somehow contain the information for lining up specific amino acids in polypeptide chains.

The hypothesis was expanded to suggest this model. Let us assume, for example, that the sequence adenine-guanine-cytosine is the code for serine. The mRNA formed on this part of the DNA molecule will be uracil-cytosine-guanine (UCG). (The mRNA molecule is large and we are now discussing one small portion of it—a single triplet.) This messenger RNA molecule, with its UCG triplet, becomes attached to a ribosome. Somehow the serine tRNA, with its attached serine, reaches that portion of the ribosome containing the UCG triplet of the mRNA. The serine becomes detached from the transfer RNA and then attaches to the amino acid on the adjacent tRNA molecule on the messenger RNA. This process is continued and other amino acids are added one by one to the growing polypeptide chain. When the chain is complete it becomes detached. In this manner the code of DNA becomes reflected in the specific amino-acid sequence of the protein.

Hypotheses suggested by this model could not be tested directly by the methods then available. As is often the case, indirect methods suggested the answer.

It had been discovered by M.Grunberg-Manago and S.Ochoa (of New York University School of Medicine) that RNA could be made synthetically from mixtures of the four ribonucleotides and the enzyme polynucleotide phosphorylase. Any combination of nucleotides, or even one kind alone, can be used. Thus the enzyme, plus uracil nucleotides, will form a synthetic RNA composed solely of a long chain of these nucleotides. This particular synthetic RNA is called poly U.

Methods had been perfected, as mentioned earlier, for obtaining the synthesis of proteins in cell fractions. The basic ingredients were the ribosomes and the supernatant. These methods were further refined by W.M.Nirenberg and J.H.Matthaei, of the National Institutes of Health. They were able to obtain a cell-free system, from fractionated *E. coli* cells, that would readily combine amino acids to form proteins.

Available theory plus available techniques suggested a critical experiment. What would happen if the cell-free system, which could synthesize proteins, was given a synthetic RNA? Could this RNA serve as a messenger?

Nirenberg and Matthaei added poly U to their system plus an abundance of each of the 20 amino acids. Protein was formed, but *it consisted solely of long chains of the amino acid phenylalanine*. The other 19 amino acids were not used. Thus it seems that a sequence of uracil alone carries all the information needed to 'tell' the ribosomes to join phenylalanines together. These experiments did not indicate how many uracils were needed but, if the code is a triplet, uracil-uracil-uracil (or UUU) is the code for phenylalanine. Subsequent experiments proved that triplets of the RNA bases, which were named *codons,* constitute the code and, finally, it became possible to identify the amino acids specified by each codon (Table 8–1).

Seemingly it requires only 20 codons to specify 20 amino acids but nature has not been content with this minimum number. Of the 64 possible permutations of three bases, 61 appear to be functional in coding. Only UAA, UAG, and UGA are not (later we will learn that these triplets have other functions). This means that some of the amino acids must be specified by more than one codon. The actual number varies: for some amino acids there may be six codons and for others only one. Thus UCU, UCC, UCA, UCG, AGU, and AGC all code for serine. There are five amino acids coded by four codons each. Three codons serve for one, isoleucine. Nine amino acids are coded by two codons each. Finally, tryptophan and methionine have a single codon each.

Thus all but two amino acids have more than one codon but no codon codes for more than one amino acid. This has been one of the most important discoveries in molecular biology. The code is said to be *unambiguous* since each codon specifies only one amino acid; it is said to

Table 8–1

The 64 triplet codons that can be formed with the four RNA bases together with the amino acids they specify. U=uracil, C=cytosine, A=adenine, and G=guanine.

TRIPLET	AMINO ACID CODED	TRIPLET	AMINO ACID CODED
UUU	phenylalanine	CUU	leucine
UUC	phenylalanine	CUC	leucine
UUA	leucine	CUA	leucine
UUG	leucine	CUG	leucine
UCU	serine	CCU	proline
UCC	serine	CCC	proline
UCA	serine	CCA	proline
UCG	serine	CCG	proline
UAU	tyrosine	CAU	histidine
UAC	tyrosine	CAC	histidine
UAA	(none)	CAA	glutamine
UAG	(none)	CAG	glutamine
UGU	cysteine	CGU	arginine
UGC	cysteine	CGC	arginine
UGA	(none)	CGA	arginine
UGG	tryptophan	CGG	arginine
AUU	isoleucine	GUU	valine
AUC	isoleucine	GUC	valine
AUA	isoleucine	GUA	valine
AUG	methionine	GUG	valine
ACU	threonine	GCU	alanine
ACC	threonine	GCC	alanine
ACA	threonine	GCA	alanine
ACG	threonine	GCG	alanine
AAU	asparagine	GAU	aspartic acid
AAC	asparagine	GAC	aspartic acid
AAA	lysine	GAA	glutamic acid
AAG	lysine	GAG	glutamic acid
AGU	serine	GGU	glycine
AGC	serine	GGC	glycine
AGA	arginine	GGA	glycine
AGG	arginine	GGG	glycine

be *degenerate* because usually more than one codon codes for each amino acid. The data so far available indicate that the code is *universal,* that is, the specific relations between codons and amino acids are the same for all organisms.

The specificity of the code depends not only on the base composition of the codon but on the sequence of these bases as well. One might not have expected a cell to distinguish between GGC and the reverse, CGG,

but it does. That portion of messenger RNA with GGC instructs the cell to incorporate glycine into the protein but, when the sequence is CGG, arginine is incorporated.

Perhaps you have noticed that the argument being developed is incomplete in several important ways: it has not been explained how the sequence of codons in mRNA ensures a certain sequence of amino acids; neither has it been explained how it comes about that the proper mRNA is formed by a gene when, according to the Watson-Crick model, the gene should consist of a pair of complementary nucleotide strands. Each strand should produce a different mRNA and, hence, a different protein.

The Anticodons of tRNA. If a specific sequence of amino acids in a polypeptide chain is the reflection of the sequence of codons in mRNA, the tRNAs with their attached amino acids must line up on the mRNA in one, and only one, sequence. The mechanism that assures this appears to be another case of complementarity.

We have already learned of two types of complementarity. The replication of a strand of DNA involves the production of a complementary strand, not a duplicate. Similarly, the mRNA produced on the DNA template is also complementary.

The relation of tRNA and mRNA appears to be a third example. Among the approximately 80 nucleotides of the tRNA molecules, there seems to be a triplet of bases, the *anticodon,* that is complementary to the codons of mRNA. The complementary sequence of bases of the codon and anticodon allow them to form weak bonds with one another (Table 8–2). Thus the anti-codons will be the same as the DNA triplets except that U replaces T.

The details are far from established and this account should be regarded as a highly simplified model. For one thing the unusual bases found in tRNA (p. 187) are being ignored, yet they will probably turn out to be important.

With this additional information, we can re-examine the interaction of ribosomes, mRNA, and the tRNAs that results in the synthesis of a polypeptide.

The surface of the ribosomes is the place where the polypeptide chain is synthesized. The data seem to suggest that there are two active sites on the ribosome: the first where a tRNA molecule, with its amino acid, attaches to the mRNA; the second where the amino acid joins the polypeptide chain and is released from its tRNA.

The events seem to be about as follows (Fig. 8–8). Assume that the problem is to synthesize a polypeptide that has alanine and glycine in
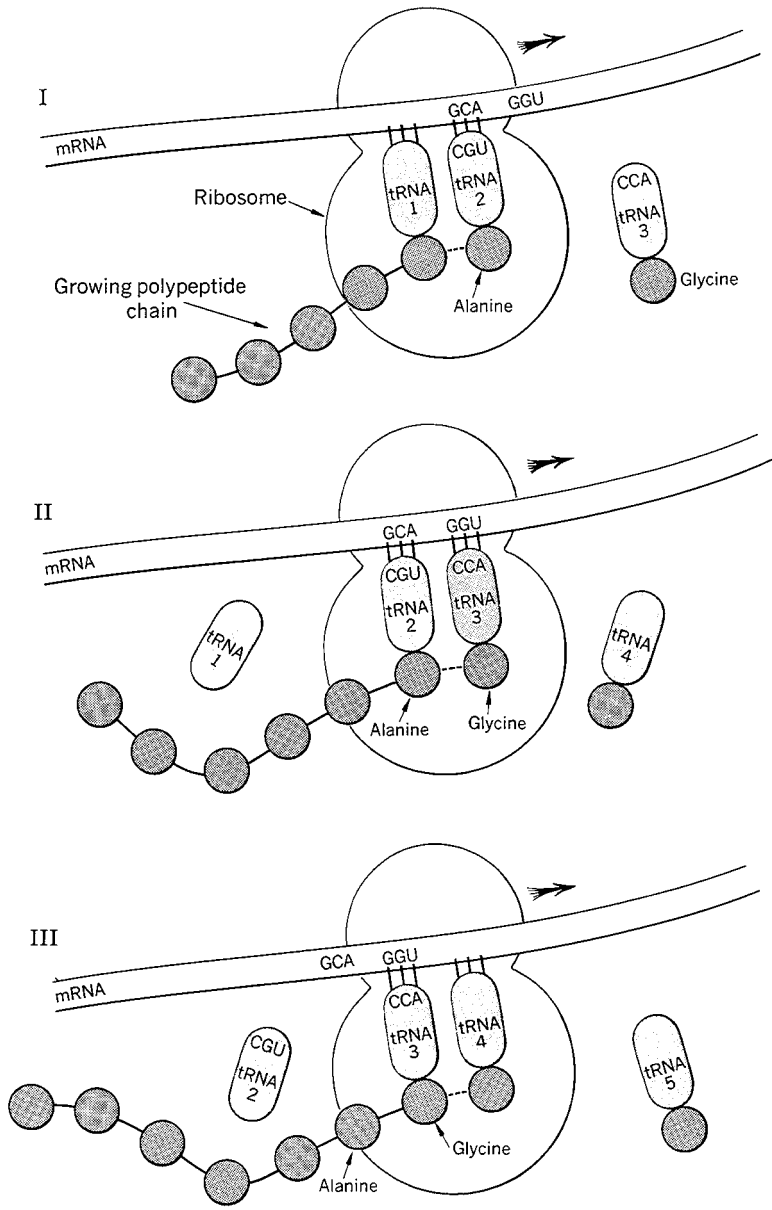
8–8 Protein synthesis. A highly schematic representation of amino acids being attached to a growing polypeptide chain. A single ribosome is shown at three different times. At I an alanine tRNA is attaching to a GCA triplet on the mRNA. A glycine tRNA is shown at the right in the cytoplasm. The ribosome

Table 8–2

A simple model of the complementary relations of the DNA, mRNA, and tRNA triplets that code for alanine and glycine.

IF CODING FOR…	THE DNA TRIPLETS WILL BE…	THE mRNA CODONS WILL BE…	AND THE ANTI-CODONS OF tRNA WILL BE…
Alanine	C	G	C
	|	|	|
	G	C	G
	|	|	|
	T	A	U
Glycine			C
	|	|	|
	C	G	C
	|	|	|
	C	G	A
	|	|	
	A	U	

this sequence near the middle of the molecule: A—G—G—A—G. We will assume that the cell has an ample pool of alanine that has been activated and attached to alanine tRNA and of glycine that has also been activated and attached to glycine tRNA. When one of the mRNA codons for alanine, such as GCA, is on the first site of a ribosome, the anticodon on an alanine tRNA recognizes the codon on mRNA. The mRNA and alanine tRNA are briefly held together by the attraction of codon for anticodon. The alanine is then attached to the polypeptide chain being formed. The alanine tRNA, now attached to the polypeptide chain by its alanine, moves to the second site on the ribosome. The first site of the ribosome is then in contact with the glycine codon, GGU. A molecule of glycine tRNA, with its attached glycine, will link to the mRNA—again the bond being between codon and anticodon. The glycine then becomes attached to the alanine at the growing end of the polypeptide. A shift now occurs: the glycine tRNA moves to the second position on the ribosome while the alanine tRNA is both ejected from

moves to the right and the alanine tRNA is moved to the second site on the ribosome, as shown in II. The alanine molecule becomes attached to the polypeptide chain. The glycine tRNA now occupies the first site on the ribosome and attaches to the GGU triplet on the mRNA. The ribosome moves again to the right shifting the glycine tRNA to the second site (III). Its glycine becomes attached to the alanine on the polypeptide chain. The alanine tRNA loses its connection with alanine and the ribosome and becomes free in the cell.

this site and loses its connection with its alanine. This process continues until the message of mRNA is translated into a specific sequence of amino acids in a polypeptide.

Is One Strand Read, or Two? In Table 8–2 the DNA triplets that code for alanine and glycine are given as CGT and CCA (there are other possible triplets; see Table 8–1). This is the sequence in one of the two DNA strands. The complementary sequence in the other DNA strand would be GCA GGT. If mRNA is made on this strand, the codons will be CGU CCA. The first codon would carry the message for arginine; the second for proline.

Since the gene *is* composed of two complementary strands of DNA, one might expect it to produce two quite different mRNA molecules. The result would be two entirely different proteins associated with each gene. It seems most unlikely that such a system would work, so the hypothesis was advanced that only one of the two DNA strands can serve as a template for mRNA synthesis.

There are data to support this hypothesis. One of the viruses, known as *ø* X 174, is unusual in having its DNA in a single strand rather than the typical double helix—at least for part of its life cycle. The virus is single stranded when it invades its host bacterial cells, but once inside a complementary DNA strand is formed. The double-stranded virus then makes mRNA. One could surmise that the mRNA might be made:

1. Only from the original DNA strand that entered the bacterium.
2. Only from the complementary DNA strand.
3. From all or parts of both strands.

Let us suppose that the single-stranded DNA that first enters the bacterial cell has a DNA sequence shown as the *left* DNA strand in Table 8–3. If so, then the newly synthesized complementary strand will have the sequence shown as the *right* strand. The two strands will form completely different mRNA molecules, as the table shows.

Methods for extracting mRNA and comparing it with the virus DNA are available. When the experiment was done, the data showed that the mRNA was formed only by the DNA strand that was synthesized in the cell. This is interesting in itself but the important conclusion for us is that only one of the two strands serves as the template for mRNA synthesis. It is not fully known how this comes about but it does serve to prevent biochemical chaos in the cell. The controlling mechanism must be at the level of mRNA synthesis and involve the participating enzymes.

RNA Polymerase. In 1960 an enzyme was discovered that could catalyze the synthesis of RNA *in vitro*. It was named RNA polymerase. The raw

Table 8–3

If both strands of DNA were involved in protein synthesis, this is what would happen:

AMINO ACID SEQUENCE	mRNA	THIS STRAND WOULD PRODUCE	THIS STRAND WOULD PRODUCE	mRNA	AMINO ACID SEQUENCE
	G	C	G	C	
Alanine	C	G	C	G	Arginine
	A	T	A	U	
	G	C	G	C	
Glycine	G	C	G	C	Proline
	U	A	T	A	
	U	A	T	A	
Tyrosine	A	T	A	U	Methionine
	C	G	C	G	
	G	C	G	C	
Valine	U	A	T	A	Glutamine
	C	G	C	G	

(Heading: DNA — THIS STRAND WOULD PRODUCE / THIS STRAND WOULD PRODUCE)
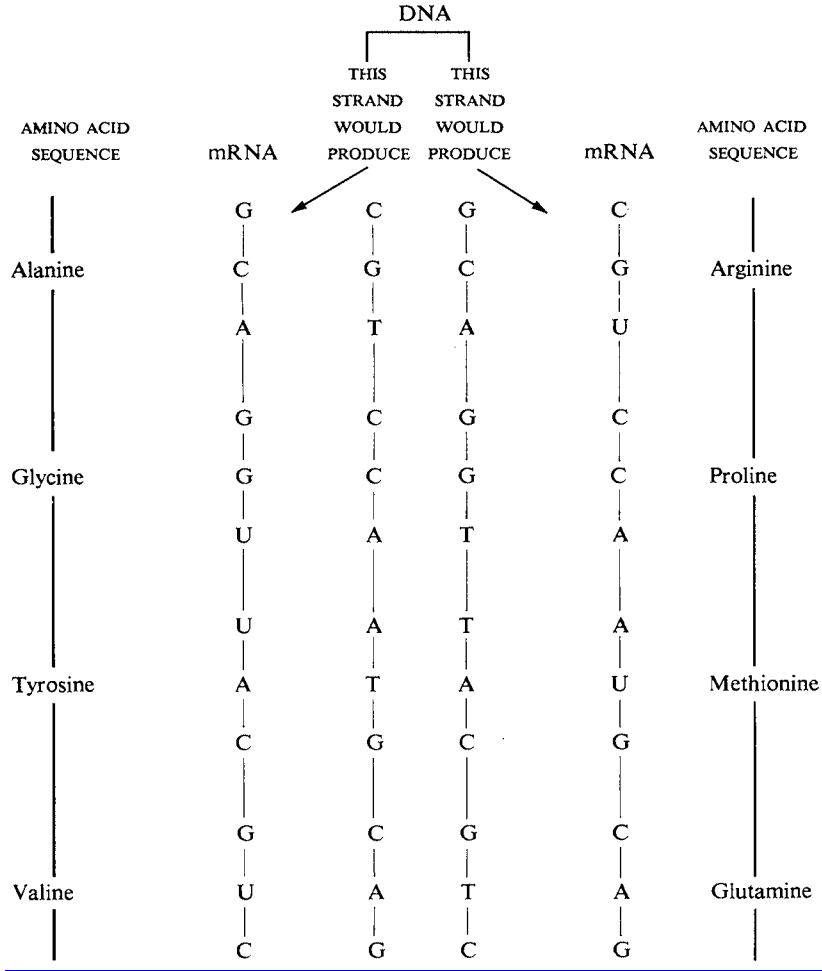
materials required for this synthesis are the four ribonucleoside triphosphates. (A ribonucleoside is composed of one of the four RNA bases, guanine, cytosine, uracil, or adenine combined with ribose; this plus three phosphate groups makes a ribonucleoside triphosphate; a nucleotide is a nucleoside plus one phosphate group.) The four triphosphates are not enough: some DNA must also be present if the reaction is to occur, as well as traces of other substances.

When RNA polymerase, the four ribonucleoside triphosphates, and DNA are together, the enzyme splits off two phosphates from the triphosphate nucleosides (making them nucleotides) and joins them to form a long RNA molecule.

RNA polymerases (there are different kinds) seem to be the key enzymes in the synthesis of the three kinds of RNA: mRNA, rRNA, and tRNA. The template in all three cases is a single strand of DNA. However, RNA polymerase seems to function better with double-stranded DNA even though it copies only one.

The details of this reaction are not fully known but possibly the events are something like this. A RNA polymerase molecule moves along double-stranded DNA transcribing one of the strands into the language of RNA. That is, if the polymerase finds an adenine nucleotide at one site on the DNA, it will add uracil in the RNA that is being synthesized. If the sequence of nucleotides in DNA is TAGCGGA, the sequence in the RNA will be AUCGCCU.

DNA Polymerase. There is a similar mechanism for the replication of DNA. When Watson and Crick proposed their model for DNA replication, it was assumed that enzyme(s) must be involved. About five years later such an enzyme was discovered by Arthur Kornberg and his associates at Stanford University. Here again the raw materials are triphosphates: in this instance the four deoxyribonucleoside triphosphates. In addition, double-stranded DNA is required as a primer. With all of these, new DNA can be synthesized *in vitro*. The two end phosphates are split from the nucleoside triphosphates, thereby producing nucleotides, which are joined to form the DNA molecules. The primer DNA serves as a template and the newly synthesized DNA is identical to it, as one would expect from the Watson-Crick scheme.

Thus Kornberg was able to reproduce *in vitro* one of life's most fundamental *in vivo* events. A dramatic confirmation of this work came from his laboratory in 1967: If the DNA of a virus is used as the primer, DNA polymerase joins the nucleotides in such a way as to produce strands of DNA identical to that of the virus. This can be tested by adding some of the synthetic viral DNA to bacteria: they are invaded and the consequences are identical to natural infections.

There are other enzymes, less well known, that are also involved in DNA replication. Some, for example, seem to be able to repair broken strands of DNA and even replace missing or incorrect nucleotides—abilities which may go far to explain why mutations are so rare, if we think of mutations as injuries to DNA that do not get repaired.

Interim Summary: Structure and Function of Genes. We have reached the end of an important episode in the intellectual history of mankind.

The accomplishments that began with Griffith's discovery of transformation in Diplococcus and led to the cracking of the genetic code by Nirenberg and Matthaei are without parallel in biology and are difficult to match in any science. With a high degree of assurance, we can give a general definition of the molecular composition of the gene and explain how it performs its controlling function in the cell.

The gene is composed of a double helix of DNA, two long strands of nucleotides wound around one another. The uniqueness of any gene is a consequence of the sequence of these nucleotides.

DNA has four main functions:

1. It serves as a template for making copies of itself.
2. It serves as a template for making transfer RNA.
3. It serves as a template for making ribosomal RNA.
4. It serves as a template for making messenger RNA.

The fact that the gene can be duplicated exactly is the basis of constancy of inheritance. The rare errors in duplication are mutations.

The three types of RNA are concerned with the synthesis of proteins, long chains of precise sequences of amino acids. The sequence of amino acids is a reflection of the sequence of nucleotide bases in DNA. Three bases are required to specify a single amino acid. If the sequence is guanine-thymine-adenine, that portion of messenger RNA formed by this triplet will be the codon cytosine-adenine-uracil. The messenger RNA molecule moves to the cytoplasm and becomes associated with ribosomes. Amino acids combine with specific tRNA molecules. The tRNA then attaches to the mRNA probably by means of a specific region that is complementary to the codon of the mRNA. In our example, the tRNA would probably have the sequence guanine-uracil-adenine as the active site.

Thus, opposite each codon of the mRNA molecule there would be a tRNA with its appropriate amino acid. The amino acids then join one another and sunder their connections with the tRNA molecules. Thus, a protein is synthesized.

These events are summarized in Table 8–4 where it is shown how a short length of a polypeptide chain is formed. A more visual representation is shown in Figure 8–8.

This has been a summary of some of the mechanisms by which DNA can supervise the synthesis of specific proteins. Nothing has been said about the control of this supervision, that is, whether a given section of DNA will be active.

Regulation of Gene Function. A healthy, rapidly growing *E. coli* cell has about 10,000,000 protein molecules, representing a huge array of

Table 8–4

The relation of specificity in DNA, messenger RNA, and protein.

IF THE SEQUENCE OF BASES IN ONE DNA STRAND IS…	…THEN THE SEQUENCE OF BASES IN MESSENGER RNA WILL BE…	…AND THE PROTEIN WILL HAVE THE SEQUENCE OF AMINO ACIDS
G	C	
T	A	HISTIDINE
A	U	
C	G	
A	U	VALINE
A	U	
A	U	
A	U	LEUCINE
T	A	
A	U	
A	U	LEUCINE
C	G	
T	A	
G	C	THREONINE
G	C	
G	C	
G	C	PROLINE
G	C	
C	G	
A	U	VALINE
A	U	
C	G	
T	A	GLUTAMIC ACID
T	A	
T	A	
T	A	LYSINE
T	A	

types, and each made according to the general scheme already described. Some kinds are abundant, others are rare. Such cells can divide into two daughter cells in half an hour, so in one hour the descendants of the original cell will have a total of 40 million protein molecules—an incredible synthetic feat.

Think also of the complexity of cells in higher organisms. Your blood cells synthesize hemoglobin. Other cells do not synthesize hemoglobin but they do make many different kinds of proteins.

What controls these vast and precise biochemical events? Clearly there must be mechanisms that can start a gene making RNA and others that can stop it. The genes responsible for hemoglobin synthesis are 'turned on' in red blood cells but, presumably, they never function in other cells.

The answers to these questions are not completely known but there is a useful working hypothesis.

The Operon Hypothesis. In *Escherichia coli* there is one gene that controls the synthesis of the enzyme tryptophan synthetase, and another that controls the synthesis of the enzyme *β*-galactosidase. Presumably the mechanism is the usual one: the tryptophan synthetase gene transmits through messenger RNA the code for joining amino acids in the specific way that makes tryptophan synthetase. The events beginning with the *β*-galactosidase gene and ending with the formation of the specific enzyme *β*-galactosidase are apparently the same.

Tryptophan synthetase has a specific role in the cell. Under its catalytic influence, indole and serine are combined to form tryptophan. The amino acid tryptophan is one of the 20 found in all living organisms.

E. coli cells normally synthesize tryptophan synthetase but if they are grown in a medium containing tryptophan the enzyme is no longer made. Thus the gene functions when there is no tryptophan in the medium, but is inhibited when tryptophan is present. This is a clear example of a gene's action being controlled by a non-nuclear substance.

The situation with respect to the *β*-galactosidase gene is almost the reverse. *E. coli* can use either glucose or lactose as a carbon source for intracellular syntheses. When there is no lactose in the cell's environment, no *β*-galactosidase is synthesized. If lactose is added to the medium in which the cells are growing, synthesis of the enzyme begins. *β*-galactosidase hydrolyzes lactose to glucose and galactose. We must assume that the gene does not function in the absence of lactose but functions when lactose is present. Once again, this is an example of non-nuclear control of a gene's activity.

Experiments of this sort were the basis of the *operon hypothesis* for the regulation of the functioning of genes. This hypothesis was proposed in 1961 by F.Jacob and J.Monod of the Pasteur Institute in Paris. It has stimulated a large amount of fruitful work and there are enough data to suggest that it is essentially correct. Possibly its relative precision can be compared to Mendelism in 1900—correct in broad outline, useful for organizing data, testable by experimentation, but destined to be greatly extended by future discoveries.

An essential element of the operon hypothesis is that genes do not

control themselves. A gene does not suddenly decide, 'It is time to make β-galactosidase' and then proceed to produce the mRNA that brings this about. The stimulus for activity comes ultimately from the biochemical environment in which the gene is situated.

An *operon* is thought of as a portion of a DNA strand consisting of genes that serve as templates for the synthesis of one or more types of mRNA together with other areas that regulate this synthesis. Figure 8–9 shows the lactose operon (lac operon) of *E. coli.* It consists of three genes, **z, y,** and **a;** a *promoter* site, where RNA polymerase attaches to the DNA and begins the transcription of mRNA; and the *operator,* which either allows the RNA polymerase to move along the DNA strand, or blocks its passage.

Each operon is assumed to have its own *regulator gene,* which produces a mRNA that controls the synthesis of a *regulator protein.* The regulator protein exists in two forms, active and inactive, a difference apparently due to the shape of the molecule. The active form of the regulator protein combines with the operator and, as we shall see, blocks transcription of mRNA.

When there is no lactose in the cell, no β-galactosidase is synthesized (Fig. 8–9). The regulator gene for the lac operon makes mRNA, and this leads to the synthesis of the regulator protein in the active shape. The regulator protein then binds at the operator site of the lac operon. If the RNA polymerase attaches to the promoter site to begin the transcription of mRNA, its passage along the DNA molecule is blocked by the regulator protein attached to the operator. No mRNA can be transcribed from the **z** gene, the one for β-galactosidase mRNA.

In the presence of lactose, however, mRNA is synthesized. The regulator protein is still formed but its shape is altered in the presence of some metabolite(s) associated with lactose (possibly the metabolite combines with the regulator protein; this would certainly alter its shape). It can no longer combine with the operator. The RNA polymerase attaches to the promoter site and begins to move down the DNA strand. The operator site is not blocked so the RNA polymerase passes along the **z** gene transcribing it into the mRNA for β-galactosidase. It continues on to make the mRNA from the **y** and **a** genes. The mRNA for the **y** gene serves as a template for the synthesis of a protein that makes the cell membrane of *E. coli* more permeable to lactose. The protein associated with the **a** gene is also related to lactose metabolism but its precise role is unknown.

Thus, whether a gene makes β-galactosidase or not depends on an environmental condition: the presence or absence of lactose. The biochemical economy of this is obvious: β-galactosidase has the function of

NO LACTOSE

I
The regulator gene for the *LAC* operon produces mRNA.

II
This mRNA joins with ribosomes and regulator protein is synthesized.

III
The regulator protein combines with the operator and prevents RNA polymerase from moving along the DNA strand to make mRNA from genes z, y, and a.

I — mRNA
II — Protein
III

Regulator gene | Promotor site | Operator | Gene z | Gene y | Gene a

RNA polymerase

IV

I — mRNA
II — Protein
III — Inhibitor

LACTOSE

I
The regulator gene for the *LAC* operon produces mRNA.

II
This mRNA joins with ribosomes and regulator protein is synthesized.

III
The regulator protein is inactivated when lactose is present.

IV
RNA polymerase moves along the DNA and makes mRNA from the z gene.

V
The mRNA of the z gene, and ribosomes, synthesize β-galactosidase.

V — β galactosidase

8–9 A highly schematic representation of the *lac* operon and its functioning. The upper portion of the diagram shows what happens when there is no lactose in the cell: no mRNA can be made from genes z, y, and a and, hence, their specific proteins cannot be synthesized. When lactose is present, as shown in the lower portion of the diagram, mRNA can be made from genes z, y, and a and their specific proteins will be synthesized. The z gene is responsible for β-galactosidase.

catalyzing the hydrolysis of lactose to glucose+galactose. In the absence of lactose and, hence, of the need to hydrolyze it, no β-galactosidase is synthesized. The cell can do other things with those amino acids that might have been used for β-galactosidase.

The contrasting effects of lactose and tryptophan were mentioned at the beginning of this section (The Operon Hypothesis). The presence of lactose stimulates the production of the enzyme that hydrolyzes this sugar. When there is tryptophan in the medium where *E. coli* cells are growing, synthesis of tryptophan is repressed. Here again the economy is obvious: when adequate concentrations of tryptophan are present in the cell, there is no need to synthesize more.

The tryptophan operon is also controlled by a regulator gene, which produces a regulator protein. In contrast to the situation with the regulator protein for the lac operon, which is synthesized in an active form, the regulator protein for the tryptophan operon is synthesized in an inactive form. Hence, it cannot combine with the tryptophan operator and so prevent the formation of mRNA. Therefore, mRNAs will be produced that result in tryptophan synthesis. When there is an excess of tryptophan in the cell, however, the regulator protein assumes the active form. It then combines with the tryptophan operator site and prevents the formation of mRNA. The result: no tryptophan synthesis.

There is a third type of gene action. Some operons seem to form mRNA molecules at a fixed rate that appears to be uninfluenced by the exact molecular composition of the cell. The molecules controlled by these operons are called constitutive proteins.

The Need for Nonsense. Recall that 61 of the 64 possible codons specify an amino acid (Table 8–1). Three do not: UAA, UAG, and UGG. These were given the flattering name nonsense codons. It was not long until they began to make sense. Figure 8–8 suggests that a single long mRNA molecule is made from the **z, y,** and **a** genes of the lac operon. If this mRNA is translated into protein, the result will be one very long polypeptide chain, not three. It has been discovered that the nonsense codons have the function of ending a polypeptide chain and therefore of permitting a single mRNA to make more than one polypeptide. They are now called *terminating codons.* The last triplet in the **z** gene and the **y** gene (and possibly the **a** gene also) seems to be one of the nonsense codons. When the ribosomes are reading the mRNA, they add the amino acids called for by each codon until they reach one of the nonsense codons. At this point no amino acid can be added to the polypeptide chain, and the chain is terminated. Synthesis of a new molecule then begins again with the sequences beyond the nonsense codon. The

initiation codon for the new mRNA molecule generally seems to be AUG but some evidence suggests that GUG can also serve.

Summary. The gene was a 'black-box' for most of its history, having started life as an abstract concept that helped geneticists think about the results of their experiments. Later it became a 'thing' that could be associated with chromosomes and share their odyssey during mitosis, meiosis, and fertilization. This level of understanding was sufficient to answer most of the non-chemical questions about variation and heredity and to serve as a powerful method for improving man's food animals and food plants. There was even enough understanding to enable him to improve himself, had there been the desire to do so.

In the 1940s the gene emerged from the black-box as DNA and in the 1950s its chemical nature was more precisely defined. In the 1960s a generally satisfying model was formulated for the specific nature of the gene and for its mode of action in the cell.

Some geneticists now feel that the broad conceptual framework of their science has been completed: there are no big questions remaining. To be sure there is a great amount of detail yet to be added, but the great problems remaining are the roles of genes in evolution and in differentiation.

In the last part of this book we will explore some of the problems of the genetic control of differentiation but, before we do, there are some practical things to be said about the genetics of man.

Suggested Readings

Chapter 7 of the *Readings* includes Gunther Stent's article 'DNA' and additional references.

Further information on the ideas developed in this chapter can be obtained from these books and articles.

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9 The Genetics of Man

Humiliating as it may seem, our understanding of inheritance in man is founded heavily on what was discovered in other organisms such as peas, Drosophila, corn, mice, Neurospora, and more recently, microorganisms. Man is not a very good organism for genetic research. His generation time is much too long—most geneticists would be dead before an F_3 appeared. He has so many chromosomes, 23 pairs, that it is extremely difficult to detect linkage groups. He would be most expensive to maintain as a laboratory animal and some of his mores prevent sound experimentation. For example, abundant offspring, brother-sister matings to develop highly homozygous strains, backcrosses of F_1 individuals to members of the parent generation, crosses based on the design of the experiment rather than emotion are not *de rigueur.* Matings occur in man for many reasons, all thought excellent at the time, but rarely, if ever, is one of the reasons the advancement of genetics. But thanks to more amenable organisms and the near-universality of genetic principles, a firm foundation for a genetics of man has been laid. Nevertheless, many of the statements that will be made in this chapter are more in the nature of probable hypothesis than of fact. Many patterns of inheritance, originally thought to be clear examples of autosomal recessives, autosomal dominants, sex-linked genes, and so on, have been shown by later data to be more complex.

A human geneticist attempts to deduce genotypes from the observed phenotypes of parents and offspring. He has developed a symbolism that allows one to easily recognize males, females, parents, offspring, and

individuals with and without the phenotype being studied. Thus males are represented by a ▢ and females by a O. Husband and wife are represented as ▢—O and their children on lines below them. If an individual possesses the trait being studied he is shown as either ■ or ● depending on the sex.

Figure 9–1 shows a hypothetical example of the inheritance of red hair **(r)**, which seems to be an autosomal recessive to non-red hair **(R)**. In the cross labelled 1, neither parent had red hair but two of the children did. We can assume, therefore, that each parent was **Rr**. The red-haired daughter **(rr)** married a man who did not have red hair. He could be either **RR** or **Rr** (cross 2). They had four children, none with red hair. This number of children is too small to establish the genotype of the father. If he were **RR,** none of his children could have red hair. If he were **Rr,** one would expect half of them to have red hair and half not. However, the first four children could have non-red hair and he still be **Rr.** The chance of his first child not having red hair would be one in two. For the first four children not to have red hair, the chance would be $1/2 \times 1/2 \times 1/2 \times 1/2 \times = 1/16$. Thus it is probable that he was **RR** but there is one chance in sixteen that he was **Rr**.

The red-haired son married a red-haired girl (cross 3) and all of the children had red hair, as would be expected from a cross of two homozygous recessives. Finally in our hypothetical cross 4, two of the first cousins marry and, since one of the children has red hair, the mother must have been heterozygous.
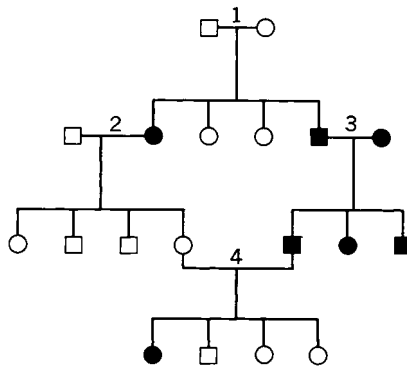
9–1 A hypothetical pedigree of the inheritance of red hair.

Autosomal Recessives. A very large number of traits in man are believed to be due to autosomal recessive genes. The following list shows some of the cases that are fairly well established.

albinism	skin and eyes without dark pigment
alkaptonuria	homogentisic acid in urine (see p. 143)
amaurotic family idiocy	retarded development of body and brain; death in infancy (Tay-Sachs Disease)
analbuminemia	lack of serum albumen
anophthalmos	lack of eyes
bird-headed dwarf	abnormally shaped head
congenital cataract	opacity of lens
color blindness	lack of cones in eye
congenital deafmutism	deaf at birth; rarely learns to speak
cretinism	abnormal development due to defective thyroid
Friedreich's ataxia	degeneration of parts of nervous system
hermaphroditism	ovary and testis in same individual
hypotrichosis	little or no hair
lactase deficiency	lactase enzyme absent in adults
microcephaly	brain greatly reduced in size
muscular atrophy	degeneration of muscles. Death in infancy
muscular dystrophy	degeneration of muscles in later life
pernicious anemia	abnormal blood cells; caused by lack of absorption of vitamin B_{12} in intestine
phenylketonuria	mental retardation due to lack of an enzyme
pituitary dwarfism	small size due to deficiency in growth hormone of the pituitary

Even this short list reveals the broad spectrum of phenotypes caused by homozygosity for autosomal genes. Some genes, such as the one causing alkaptonuria, have been implicated in biochemical reactions. Others, such as the gene causing amaurotic family idiocy, seem to have less precise effects. Even in this case, however, a reasonable hypothesis is that all genes have their primary effect at the molecular level: research has revealed this level for some genes but not for others.

The close relation of genes to metabolic pathways, first adequately demonstrated by Beadle and Tatum in Neurospora (page 144), has its counterpart in man. Figure 9–2 shows some of the reactions involving the amino acids phenylalanine and tyrosine and the abnormalities that arise if these reactions are blocked. Presumably all of the reactions shown are catalyzed by enzymes formed under the controlling action of genes. In some instances mutations have occurred altering these enzymes. When these genes are homozygous, specific abnormalities may result.
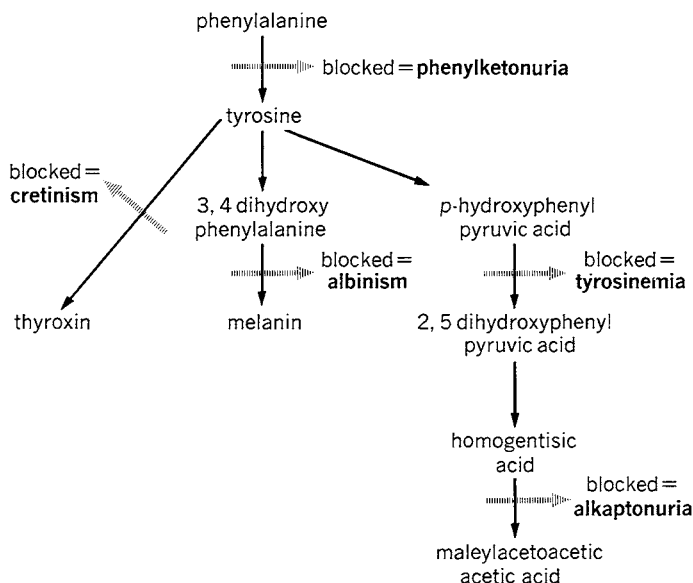
9–2 Metabolism of phenylalanine. Some of the metabolic pathways for the metabolism of this amino acid are shown. Genetic defects are known that are the result of the inhibition of specific enzymes involved in the reactions.

The abnormality may be relatively mild, as in the case of albinism. All of the other abnormalities shown in Figure 9–2 are severe and, if untreated, lead to a wasted human life.

Autosomal Dominants. Numerous dominant autosomal genes are also known for man. As you might suspect, they are much easier to study since both the homozygotes and the heterozygotes exhibit the phenotype. Here are some of them.

achondroplasia	skull and other skeletal abnormalities
aniridia	absence of iris
baldness	possibly a recessive in females
brachydactyly	short fingers
canine teeth	upper canines absent
hereditary cataract	opacity of lens
cleft palate	separation of palate and often of upper lip
corneal dystrophy	clouding of the cornea
congenital deafness	can be caused by many different genes

diabetes insipidus	excessive urine due to lack of a pituitary hormone
distichiasis	two rows of eyebrows
ear malformation	external ear curled
heart block	abnormal heart contractions
Huntington's chorea	degeneration of the nervous system
porcupine man	scaly skin (see page 9)
mid-digital hair	hair on middle digit
myopia	nearsightedness
night blindness	poor vision in the dark
PTC taster	ability to taste phenylthiocarbamide
piebald	lack of pigment in patches of hair and skin
polydactyly	extra fingers and toes
Raynaud's disease	cold fingers and toes due to poor circulation
retinoblastoma	tumors develop in retina
split hand	defect of hands
syndactyly	fusion of fingers or of toes
premature white hair	white hair developed during youth

Again one can observe a great variety of defects. One of the phenotypes, cataract, appears in the list of dominant autosomal mutations and in the autosomal recessive list as well. Cataract can be produced in many ways, probably by numerous different genes and by non-genetic factors as well (such as X-rays, trauma, old age). This is true for many of the conditions shown in these lists. One must not assume that the condition is caused only by mutations at a single locus.

Sex-Linked Inheritance. In man, as in Drosophila, the female is **XX** and the male **XY**. Numerous genes are known for the **X** chromosome but, so far, there is no well established case of a **Y** chromosome gene. The **Y** is, however, very important in sex determination. For many years it was believed that genes for the porcupine man, described by Darwin (page 9), as well as those for syndactyly (webbed toes), were carried by the **Y**. Both are now believed to be autosomal.

The pattern of transmission of the **X** and **Y** is shown in Figure 4–4 (bottom). The males inherit their **X** chromosomes solely from their mothers and transmit them solely to their daughters. Females receive one **X** from their father and one from their mother.

A recessive gene on the **X** will always be expressed in the male, which has no locus on the **Y** to mask its effect. Females are rarely homozygous for recessive mutants carried on the **X**. This is a consequence of the mutants being so rare that it is most unusual for them to come together in the same individual. Heterozygous females are known as 'carriers.'

They transmit the mutant allele to half of their sons and the normal allele to the other half. In the rare event of a cross between a male with an **X** chromosome mutant and a carrier female, half of the daughters will be homozygous for the mutant allele.

Darwin and other pre-Mendelians were aware that some traits, such as color blindness, seemed to be restricted largely to males and to appear in alternate generations: in an individual, his grandsons, and great-great grandsons but not in his sons or great grandsons (page 12). Much later it was realized that these were cases of **X** chromosome inheritance.

It is highly probable that the following genes are on the **X**. In many cases similar phenotypes seem to be caused by different genes and some of the phenotypes closely resemble conditions that do not have a hereditary basis.

ocular albinism	iris nearly colorless
albinism with deafness	little or no pigment; little or no hearing
congenital cataract	opacity of the lens
color blindness	nearly complete absence of cones in eye
congenital deafness	one of the many genes causing deafness
diabetes insipidus	excess urine related to neurohypophyseal abnormality
ectodermal dysplasia	absence of teeth, hair, and sweat glands
G6PD	absence or deficiency of the enzyme glucose-6-phosphate dehydrogenase
classical hemophilia	defective coagulation of blood
hypophosphatemia	low blood phosphorus resulting in rickets not cured by vitamin D
ichthyosis	scaly skin
megalocornea	enlarged cornea
mental deficiency	many genes, including those on the **X,** can be a cause
microphthalmia	eyes reduced or absent
muscular dystrophy	degeneration of muscles
night blindness	poor vision at night
retinoschisis	degeneration of the retina

Most of the **X** chromosome mutants behave as recessives. They will, naturally, be expressed in males whether they are dominant or recessive. One would have to study a homozygous female to decide whether the mutant is dominant or recessive and, for many of the mutants, no homozygous female has ever been discovered. This is due mainly to the rarity of the mutant genes: the chance of two appearing in the same individual is so small that it is almost never observed. Moreover some of these

mutants cause early death, or sterility, in the males. These individuals do not breed and there is no possibility of a homozygous daughter ever being formed (apart from a new mutation).

Genetics of Erythrocytes. More is known about the genetics of erythrocytes than for any other human cell. The mutants fall into two major classes: those affecting hemoglobin and those affecting the proteins and other molecules on the surface of the erythrocyte.

A hemoglobin molecule is composed of four subunits: two kinds of polypeptides, each represented twice (page 178). There are, however, five main types of polypeptides, each symbolized by a Greek letter. The most common type of hemoglobin in adults is hemoglobin A. It is composed of two alpha *(α)* and two beta *(β)* polypeptides. The other kinds of polypeptides are known as gamma *(γ)*, delta *(δ)*, and epsilon *(ε)*.

A human being, in his growth from embryo to adult, produces a sequence of different kinds of hemoglobin. The earliest to be formed is embryonic hemoglobin: composed of two α and two ε polypeptides. Later, fetal hemoglobin is formed: composed of two α and two γ chains. Finally there is the adult type with two α and two β chains. The rare adult hemoglobin, hemoglobin A_2, is composed of two α and two δ chains.

These five basic polypeptides are under the control of five different cistrons, or genes. The activation or repression of these cistrons at different periods in human development is a fascinating phenomenon. But this is only the beginning of the complexity. Earlier we saw that a change in the β cistron resulted in the substitution of one amino acid for another and the consequence was sickle cell hemoglobin (page 182). The number of similar genetic changes now known in these different cistrons is in the hundreds.

The surface of an erythrocyte has numerous gene-controlled molecules that act as antigens, that is, cause the production of antibodies under suitable conditions. The first of these to be discovered was the ABO blood group (page 119). There are two main antigens, A and B, each controlled by a gene. The **A,** or I^A, gene produces antigen A and the **B,** or I^B, gene produces antigen B. An $I^A I^B$ individual produces both antigens. There is another allele, **O** or I^O, at this locus; it produces no antigen. Subsequently it has been discovered that there is more complexity. For example, the **A** gene is now known to have three variants, A_1, A_2, and A_3.

The MN blood groups are also due to specific antigens on the surface of the erythrocyte, which are controlled by the L^M and L^N alleles. An $L^M L^N$ individual has both the M and N antigens on his erythrocytes. No allele is yet known that prevents the formation of an antigen at this

locus, corresponding to the I^O allele in the ABO blood groups. However, there are other alleles now known for the **L** locus.

Another series of alleles affecting a protein on the surface of human erythrocytes and having important medical consequences was discovered in a most interesting manner. Blood from a Rhesus monkey was injected into a rabbit and antibodies were produced in the rabbit to the antigens of the monkey erythrocytes. When serum from the rabbit was later mixed with blood of the Rhesus monkey, the monkey erythrocytes were agglutinated. This was to be expected but the results of the next experiment were not. Serum from the rabbit, with the antibodies to the Rhesus erythrocytes, was mixed with human blood. The human erythrocytes were also agglutinated, suggesting that at least one of their antigens was the same as one of the antigens of the monkey. Subsequently it was discovered that about 85 per cent of the human beings tested have the Rhesus antigen; 15 per cent do not. Genetic analysis revealed that presence of the antigen **(R)** is dominant to its absence **(r)**. It is now known that several antigens and alleles are involved. The genetics is complex but the following simplified account of the effects of this gene is correct, though incomplete.

The **R** and **r** alleles are autosomal and are inherited in a simple Mendelian manner. Any individual will have two of the various alleles at this locus. Some crosses can result in a serious disease of the newborn: erythroblastosis fetalis. This involves the destruction of the baby's erythrocytes and, if untreated, death often occurs shortly after birth.

Erythroblastosis fetalis occurs when the father is Rh-positive, that is, **RR** or **Rr** and the mother is Rh-negative, that is **rr**. If the first child conceived is **Rr,** its corpuscles will have the Rhesus antigen. If some of the baby's erythrocytes enter the mother's blood vessels, she will produce antibodies to the antigen. Normally there is no way that this could happen: the circulatory systems of the mother and the baby in the uterus are separate. However, a trauma could occur, especially at the time of birth, and some of the baby's erythrocytes enter the maternal bloodstream.

The first child is rarely affected. If a second Rh-positive child is conceived, the consequences may be grave. The mother's blood already contains antibodies to the antigen on the baby's erythrocytes. These antibodies may pass across the placental membranes and agglutinate the erythrocytes of this second baby. The agglutinated erythrocytes are destroyed, a severe anemia results, and the baby's blood-forming system is stimulated into abnormal activity.

The frequency of these events—an Rh-negative mother first conceiving an Rh-positive child, then producing antibodies against the Rhesus anti-

gen, and a second Rh-positive child being conceived and erythroblastosis fetalis occurring—is less than half a per cent of *all* pregnancies. In this type of cross, however, about 90 per cent of all Rh-positive babies are affected after the mother has produced the antibodies.

Many babies can be saved if their blood is replaced soon after birth. If there has not already been brain or other damage, the baby may develop normally. Once the baby is no longer exposed to the mother's antibodies, further damage will not occur.

An Rh-negative woman will also produce antibodies if she is given a transfusion of Rh-positive blood. If this occurs before she conceives her first Rh-positive baby, this baby could develop erythroblastosis fetalis.

Sex Determination. The remarkable analysis of sex determination in Drosophila was made possible by the spontaneous occurrence of individuals with different numbers of chromosomes (page 112). Drosophila males are **XY** and females **XX**. The question, 'Is maleness a consequence of an individual having a **Y,** or only one **X,** or both an **X** and a **Y**?' was answered when a fly was discovered with a single **X** and no **Y:** it was a male. Sex determination in Drosophila was eventually interpreted as a consequence of the balance between the number of **X** chromosomes and the autosomes (Fig. 5–8). The **Y** seemed to be of slight importance.

Many biologists at first assumed that the Drosophila pattern applied also to man. They could do little more, since it was nearly impossible to study the chromosomes of man. In fact, it was not until 1956 that the number was accurately determined for man: 23 pairs.

In recent years there has been enormous progress in the study of human chromosomes. Such a study requires large numbers of human cells in the process of mitosis. It has been found that, if a small amount of blood is placed in the proper culture medium, the leukocytes will begin to divide. The cells are then fixed, stained with a nuclear dye, and spread on a microscope slide. It is then possible to study all of the chromosomes in the diploid set. The chromosomes vary in length and in their structure. It is not possible to recognize all of them individually but they can be placed in seven easily recognized groups (Fig. 9–3), which are based on the length of the chromosomes and on the position of the centromere.

A discovery that was made in the cat was later extended to man. If the epidermal cells scraped from the inside of the cheek are stained, and studied, it is found that the resting nucleus has a small, deeply staining area, which was given the name *Barr body* after its discoverer Murray Barr. The Barr body is a condensed **X** chromosome. A normal female
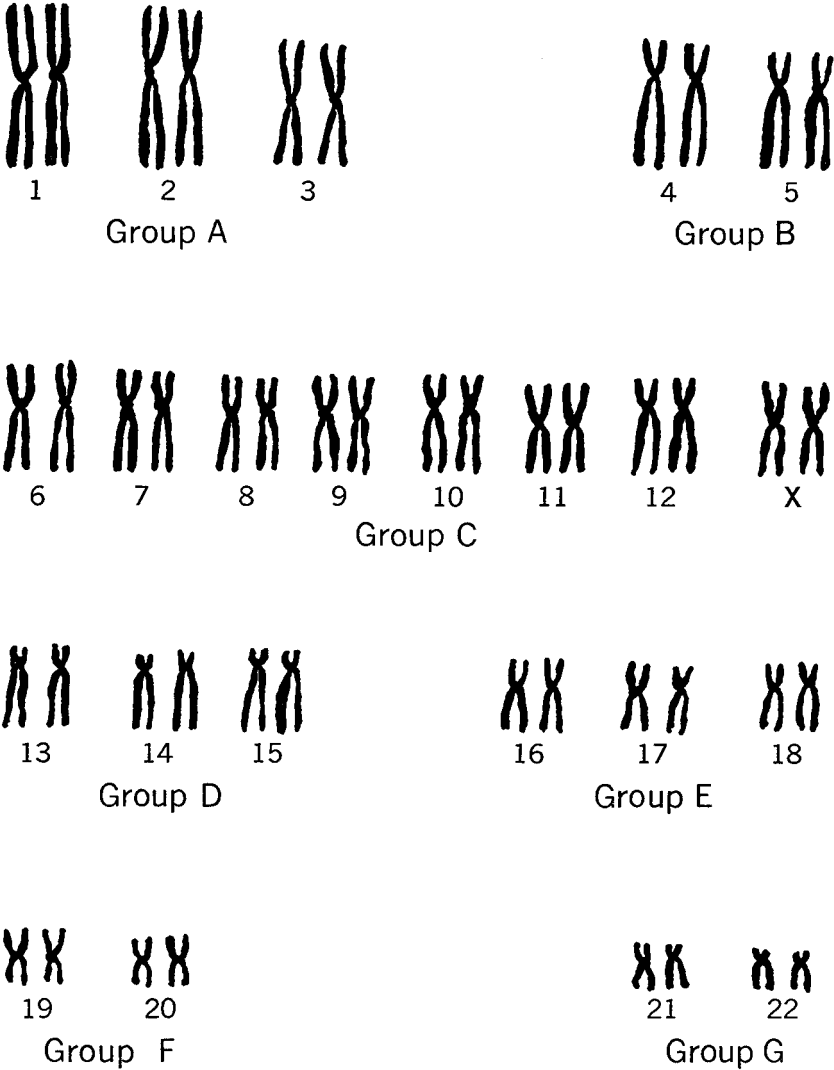
9–3 Human chromosomes. The metaphase chromosomes of a human female are grouped on the basis of size and position of the centromere. A male would have only a single **X**, that is there would be one less chromosome in Group C. His **Y** would resemble one of the G Group chromosomes.

has only one Barr body. This means that her other **X** chromosome is not condensed. There is no Barr body in the nucleus of normal males, which means that the single **X** is not condensed.

When the various techniques for the study of human chromosomes were perfected, they were used for some difficult medical problems, ones that had baffled diagnosticians. For example, there is a condition known as *Turner's syndrome*. The afflicted individuals are female on the basis of most of their characteristics. They have the external genitalia of females, but are short, have broad and flat chests, wide necks, and little or no enlargement of the breasts. When it has been possible to check, it is found that the ovaries are absent or vestigial. Menstruation never occurs.

A related condition is *Klinefelter's syndrome*. These individuals have the external genitalia of males but the testes do not mature, sterility is usual, and there may be a slight enlargement of the breasts.

The nuclei of individuals with Turner's syndrome were studied and were found to have no Barr body. Furthermore, these individuals have only 45 chromosomes instead of the normal 46. Upon closer analysis it was found that the missing chromosome was of group C—the group that includes the **X** chromosomes. The data strongly suggest that individuals with Turner's syndrome have only a single **X** chromosome. Such a condition is called **XO,** the **O** indicating the absense of the second sex chromosome.

Individuals with Klinefelter's syndrome, although males in most respects, were found to have one Barr body and 47 chromosomes. In this case there are two **X**s and one **Y**. Thus, these individuals are **XXY**.

One should compare these conditions with Drosophila (Fig. 5–8). In Drosophila an **XO** individual is male; in man, female. An **XXY** Drosophila is female; in man, male. In Drosophila the number of **X** chromosomes in relation to the autosomes is the primary determinant of sex. In man, maleness is due to the presence of a **Y,** femaleness to its absence.

Other variations in the number of sex chromosomes in human beings have been discovered. For example, females of these types have been detected: **XXX, XXXX,** and **XXXXX**. Such individuals have 2, 3, and 4 Barr bodies respectively, the rule being, for both females and males, one less Barr body than the number of **X** chromosomes. All of these females suffer some degree of mental retardation and often other defects. Some are fertile. In meiosis ova are produced with abnormal numbers of **X** chromosomes and consequently these females may be the mothers of a variety of abnormal children.

Males have their troubles, too: **XYY, XXXY, XXYY, XXXXY,** and

XXXYY types have been found. All suffer mental retardation as well as morphological and physiological defects—usually of the Klinefelter's syndrome type. Some are fertile.

The **XYY** male has stimulated considerable interest and concern. Some are apparently normal in behavior and appearance. Others are extremely tall. There is evidence to suggest that some **XYY** individuals may be abnormally aggressive and institutions for the criminally insane have a higher frequency **XYY** of males than is present in the general population.

There are not, as yet, sufficient data to give accurate figures for the percentages of individuals with abnormal numbers of chromosomes. These are some estimates:

XXY	1 per 500 male births (0.2%)
XYY	1 per 2000 male births (0.05%)
XO	1 per 3500 female births (0.03%)

In all of the cases described so far the autosomes have been normal, that is, two of each type have been present in the diploid nucleus. They too can vary in number.

Abnormal Numbers of Autosomes. Physicians have long recognized a pattern of defectiveness in children known as Down's syndrome (or Mongoloid idiocy). Growth and mental development are greatly retarded and death usually occurs in childhood. Cytological analysis has shown that these children have an extra chromosome in the G group (Fig. 9–3). The G group in a normal individual contains two pairs of small autosomes (the Y is in this group also), which are given the numbers 21 and 22. It is not possible to distinguish chromosome 21 from 22. In Down's syndrome there are five G autosomes, and by convention, it is assumed that the extra one is a chromosome 21. Thus there are two chromosomes 22 and three chromosomes 21. When one of the chromosomes is represented three times, rather than the normal two, the condition is referred to as *trisomy*. Down's syndrome, therefore, is associated with trisomy 21.

The frequency of Down's syndrome is about one per 500 live births (0.2%). Mothers over 40 are far more likely to have children with Down's syndrome than mothers in their 20's.

Trisomy is known for other autosomes as well: chromosome 16, chromosome 18, and one of the chromosomes of the D group.

These variations in chromosome number, of both sex chromosomes and autosomes, are assumed to be due to errors in meiosis. Such errors are not peculiar to man: they were discovered much earlier in Drosophila

and are known for all species that have been carefully studied. Human geneticists and physicians are beginning to believe that babies with abnormal chromosome numbers may be conceived with a fairly high frequency. About 10 per cent of all conceptions result in spontaneous abortions and many of the aborted babies have abnormal numbers of chromosomes. Whereas the frequency of Down's syndrome in newborn babies is about one in 500, the frequency in spontaneous abortions is about one in 40. Turner's syndrome is about 200 times more frequent in spontaneous abortions than in live births.

Thus, spontaneous abortion appears to be a powerful mechanism for preventing the birth of individuals with defective complements of chromosomes. At the present time about one-quarter of spontaneous abortions can be correlated with gross chromosomal defects. This is a new field of research and, as the methods of study improve, it is expected that the proportion will increase. It is also probable that many spontaneous abortions are of embryos that have specific gene defects (as distinct from the gross chromosomal defects).

The Genetic Basis of Intelligence. Geneticists have usually been successful in discovering the pattern of inheritance for those phenotypes they can easily identify. Not surprisingly, their success has been limited when the characteristics being studied are vague.

Intelligence may be taken as an example of a phenomenon that is difficult to study. If you will consult a dictionary, you will find that the term is vague. A person may be described as 'highly intelligent' if he is unusually gifted in one field, such as mathematics, even though he is hopeless in others, such as writing. Another 'highly intelligent' person may be a gifted writer who is incapable of all but the simplest mathematics.

Numerous sorts of IQ tests have been devised to measure intelligence but there are endless debates about their validity. Tests that restrict themselves to specific behaviors: abilities to recognize geometrical patterns and relationships, musical ability, manual dexterity, and so on, may have a greater validity. The fact remains, however that success in measuring intelligence will be limited until the phenomenon can be better defined.

In spite of these reservations, it is abundantly clear that differences in intelligence exist and that they may have some basis in heredity. There is also a general relation between what an individual scores on IQ tests and intellectual abilities as measured in other ways. Individuals with an IQ in the range 50–70 are classed as 'feebleminded.' Very few of these individuals can lead a fully independent life in a complex society; they must be helped and supervised. With rare exceptions, no environment,

school, or medical treatment can bring about a behavior pattern of the sort associated with high IQ.

The greatest problem for the geneticist concerned with the inheritance of intelligence is that what is measured is a consequence not only of what is inherited but also of the cultural milieu in which the person has lived. Success on an intelligence test is greatly influenced by innate ability but to this we must add the influences of the home environment, schools, mass media, books, friends, opportunities, community, and the historic period in which a person lives. Some geneticists have made a guess that heredity contributes about 80 per cent and the environment 20 per cent to one's performance on an intelligence test.

Considering the difficulties involved, you may wonder why attempts are made to measure intelligence and aptitudes. Some of the good reasons are based on a desire to provide opportunities more suitable to the individual's abilities. A young person, upon finishing school, must make the most important decisions of his life—among them the choice of a career. These choices are made, more often than not, on the basis of very incomplete information about the variety of available careers and about one's own abilities. An individual may never have the opportunity to discover for himself what he can do best and then to live a life that will allow this to come about.

It is a reasonable hypothesis that, to the extent that individuals have lives matched to their abilities, the gains to the individuals and to the society will be increased. If this is to come about, one of the things necessary will be improved methods for measuring individual differences and, on the basis of this information, improved capabilities to predict future behavior. Of course, this could be done without having exact information on the relative influences of heredity and environment on what is being measured.

In the present state of our knowledge, many individuals believe that the dangers are greater than the benefits of any attempt to guide one's future on the basis of tests—no matter how carefully constructed. Such information might be used to make opportunities available; it might also be used to deny opportunities. An individual may have superior abilities in some areas and inferior abilities in others. If he were unfortunate enough to be tested only in the areas of his lesser abilities, and his future plans made on the basis of these tests, the results might be disastrous to the individual and harmful to society.

Another situation in which notions about intelligence have had harmful consequences is where it is assumed that groups differ in their intelligence. It is often assumed that women are less intelligent than men and that blacks are less intelligent than whites, and so on. At times these beliefs are held even if the available evidence seems to suggest other-

wise. During the school years, girls generally do somewhat better than boys on intelligence tests. In this and similar cases, however, there is a very broad overlap in abilities and the differences within each group are far greater than the means between the groups. And there is abundant evidence to show that, when any group is denied opportunity, that group makes a lesser contribution to society.

In spite of the enormous amount of time and effort that has gone into attempts to measure intelligence, it is the overwhelming opinion of geneticists, psychologists, and sociologists that no innate differences in ability have been demonstrated for various races and ethnic groups. As of today, one cannot say that the intelligence of men or women or of blacks and whites is different; neither can one say they are the same.

Let us suppose that it does become possible to demonstrate true innate differences. What should be done with such information? As an example, let us suppose that the intelligence of females averages slightly higher than that of males. Remember that, on the average, the muscular development of males is superior to that of females. A program that might take advantage of these differences would be to restrict those careers that demand a higher intelligence to women and those that require a higher proportion of brawn to men. An alternative solution might be to find the bright males (black, white etc.) and bright females (black, white etc.) for the more intellectually demanding positions and leave the more muscular jobs for those best able to cope with them.

Eugenics. During the nineteenth century tremendous advances were made in selecting superior strains of domestic animals and plants. Such selection had been productive for centuries and the then current examples were impressive to individuals such as Francis Galton (1822–1911) in England. He proposed a science of *eugenics,* which was to concern itself with improving the hereditary endowment of the human race. If one could select a superior horse or cabbage, why not a superior man?

Geneticists have never doubted that this is theoretically possible. The techniques are simple and easily applied—again in theory. Let us assume, for example, that one wished to select for large size in the human population. One could proceed in exactly the same way as one would with domestic animals or plants. Large individuals would be crossed with each other. Small individuals would be prevented from crossing. If this procedure were repeated, generation after generation, it is safe to predict that the size of the individuals in the human population would gradually increase. This same procedure could be followed for any phenotype that could be recognized and had an inherited basis.

Imagine the difficulties in conducting such an experiment: Who would

decide what is 'good'? That is, what phenotypes are judged desirable and, hence, are to be increased in frequency through selection? Most human beings would regard such a program as a repugnant intrusion on the way they intend to lead their lives. There is little likelihood, therefore, that in the near future man will use the techniques of animal husbandry to improve his breed.

But if man is unwilling to select for what is 'good,' might he be willing to prevent what is 'bad'? There are some inherited characteristics of man that most individuals would agree are undesirable: congenital deafness, blindness, gross deformities of the body, idiocy, and so on. What should be done about these cases? For years it was assumed that very little could be done since many of these abnormalities are caused by recessive genes and one could never identify the heterozygotes unless they produced a defective child. Geneticists are rapidly increasing their skill in detecting harmful recessive genes in heterozygotes. Furthermore, it is becoming more and more possible to detect abnormal babies before they are born. For example, the techniques are fairly well developed for examining cells derived from the unborn and determining if there are any chromosomal abnormalities.

What would you propose to do with this information? The answer must be a human answer—but a human answer based on scientific information.

Questions

1. In man rare sex-linked mutants tend to be twice as frequent in females as in males. Can you suggest an explanatory hypothesis?
2. Individuals with Klinefelter's syndrome are **XXY** males. A gene for color blindness is carried on the **X**. There are cases recorded where a father with normal vision and a mother heterozygous for color blindness produced a color-blind son with Klinefelter's syndrome. What hypotheses can be suggested to explain this event?
3. A woman heterozygous for sex-linked hemophilia married a man who did not have hemophilia. They had three sons: one normal and two with hemophilia. They also had two daughters. One was normal. The other had hemophilia and she grew slowly and never reached sexual maturity. What explanatory hypotheses can you suggest? How might they be tested?
4. One of the original hypotheses to explain the inheritance of the ABO blood groups was based on the interaction of two loci, A and B. An A type individual could be **AA bb** or **Aa bb**. A B type individual could be **aa BB** or **aa Bb**. AB individuals could be **AA BB, Aa BB, AA Bb,** or **Aa Bb**. O type individuals could only be **aa bb**. What types of observations could you make that would prove or disprove this hypothesis?

5. Suggest an explanatory hypothesis for this pedigree, the defect being deafmutism.
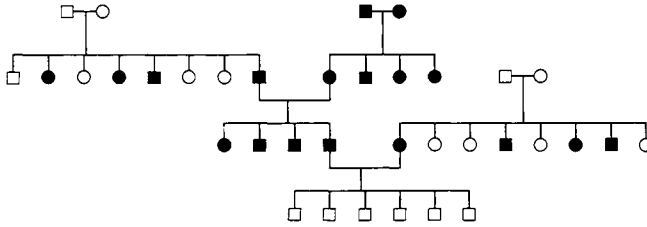
   (If you find the problem insoluble, refer to the Annals of Human Genetics *20:*177–231).

6. Assume that it is possible to measure human intelligence accurately and that the mean intelligence of Group A is 15 per cent less than the mean of Group B. How would you suggest that this information be used?

Suggested Readings

Chapter 8 of the *Readings* reproduces an article by Joshua Lederberg in which he explores some of the possibilities of using genetics for human welfare. You will also find there additional references on human genetics and genetic engineering.

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CONCEPTS IN EMBRYOLOGY

INTRODUCTION

Embryology is the branch of biology dealing with the events associated with the formation of an adult individual from a fertilized ovum. The magnitude of the events during this period can be inferred from a consideration of the differences between a fertilized ovum and the adult of an animal such as the frog. The fertilized ovum consists of a single cell, the frog of many billions; and the differences are more profound than mere cell number. A billion frog eggs, no matter how arranged, do not constitute a frog. The cells derived from the single-cell zygote differentiate along many separate pathways between the fertilized ovum and the adult. Some form muscle cells, others neurons, and still others the specialized cells of the liver, stomach, kidney, endocrine glands, and gonads. All the diverse cell types of the adult are derived from a single cell, the fertilized ovum.

There are two man processes involved in development. One is a tremendous *increase in cell number.* This is accomplished by mitotic division of already existing cells, or as Virchow expressed it, *omnis cellula e cellula.* The increase in cell number involves an increase in total mass and this, in turn, necessitates the utilization of food from some extraneous source.

The second process is *differentiation.* The term probably suggests the nature of the process. During the course of development, the cells change in structure and function: they become different from their earlier forms and from one another.

Our aim in the study of embryology shall be to ask and attempt to

answer some of the pertinent questions concerning development. The 'whys' and 'hows' of development are among the most exciting questions that a biologist can ask. We must begin our study of embryology, however, with a brief description of early development. It is necessary that we have this background before we can ask intelligent questions about the dynamics of the embryological processes. The frog will be used as a type since more is known about its development than about that of any other animal.

10 A Synopsis of Development of the Amphibian Embryo

Frogs of one species or another are found on all of the major land masses. Their embryos are usually easy to collect and for more than a century they have been a favorite material for embryologists. In the cooler regions of the temperate zones there is usually one breeding season each year. *Rana temporaria* of Europe and *Rana pipiens* of North America lay their eggs in ponds during the spring.

Those seeking to answer embryological questions by experimenting on living embryos previously could work only during the relatively short breeding season. Since the 1930s, however, it has been possible to obtain eggs from some species by injecting them with hormones. Thus eggs can be obtained from *Rana pipiens* females by injecting them with pituitary glands or with purified preparations of hormones. Most of the frogs used in this way, in the United States, are collected in the autumn, before they begin their hibernation, in northern Vermont or Wisconsin.

Meiosis and Fertilization. When the ovum of the frog leaves the ovary, meiosis begins. The first meiotic division occurs and the first polar body is formed while the ovum is passing through the body cavity, or when it is in the upper portion of the oviduct. Metaphase of the second division is reached by the time the ovum enters the uterus. There are no further nuclear changes until fertilization.

Fertilization occurs as the ova leave the body of the female. A single sperm enters each ovum. The head of the sperm contains the paternal nucleus with its haploid set of 13 chromosomes. Immediately behind the sperm head is a centriole. This will form an essential part of the mitotic apparatus of the embryo's cells.

Meiosis in the egg, which had stopped at metaphase of the second division, is resumed after fertilization. The second polar body is pinched off, leaving the maternal nucleus with the haploid set of 13 chromosomes. The maternal nucleus and the paternal nucleus then fuse to form the diploid zygote nucleus with 26 chromosomes. This nucleus divides by mitosis to form all the nuclei of the embryo and adult.

Once fertilization has taken place, the development of the embryo begins. A sequence of definite stages is passed according to a timetable which is dependent on temperature. Our description will be based on *Rana pipiens* embryos developing at 20°C. At 25°, development would take approximately half as long and at 15° nearly twice as long.

The Uncleaved Zygote. The just-fertilized ovum is a sphere approximately 1.7 mm in diameter (Fig. 10–1, this figure and all of those in this chapter show the embryos magnified 25 times). Somewhat more than half of the embryo is a dark chocolate-brown and the remainder is almost white. The center of the dark area is the *animal pole,* the site where the polar bodies formed. The *vegetal pole* is 180° from the animal pole and in the center of the unpigmented area. The *animal hemisphere,* with the animal pole in its center, is the pigmented half of the embryo. The *vegetal hemisphere* is the unpigmented half of the embryo that has the vegetal pole in its center.

The entire embryo is surrounded by membranes of a jelly-like substance, which were secreted by the oviduct. (The jelly has been removed from the embryos used in the photographs.)

Shortly after fertilization, the embryo rotates within its membranes so
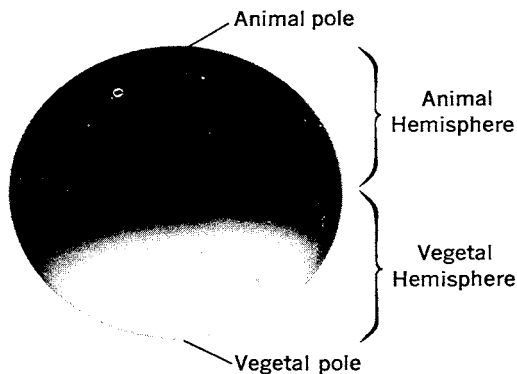
10–1 0-hour embryo. 1-cell stage.

that the animal hemisphere is uppermost. The orientation that one observes when examining an embryo under a microscope is shown in Figure 10–2. The animal pole is in the center. Since the pigmented area occupies slightly more than the animal hemisphere, a top view of the embryo shows only the heavily pigmented zone.

The Early Cleavage Stages. Two and one-half hours after fertilization, the first spectacular event in development occurs (Fig. 10–3). A tiny groove appears in the animal hemisphere and this gradually enlarges to form the *first cleavage furrow*. This furrow slowly extends through the embryo until it is divided into two cells. Preceding this external indication of mitosis, the nucleus had gone through the usual prophase, metaphase, anaphase, and telophase stages, and each daughter cells receives a diploid set of chromosomes.

The *second cleavage* occurs about 3 1/2 hours after fertilization (Fig. 10–4). The plane of this cleavage is vertical and at a right angle to the plane of first cleavage. It also begins at the animal pole and extends through the embryo to the vegetal pole. When this cleavage is complete, the embryo consists of four cells.

The *third cleavage* occurs about 4 1/2 hours after fertilization (Fig. 10–5). The plane of this cleavage is perpendicular to the first two. Its position is somewhat above the equator of the embryo, with the result that there are produced four smaller animal-hemisphere cells and four larger cells, which contain the lower part of the animal hemisphere and all of the vegetal hemisphere.

The process of cleavage continues. The embryo becomes divided into smaller and smaller cells (Fig. 10–6). With each division of a cell there is a concomitant division of the nucleus, every daughter cell receiving the diploid set of 26 chromosomes.
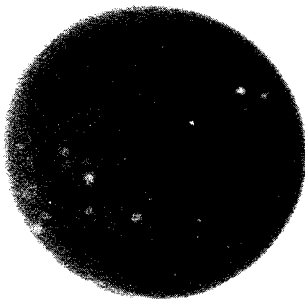
10-2 1-hour embryo. 1-cell stage.   **10-3** 2½-hour embryo. 2-cell stage.
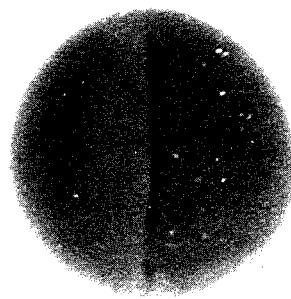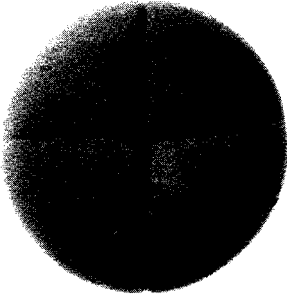
10-4   3½-hour embryo. 4-cell stage.
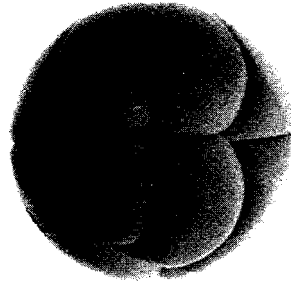
10-5   4½-hour embryo. 8-cell stage.
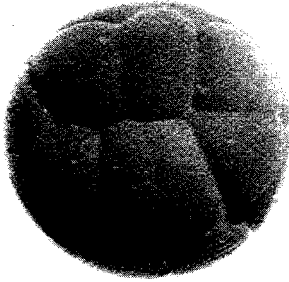
10-6   5½-hour embryo. 16-cell stage.
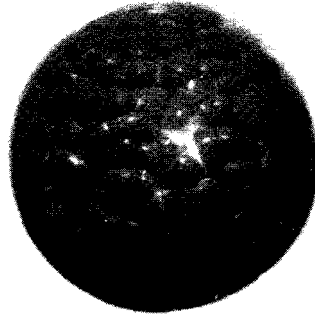
10-7   9-hour embryo. Early blastula.

The Blastula Stages. The 9-hour embryo (Fig. 10–7) is an early *blastula*. The blastula stage is characterized by an internal cavity, the *blastocoel,* which cannot be seen from the exterior. More will be said about it later when we consider the internal events during early development.

At 14 hours (Fig. 10–8), the embryo is a middle blastula. The rate of mitosis of the cells of the animal hemisphere is more rapid than that of the cells of the vegetal hemisphere, so they are more numerous and smaller. If the embryo is turned upside down, the much larger vegetal hemisphere cells are visible (Fig. 10–9).

During the next few hours, continuing cell division is the only visible event. The animal hemisphere cells become so small that they can be distinguished only with difficulty (Fig. 10–10). In this 22-hour late blastula, the next major event of development is foreshadowed. If this embryo is rotated slightly and examined from the side, a special area of pigmentation will be observed (Fig. 10–11) slightly below the embryo's equator. The blastopore will form at this point.

10-8 14-hour embryo. Middle blastula. Top view.
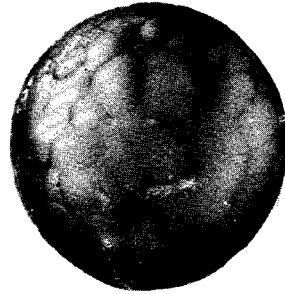
10-9 14-hour embryo. Middle blastula. Bottom view.
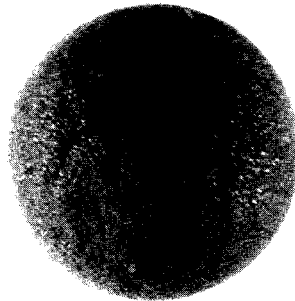
10-10 22-hour embryo. Late blastula. Top view.

Gastrulation. The special pigmented cells noticed at 22 hours gradually become a groove in the surface of the embryo (Fig. 10–12). This groove is the *blastopore* and its appearance marks the beginning of gastrulation. Gastrulation is a process of development that leads to a complete reorganization of the embryo. All of the cells of the area corresponding roughly to the vegetal hemisphere move to the interior of the embryo. The cells of the remainder of the embryo, corresponding roughly to the animal hemisphere, spread and cover the entire outer surface of the embryo. This process of cells turning into the interior is known as *imagination.* The cells are invaginated through the blastopore. The area immediately above the blastopore is known as the *dorsal lip* of the blastopore.

In a few hours the blastopore has changed from a small curved groove to a full semi-circle (Fig. 10–13). Cells are invaginating along the entire length of the blastopore. The overgrowth of the pigmented cells is re-
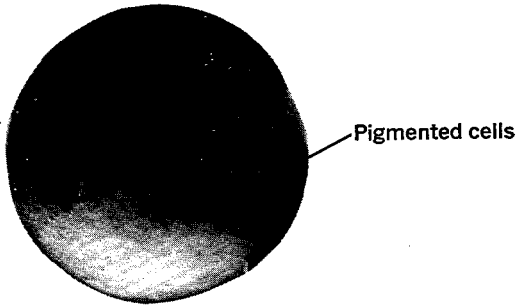
10-11 22-hour embryo. Late blastula. Side view.
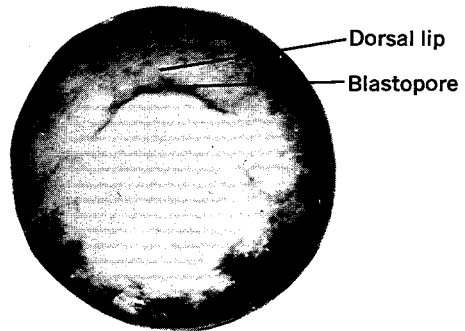
10-12 25-hour embryo. Early gastrula. Bottom view.
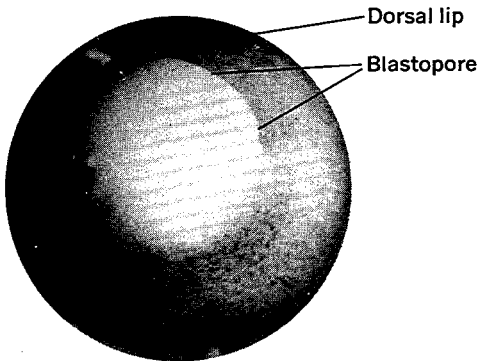
10-13 27-hour embryo. Early gastrula. Bottom view.

stricting the area of light-colored cells to a zone of continually decreasing size.

By 30 hours the blastopore is complete (Fig. 10–14). It is a 360° groove into which material is invaginating. The light-colored cells, which are now entirely surrounded by the blastopore, form the *yolk plug*.

The blastopore constricts rapidly and the yolk plug becomes correspondingly smaller as gastrulation proceeds (Fig. 10–15). By 36 hours the yolk plug is very small and the embryo is nearly covered by the overgrowth of cells that were originally restricted to the animal hemisphere (Fig. 10–16). At the end of gastrulation, the yolk plug is drawn into the embryo and the blastopore remains as a tiny slit. At this time the entire outer surface of the embryo is covered by material that was part of the animal hemisphere at the beginning of gastrulation.

The Neurula. The next prominent external change is the development of the nervous system. Approximately 40 hours after the beginning of development, the *neural folds* make their appearance on the top of the embryo (Fig. 10–17). These folds extend, as paired structures, from the blastopore region across the top of the embryo to a point where they join one another. These folds will eventually grow together, and in so doing they will form the *neural tube*. The neural tube will develop later into the brain and spinal cord. In the anterior region the neural folds are widely separated. This area will form the brain and the narrower posterior part will form the spinal cord. In Figure 10–17 the blastopore is not visible, being below the posterior edge of the embryo.

The growth of the neural folds is a rapid process and by 47 hours the folds are much better developed (Fig. 10–18). The section that will form the brain is clearly separated from the section that will form the spinal cord. The area between the folds is the *neural groove*. The embryo has begun to elongate.

At 50 hours, the two neural folds have come together and the neural groove closes off as an internal neural tube (Fig. 10–19). On the ventral side of this same embryo the area that will form the *mucus glands* has made its appearance (Fig. 10–20). The position of the blastopore is indicated by a posterior cleft. Later the cloacal opening will form where the blastopore closed.

The Tailbud Stage. Figures 10–21 to 10–23 show three views of a 70-hour embryo of the tailbud stage. The dorsal view (Fig. 10–21) should be compared with the same view of the late neurula (Fig. 10–19). The neural folds have closed completely and a number of interesting looking bumps have made their appearance. Near the anterior end of the embryo there are prominent swellings in which the *eyes* are forming.
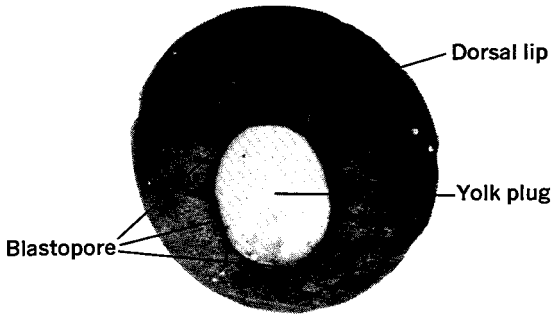
10-14 30-hour embryo. Middle gastrula. Bottom view.
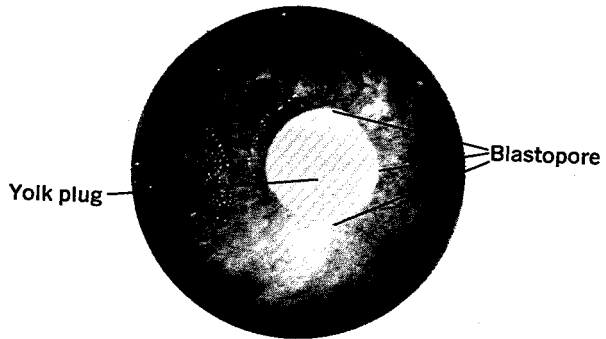
10-15 34-hour embryo. Late gastrula. Bottom view.

10-16 36-hour embryo. Late gastrula. Bottom view.

Anterior

Neural folds
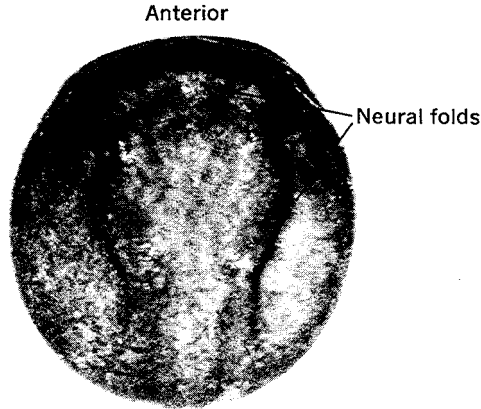
Posterior

10–17 42-hour embryo. Early neurula. Dorsal view.

Anterior

Brain region of neural folds

Neural groove

Spinal cord region of neural folds
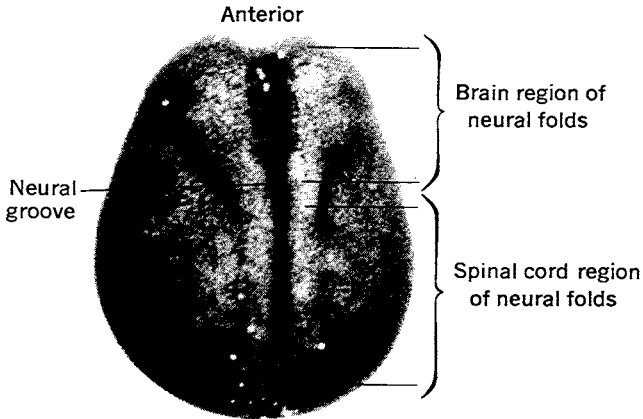
Posterior

10–18 47-hour embryo. Mid neurula. Dorsal view.

Posterior to this is an area that will form the *gills*. Smaller bumps represent the beginnings of the *pronephros,* which is the embryonic kidney. A tiny tail is forming. In the lateral and ventral view, the mucus glands are seen as prominent structures. The cloacal opening (Fig. 10–23) is indicated by a mass of white material that is being extruded.

The 100-hour Embryo. An embryo of 100 hours is the last stage we shall describe (Fig. 10–24) for by this time all of the main internal organ

Anterior

Neural folds

Posterior
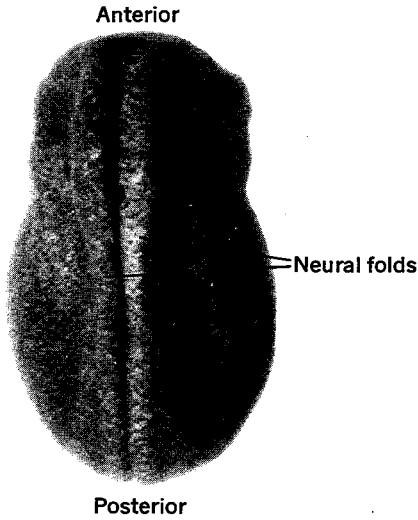
10-19   50-hour embryo. Late neurula. Dorsal view.

Anterior

Mucus gland

Blastopore
Posterior
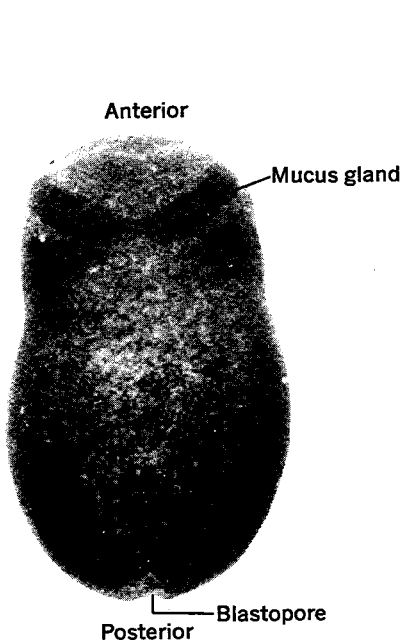
10-20   50-hour embryo. Late neurula.
Ventral view.

Anterior

Eye region

Gill plate

Pronephros

Tailbud

Posterior
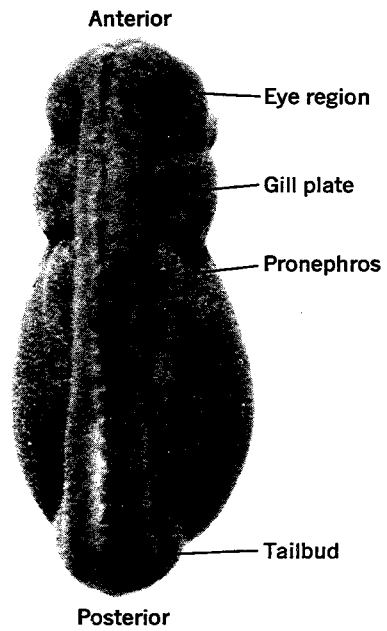
10-21   70-hour embryo. Tailbud. Dorsal view.
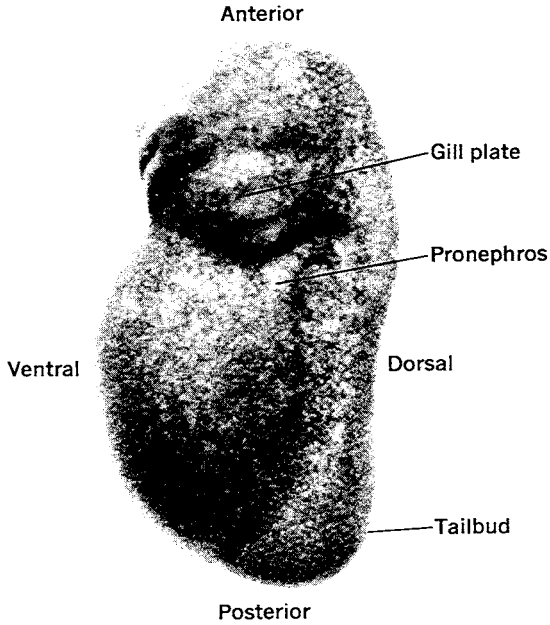
10–22 70-hour embryo. Tailbud. Lateral view.
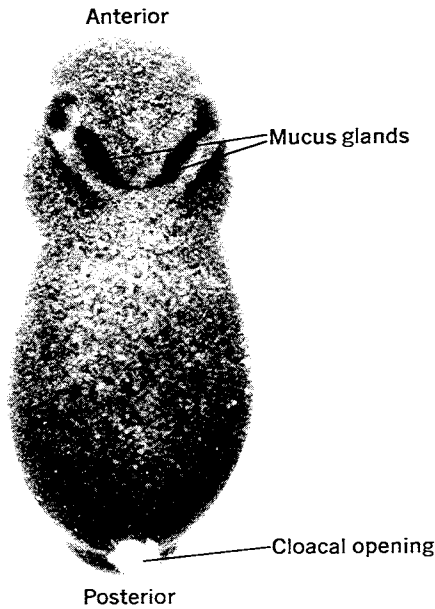
10–23 70-hour embryo. Tailbud. Ventral view.

10-24 100-hour embryo. Lateral view.
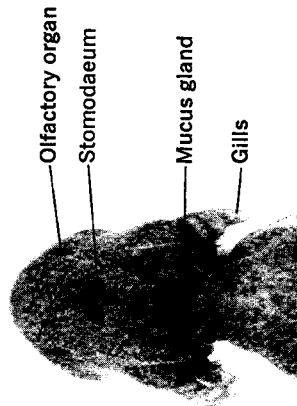
10-25 100-hour embryo. Ventral view of head end.

systems have begun to form. Externally, the embryo has begun to resemble a tadpole. The eyes are present as bumps and the *olfactory organs* are pits on either side of the head. The gills have formed, and in a living embryo we would see blood corpuscles streaming through them. The embryo has a well-formed *tail*. It is at approximately this stage that the young tadpole hatches from the jelly membranes, in which it has been encased up to this time but not shown in the photographs. A ventral view of the head (Fig. 10–25) shows the paired olfactory organs, the mucus glands, the gills, and a median depression, the *stomodaeum*. Somewhat later the *mouth* forms at the inner end of the stomodaeum.

In a period of four days the single-cell fertilized ovum has become a small tadpole with its organ systems functional. Many—in fact most—of the events that have occurred have been internal. Now that we have some knowledge of the external aspects of development, we can turn to the more complex internal events.

Suggested Readings

These books give more complete information on normal development of the amphibian embryo. These references apply to Chapter 11 as well.

BALINSKY, B.I. 1970. *An Introduction to Embryology*. Third Edition. Philadelphia: W.B. Saunders.

BODEMER, CHARLES W. 1968. *Modern Embryology*. New York: Holt, Rinehart and Winston.

RUGH, ROBERTS. 1964. *Vertebrate Embryology. The Dynamics of Development*. New York: Harcourt, Brace & World.

11 Gastrulation and Organ Formation

In this chapter we shall investigate the processes in *Rana pipiens* that convert a single-cell zygote into an embryo having the rudiments of the various organ systems.

Structure of the Blastula. During the first day of development, the most obvious process that occurs is cell division. The mass of the embryo, which was originally in a single cell, is divided into many thousands of smaller cells. The result is a blastula, which is a spherical embryo with an internal cavity, the *blastocoel* (Fig. 11–1). The blastocoel is restricted to the animal hemisphere, and in the living embryo it contains a fluid. The cells near the animal pole are the smallest ones in the embryo and they become increasingly larger toward the vegetal pole.

All cells of the blastula contain *yolk granules,* which are deposited in the ovum while it is being formed in the ovary. They serve as the food supply until the young tadpole is able to feed. The number and size of the yolk granules per cell increase in relation to the nearness of the cell to the vegetal pole—the large cells of the vegetal hemisphere have their cytoplasm packed with yolk granules. Yolk granules are denser than ordinary cytoplasm and, as a result, the vegetal hemisphere, with its abundant supply of yolk granules, is heavier than the animal hemisphere. As a consequence of this density difference the animal hemisphere is uppermost throughout the cleavage and blastula stages.

The Three Embryonic Layers. We have learned previously that gastrula-
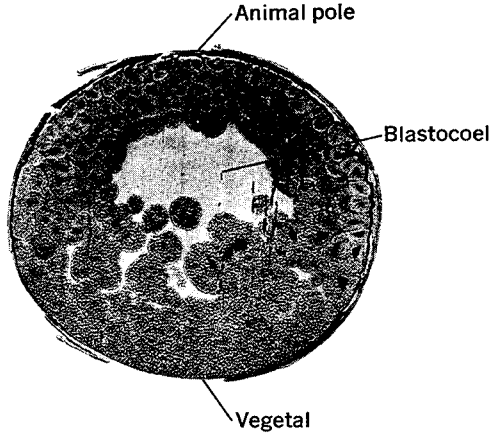
11–1 12-hour embryo. Early blastula. Cross section.

tion involves a complete rearrangement of the parts of the embryo. The vegetal hemisphere cells invaginate through the blastopore and the animal hemisphere cells spread over the entire embryo to form its outer covering. At the end of gastrulation, the cells frequently become arranged in three concentric layers, which from the outside to the center are the ectoderm ('outer skin'), mesoderm ('middle skin'), and endoderm ('inner skin').

The *ectoderm* is the outer covering of the late gastrula in the frog. The epidermis and the nervous system develop from the ectoderm.

The *mesoderm* is the second layer of the late gastrula in the frog, located under the ectoderm. It gives rise to muscles, the skeletal system, the dermis or inner layer of the skin, the circulatory, excretory, and reproductive systems.

The *endoderm* is the inner layer of the late gastrula in the frog. It gives rise to the inner lining of the alimentary canal and the structures derived from it, such as lungs, the liver, pancreas, and the bladder.

Fate Maps. The cells that form the ectoderm, mesoderm, and endoderm at the close of gastrulation can be located in the earlier stages. It is possible, for example, to map the early gastrula with reference to what its various parts *will produce*. We might call such a map a 'fate map,' since it will show the developmental fate of the parts of the gastrula.

The technique for making a fate map of the amphibian gastrula was perfected by the German embryologist W.Vogt. His method was as follows: Tiny pieces of agar are stained with vital dyes, which will not

harm living cells (Fig. 11–2). The stained agar pieces are then held against the embryo until some of the stain is absorbed by the surface cells. The result is a small colored spot on the embryo. The movement of this colored spot is then traced through development. If the spot is part of an area that invaginated, it is necessary to dissect the embryo to determine where it goes.

Figure 11–3 is taken from Vogt's work. In the first embryo there are eight zones indicated. These would have been colored in the living embryo, but are represented by dots of different sizes in the diagram. Embryo *a* is an early gastrula with the dorsal lip of the blastopore just formed; *b* is a middle gastrula with a large yolk plug; *c* is a late gastrula with a small yolk plug. The eight colored areas undergo extensive movements during gastrulation. Regions 5, 6, and 8 are all invaginated to the interior of the embryo, and 1, 2, 3, and 4 are stretched to cover a large area of the surface.

After performing numerous experiments of this sort, Vogt was able to prepare a fate map for the European toad, Bombinator (Fig. 11–4). The *presumptive ectoderm,* that is, the area that will form the ectoderm later in development, occupies nearly half of the early gastrula. Two main subdivisions of the presumptive ectoderm are recognized: (1) the *presumptive neural tube,* which will form the brain, spinal cord, nerves, and some of the sense organs, and (2) the *presumptive epidermis,* which forms the outer layer of the skin.
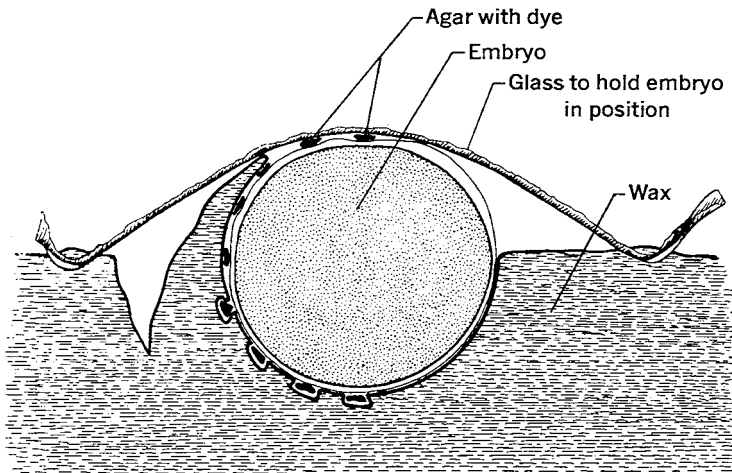
11–2 Vogt's diagram showing how embryos are stained (modified from *Roux Arch.* 106:565).
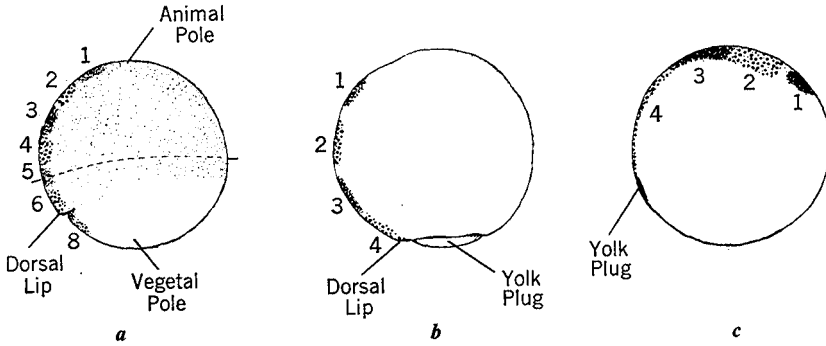
11–3 One of Vogt's experiments with vital stains. *a* is an early gastrula with the dorsal lip below 6. *b* is a mid gastrula. Spots 5, 6, 7, and 8 have been invaginated. The yolk plug is on the ventral side. *c* is a late gastrula. Spots 1, 2, 3, and 4 have spread to cover more of the surface than they occupied at the beginning of gastrulation (modified from *Roux Arch.* 120:568).

The *presumptive mesoderm* forms a band surrounding the embryo near the equator. It also is divided into two main areas. The *presumptive notochord* is composed of cells that will form the notochord, a rod of tissue that extends along the dorsal side of the embryo beneath the neural tube. The great importance of the notochord for development will be explained at a later time. The remainder of the presumptive mesoderm will form the other structures derived from this layer, such as the muscular, skeletal, circulatory, reproductive, and excretory systems.

The *presumptive endoderm* is restricted to the ventral portion of the vegetal hemisphere. This area will form the lining of the alimentary canal and structures derived from it, such as the liver, pancreas, and bladder.

Gastrulation Movements. During gastrulation all of the material below the line separating the ectoderm and mesoderm is invaginated. The cell movements involved can be understood by referring to the diagrams of sections of progressively older embryos (Figs. 11–5 to 11–10).

A convenient stage with which to begin is a very late blastula that has the faintest indication of the place where the dorsal lip will appear. An embryo of this stage is shown in Figure 10–11. A median longitudinal section of such an embryo is shown in Figure 11–5a, and an interpretative diagram is given in Figure 11–5b. The roof of the blastocoel consists of two regions of ectoderm: the presumptive epidermis and the presumptive neural tube. The portion of the blastocoel roof above the point where the dorsal lip of the blastopore will form is the
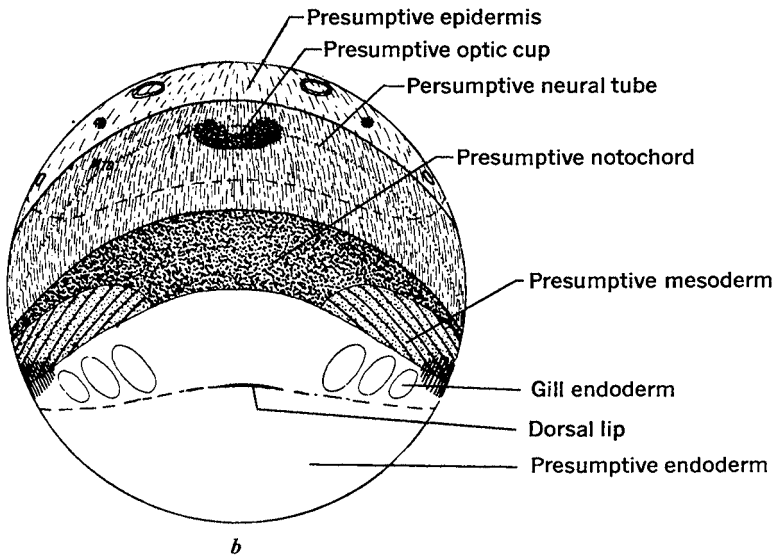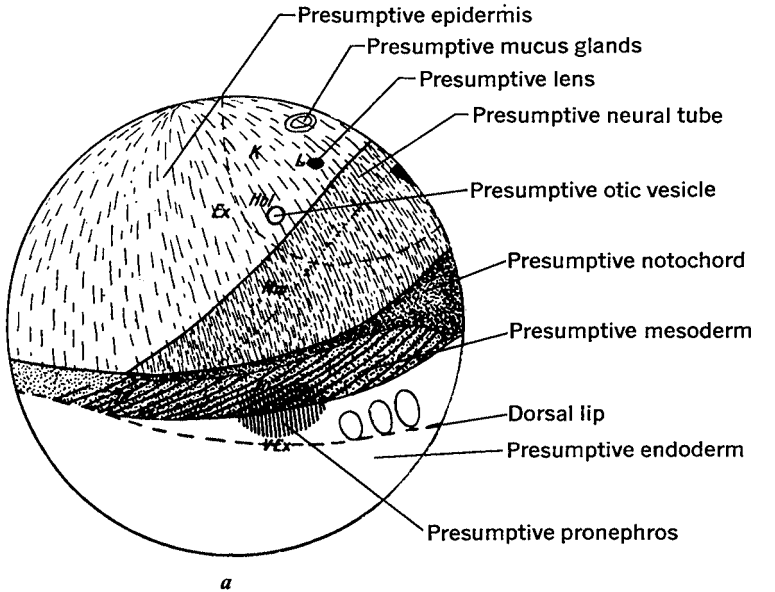
11–4 Vogt's fate map for Bombinator. *a* is a lateral view. *b* is a view toward the dorsal lip (modified from *Roux Arch.* 120:638).
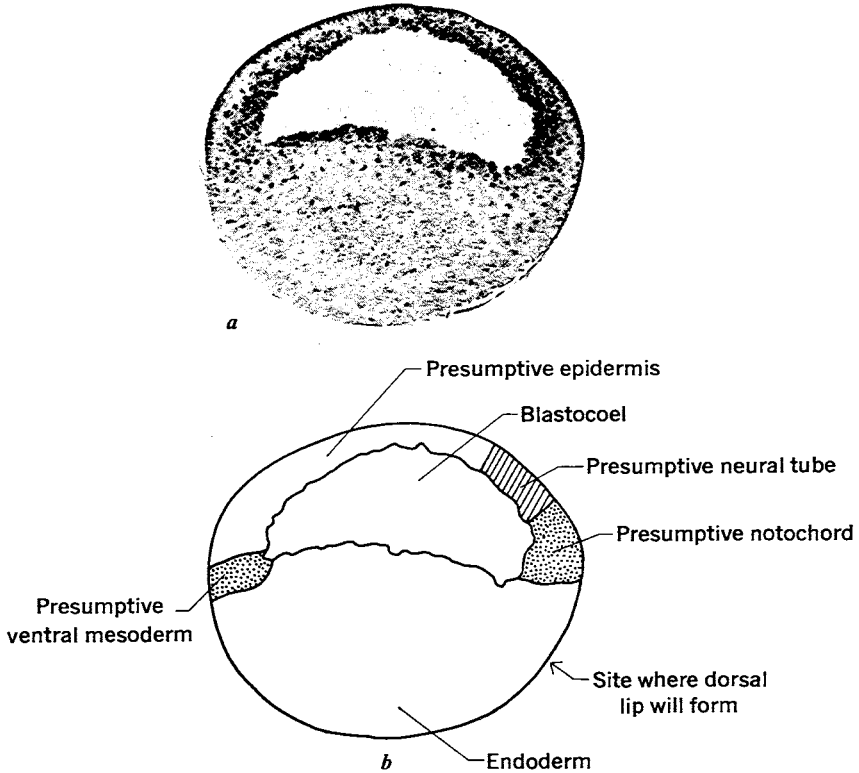
11–5 22-hour embryo. Late blastula. Cross section *(a)* and diagram showing the presumptive regions *(b)*.

presumptive notochord. During gastrulation one should pay special attention to the movements of the presumptive notochord and the presumptive neural tube areas.

Gastrulation begins with the formation of the dorsal lip of the blastopore at about 22 hours after fertilization. Figure 11–6 shows a section of a somewhat older 30-hour gastrula. This should be compared to Figure 10–14 of the whole embryo. Invagination has produced a tiny cavity, the *archenteron* ('primitive gut'). The opening of the archenteron to the outside is the blasto-pore. The invaginating cells are beginning to obliterate the blastocoel. In this embryo the blastopore is complete, so the ventral lip is seen as a slight imagi-nation. Part of the presumptive notochord cells have turned in to form the roof of the archenteron. These are rolled in over the dorsal lip in a manner analogous to a rope moving
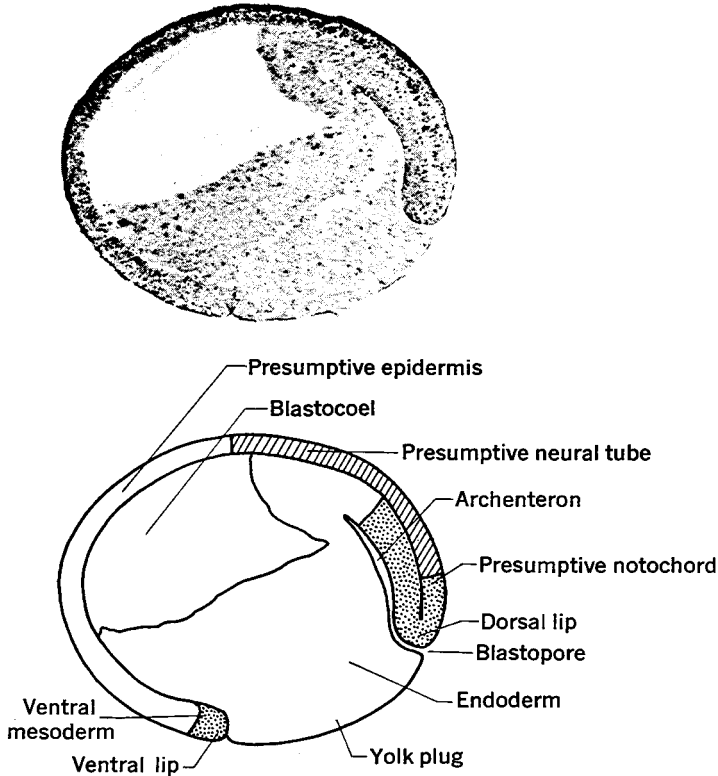
11–6 30-hour embryo. Mid gastrula. Longitudinal section and diagram of the presumptive regions.

over a pulley. The presumptive neural tube and presumptive notochord areas are expanding to cover larger portions of the surface.

Four hours later, the gastrula shows important changes (Fig. 11–7, which should be compared with the whole embryo in Fig. 10–15). The archenteron has become larger and there is a corresponding reduction in the blastocoel. (Note that in this figure, as in many others, there are spaces such as the one in the endoderm of the yolk plug. This was not present in the living embryo, but is an artifact resulting from the technique employed in making the preparation.)

In the 36-hour embryo the archenteron has nearly reached its full size (Fig. 11–8, which should be compared with the whole embryo in Fig. 10–16). The embryo is now covered entirely by ectoderm, except for a small amount of endoderm protruding as the yolk plug. All of
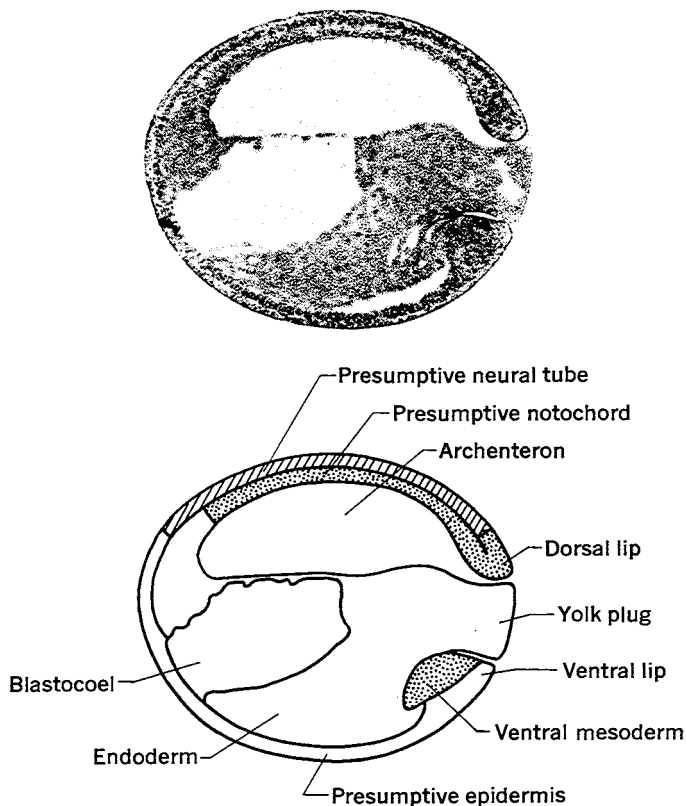
11–7 34-hour embryo. Late gastrula. Longitudinal section and diagram of the presumptive regions.

the presumptive notochord cells have invaginated. They form the roof of the archenteron and are situated beneath the portion of the ectoderm that later will form the neural tube. The section shown is not exactly along the mid-line of the embryo, which is why the blastocoel does not show: its position is indicated by dashed lines.

The Neurula. The further history of the presumptive regions will be shown in two older embryos. Figure 11–9 shows a neurula of the same stage as the whole embryo of Figure 10–18. This is a section along the mid-line, so the neural folds are seen only in the anterior portion of the embryo. The archenteron occupies nearly half of the embryo. It has a ventral outgrowth, the liver diverticulum, which will form the liver. The blastopore does not appear in the section, but its position is indicated in
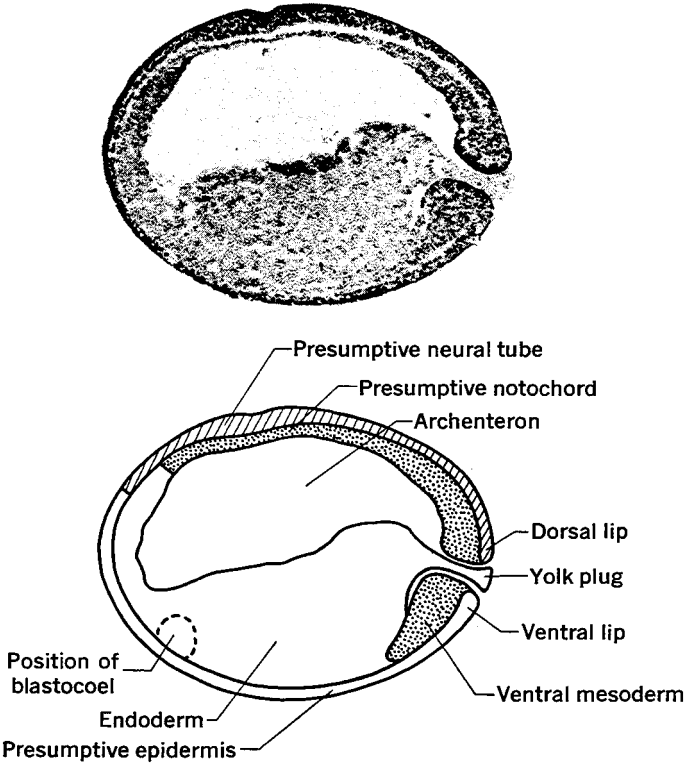
11–8 36-hour embryo. Late gastrula. Longitudinal section and diagram of the presumptive regions.

the diagram of the presumptive regions. The yolk plug has been incorporated in the main mass of endoderm. The blastocoel is but a shadow of its former self.

In the late neurula (Fig. 11–10) the neural tube has formed and the anterior portion is enlarged and bends ventrally. This is the brain. The neural tube is hollow throughout its length, as shown in the diagram. Only a portion of the cavity of the neural tube is visible, because the section is not exactly longitudinal and median. The epidermis covers the entire embryo. The mesoderm is represented in this section by the notochord and the ventral mesoderm. The archenteron is now bounded by endoderm on all sides. Earlier, the presumptive notochord formed the archenteron roof, but by the late neurula stage the endoderm has moved up from the sides and formed a layer beneath the notochord.

Some of the details of neural tube formation can be illustrated better

with cross sections of the embryos. Figure 11–11 shows an embryo shortly before the closure of the neural folds. The ectoderm on the dorsal side has formed two ridges, the neural folds, with the neural groove between them. The remaining surface of the embryo is covered with epidermis, which is also ectoderm. Beneath the ectoderm there is a continuous layer of mesoderm, which forms the notochord on the dorsal mid-line. The remaining portion of the mesoderm is a thin layer, which is difficult to see in the photograph, but is recognizable by the numerous darkly stained nuclei. Most of the embryonic mass is endoderm. This innermost layer forms the thin sides and roof of the archenteron and the thick ventral portion.

The embryo just described shows the characteristic distribution of
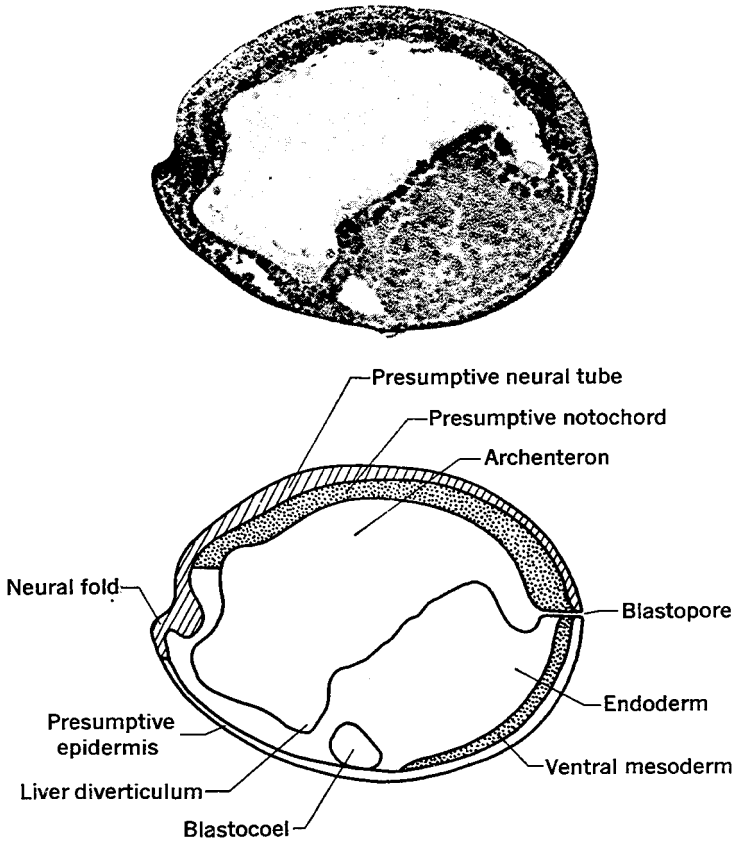
11–9 47-hour embryo. Mid neurula. Longitudinal section and diagram of the presumptive regions.
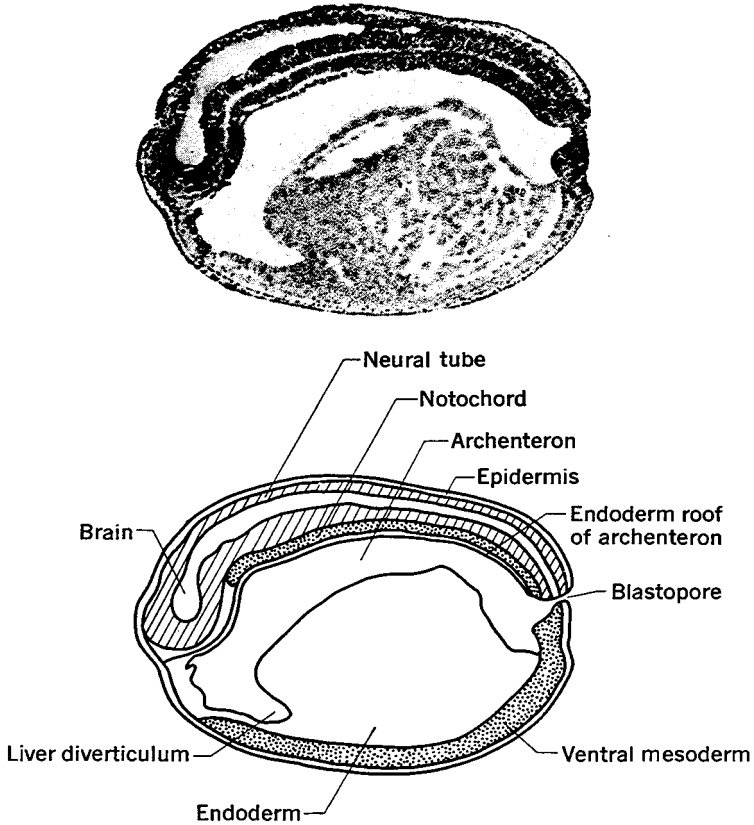
11–10 55-hour embryo. Late neurula. Median longitudinal section and diagram of the parts.

the three embryonic layers. The outer layer is the ectoderm, beneath this is the mesoderm, and the innermost one is the endoderm. We might visualize them as three tubes of different sizes fitting into one another.

A few hours later the two neural folds meet and fuse, forming the neural tube with its central cavity (Fig. 11–12). This cavity extends throughout the neural tube, being especially wide in the brain region. Apart from the closure of the neural folds, this stage is not much different from the preceding one. The embryo consists of the three embryonic layers with little cell differentiation.

The 80-hour Embryo. For our purposes it will be necessary to consider one older stage, an embryo of 80 hours. A section in the anterior end

Neural groove
Neural folds
Notochord
Mesoderm
Archenteron
Endoderm
Epidermis
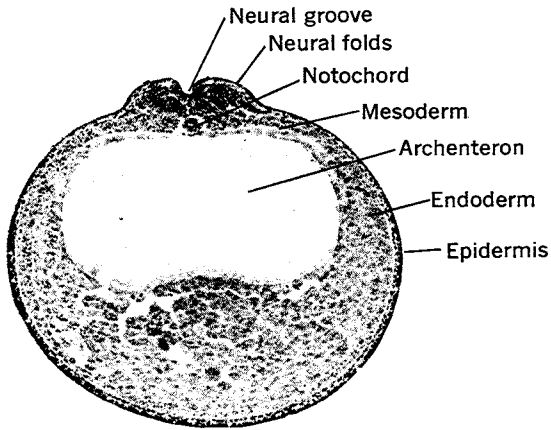
11–11 47-hour embryo. Late neurula. Cross section.

Neural tube
Notochord
Mesoderm
Endoderm
Epidermis
Archenteron

11–12 50-hour embryo. Late neurula. Cross section.
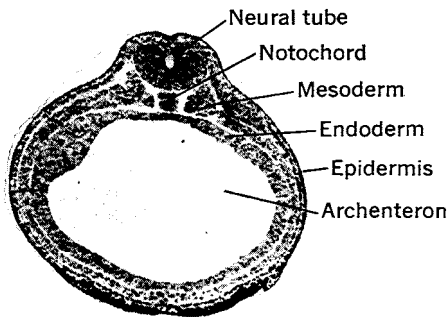
Brain
Epidermis
Optic cup
Lens
Mesoderm
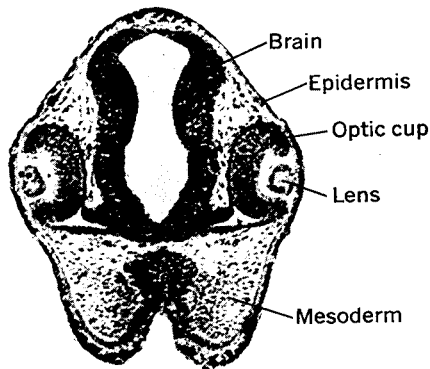
11–13 80-hour embryo. Cross section in eye region.

of an embryo of this age shows interesting changes in the neural tube (Fig. 11–13). This structure has enlarged to form the brain, and from its ventro-lateral portion the optic cups have grown out. The optic cups will form the retina, which is the portion of the eye that is sensitive to light. The epidermis adjacent to the optic cup forms the lens. Both the optic cup and lens are derived from ectoderm. Note that this section is anterior to both the archenteron and notochord. A study of Figure 11–10 will show why this is so.

Figure 11–14 is a more posterior section of the same embryo showing other structures. The neural tube at this level is the hindbrain, which is the portion that forms the medulla later in development. The otic vesicles, which were pinched off from the layer of ectoderm covering the embryo, are lateral to the hindbrain. They will be the ears. The archenteron is present in this section, surrounded by its layer of endodermal cells. The heart is forming from mesodermal cells below the archenteron. The epidermis on the ventral side has formed the mucus glands.

A section in the mid-region of the body shows the beginnings of the excretory system (Fig. 11–15). The mesoderm at the sides is forming the pronephros. The mesoderm above the pronephros, which is known as the myotome, will form most of the voluntary muscles of the body. Later the mesoderm ventral to the pronephric region will split to form a double layer and the space between will be the coelom.
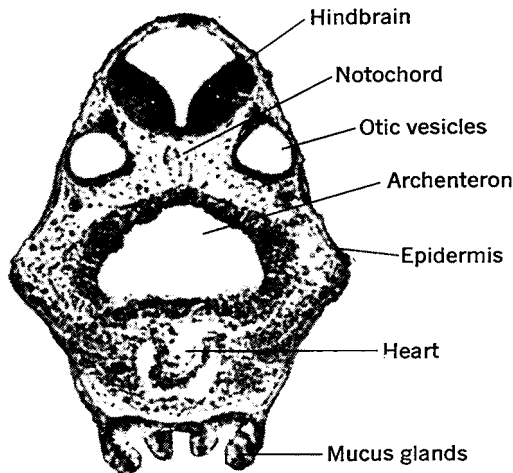
11–14 80-hour embryo. Cross section of heart region.
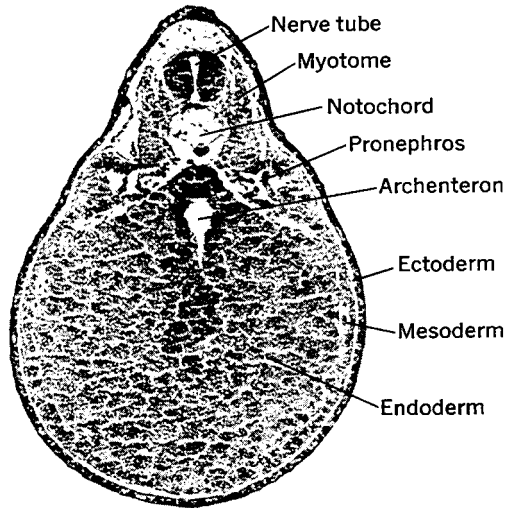
11–15 80-hour embryo. Cross section of mid body region.

The coelom was mentioned earlier, but not defined. Now we have the background for an adequate definition. A coelom is a body cavity lined by thin membranes (epithelia) derived from mesoderm.

This brief survey of early development in the amphibian embryo was designed to provide a background for the consideration of the problems of embryology. Now that we have learned something of *how* an embryo develops we can consider some of the controlling processes that are responsible for embryonic differentiation.

Suggested Readings

The readings suggested for Chapter 10 will serve for this chapter as well.

12 Differentiation

The Problem of Differentiation. The main problem of embryology is this: How, in the course of development, does a cell of one type change into other types of cells? Cell division divides the fertilized ovum into the many cell types of the adult: eye cells, heart cells, kidney cells, nerve cells, and so on— all traceable to an identical beginning. How can the fact of differentiation be made to conform to the fact of mitotic cell division, a process that seems to give daughter cells identical with each other and with the parent cell?

Development has fascinated human beings for a very long time. The growth of a huge tree from a tiny seed, the conversion of an egg into a chick, and, of course the development of man himself—all the more mysterious because the early stages are hidden from view. How can an egg, apparently formless and often so small as to be nearly invisible, have the potential for producing a large and complex organism? Interest in this problem is not new. Aristotle was fascinated with development. He studied many sorts of embryos, especially chick embryos, and his descriptions and speculations were not improved upon for 2000 years.

PREFORMATION AND EPIGENESIS

We can begin our study of embryological concepts at the close of this long and sterile period. Much of the speculation of the seventeenth and eighteenth centuries concerned two rival concepts of development, preformation and epigenesis. *Preformation,* as the term implies, means that all the parts of the adult, including the most minute ones, are already perfectly formed at the very beginning of development. *Epigenesis* means

that the adult parts are not present at the beginning, but are developed during embryonic life.

Preformation. Preformation was the generally accepted doctrine during the seventeenth and eighteenth centuries. The basis for this belief was largely philosophical and, to a lesser extent, theological. In addition there were some fascinating 'observations' that seemed to support the concept of preformation.
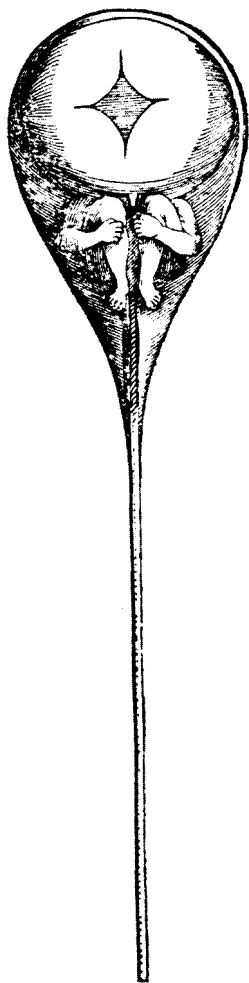
12–1 Homunculus in human sperm (from Hartsoeker, *Essai de dioptrique,* Paris, 1694, p. 230).

Some of the greatest scientists of the time, such as Swammerdam, Malpighi, Leeuwenhoek, Leibnitz, Reaumur, Spallanzani, and Bonnet, were preformationists. They were divided into two schools: the 'ovists,' who believed that a tiny preformed body was present in the ovum, and the "spermatists,' who believed that a tiny body was present in the sperm. Swammerdam, an ovist, believed that the animal hemisphere of the frog's egg contained a tiny frog. This subsisted on the food material of the vegetal hemisphere. Embryonic development was simply the increase in size of this tiny frog. Others thought they saw little chickens in the unincubated hen's egg.

The spermatists reported tiny animals in sperm. Figure 12–1 shows what one observer thought he would find if he could see the inner detail of the human sperm. Many others claimed to confirm him, not only on human sperm but on animal sperm as well.

There is no problem of differentiation for the preformationists: the adult structures are already differentiated at the beginning of embryonic life; development consists only of growth.

The theory of preformation had one interesting corollary. Let us adopt the spermatist position and consider the tiny creature curled up in the sperm head of Fig. 12–1. Let us further suppose that this gametic Lilliputian is a male. If so,
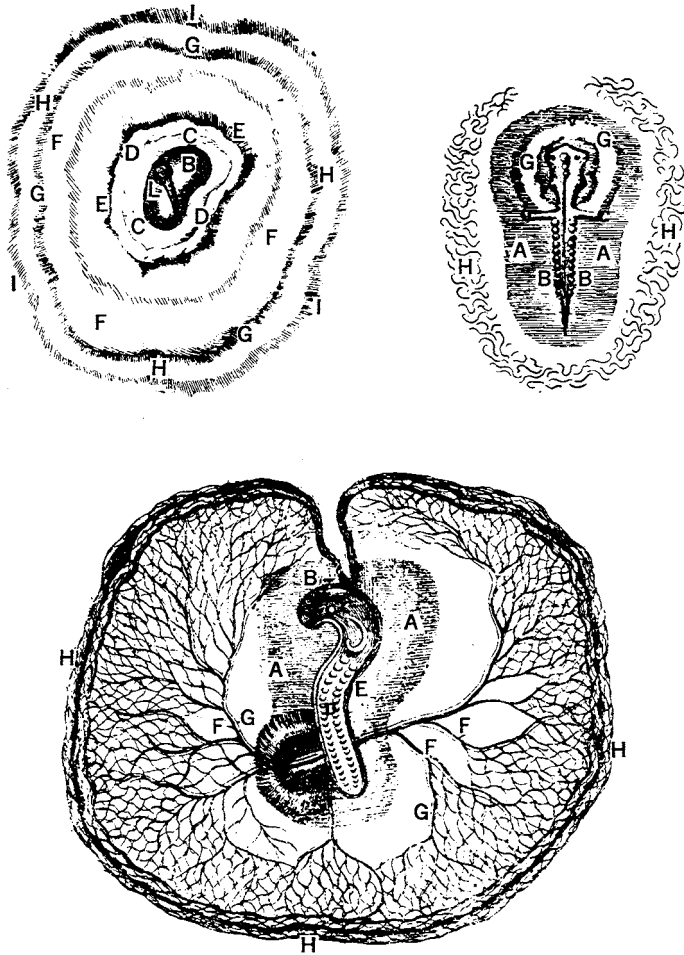
12–2 (legend on facing page).

his testes must be fully formed and contain sperm. These sperm would contain fully formed creatures as well. They would be the potential children of the homunculus. These children would contain the grandchildren, and the grandchildren would contain the great-grandchildren, all encased like a set of Russian dolls.

Ridiculous as this may seem today, speculations of this sort were made in all seriousness. It was stated by some that Adam or Eve (depending on whether the author was a spermatist or an ovist) must have had the 'seeds' for all future generations of mankind in his or her
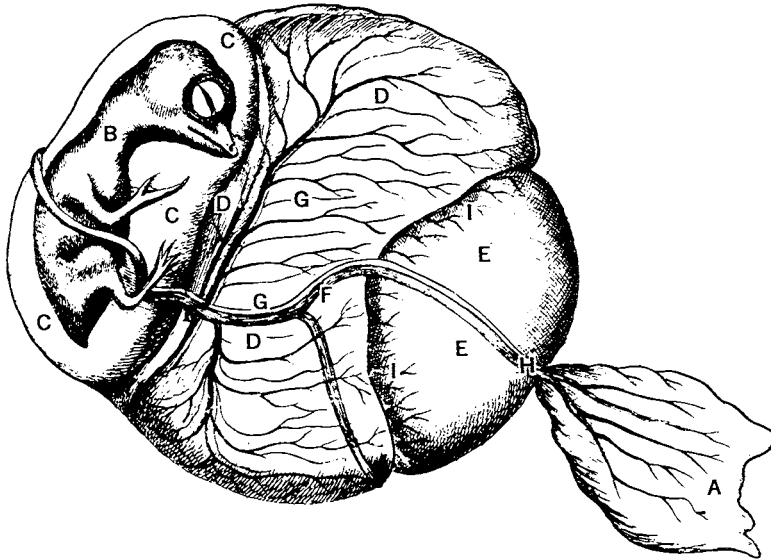
12–2 Four stages in the development of the chick (from Malpighi, 'De Formatione pulli in ovo,' in *Opera Omnia,* Scott and Wells, London, 1686).

gonads. The human race would come to an end when the supply of successively encased homunculi was exhausted.

It was probably a philosophical difficulty that made men believe in the theory of preformation when observation might have convinced them of epigenesis. How was one to understand that something entirely new could appear during development? How could the heart, or the brain, suddenly appear where only formless protoplasm existed before? If the egg of the frog was a structureless body detached from any influence of the parent, how was it able to develop into an exact replica of its species? Why did it not form a toad, or a fish, or an elephant? Clearly something 'preformed' must be transmitted from parent to offspring, or the frog's egg would not develop into a frog. No doubt it was easier for the embryologists to imagine that structure, rather than anything else, was transmitted. For this reason it was assumed that the earliest embryo must be a miniature adult.

As an example of how reason can lead one to deny what his senses reveal, we should say a few words about Marcello Malpighi (1628–94), an outstanding seventeenth-century Italian biologist. (Malpighi made many discoveries. He was, for example, the first to observe capillary con-

nections between arteries and veins. William Harvey, who is generally credited with the theory that blood circulates, assumed that connections must exist, but he did not see them.) Malpighi was a preformationist, yet his work on the hen's egg showed that development was epigenetic. The figures in 12–2 are his drawings of the developing chick. In the unincubated egg there is a tiny embryo. (In the chicken, fertilization is internal; some development occurs before incubation begins.) In increasingly older embryos new structures make their appearance, and gradually a chicken-like creature is produced. Malpighi believed that this epigenetic phenomenon was an illusion. To him, the structures that made their appearance were there all the time; he was just not able to see them. If Malpighi had not let his beliefs overrule his observations, posterity would have hailed him for presenting the first well-documented case for epigenesis.

Malpighi should not be criticized for disbelieving what he saw. Scientists throughout the ages have had to face conflicts between preconceived beliefs and observations. Not infrequently the observations are made to fit the preconceived beliefs. Sometimes this has been proved, by subsequent events, to be the correct thing to have done. For example, consider Flemming's belief, mentioned in Chapter 2, that chromosomes are constant cell structures. He held this view in spite of the fact that the chromosomes seemed to disappear between successive divisions. At other times the acceptance of beliefs contrary to observation has retarded the progress of science for years. In Malpighi's case, observation should have triumphed over reason. It did not. We must realize, of course, that a verdict of this sort can be given only in retrospect. It is not intellectually dishonest to fit observations to ideas: a scientist must seek to fit observations into reasoned order.

Epigenesis. In spite of an almost universal belief in preformation, there were always men of renown who thought that development was epigenetic. They believed that adult structures were absent from the early embryo, and that they made their appearance during the course of embryonic life. This was the belief held by Aristotle and, two millennia later, by the English biologist William Harvey (1578–1657).

In 1759 the dissertation of Caspar Friedrich Wolff (1733–94), a German zoologist, was published, the most careful description of the developing chick that had been made. Wolff believed in epigenesis, and the observations reported in his dissertation appeared to confirm his belief, but during his lifetime he was unable to convince many of his contemporaries. In fact it was not until the early part of the nineteenth century that a majority of biologists finally accepted the concept. The

careful observations of Wolff and those who followed him finally convinced embryologists that new structures do make their appearance in development.

Epigenesis is, of course, the view that we hold today. The study of the photographs of frog embryos (Figs. 10–1 to 10–25) will indicate that the fertilized ovum is not an adult in miniature. Development begins in a relatively structureless and homogeneous mass, and gradually the organs and parts differentiate to produce the adult body. The problem of differentiation, which would be non-existent if preformation were true, returns in full force and must be explained.

MOSAIC AND REGULATIVE DEVELOPMENT

The Mosaic Theory of Development. The next major attempt to solve the problem of differentiation was made by the German embryologist Wilhelm Roux (1850–1924) in the last two decades of the nineteenth century. He was working at a time when spectacular discoveries of chromosome movements in mitosis and meiosis were being made (Chapter 2). Roux attempted to apply the cytological information to embryological problems. According to him, the zygote nucleus contains the *determinants* for differentiation. These determinants were localized in the chromosomes, and during cleavage they were parcelled out to separate cells. Finally, each cell would be left with only the determinants of a single sort, such as heart-cell determinant, muscle-cell determinant, and so forth. The theoretical biologist, August Weismann (1834–1914), also had a prominent role in the development of this theory.

Cleavage and the Polarity of the Frog Embryo. Roux was led to this hypothesis by some observations he made on early development in the frog. Shortly after fertilization, and before first cleavage, some of the black pigment of the animal hemisphere, near the equator, disappears, producing the *gray crescent*. The plane of first cleavage cuts through the center of the gray crescent. Still later the dorsal lip of the blastopore forms in the center of the area where the gray crescent had been. The anus forms where the blastopore closes. Thus the position of the gray crescent corresponds to the posterior end of the embryo. Since the plane of first cleavage runs through the gray crescent, it can be looked upon as dividing the embryo along what will be the future midline. Thus one of the two cells formed will become the right side of the body and, the other, the left side.

Roux believed that the first mitotic division of the embryo resulted in the determinants for all structures of the right side going into the right cell and those for the left side going into the left cell (Fig. 12–3).

The plane of the second division is at right angles to the first, cutting across the body of the future embryo. The four cells will represent the right anterior, left anterior, right posterior, and left posterior sections of the future embryo. At this second division, Roux believed, the determinants for the four regions of the future embryo are segregated. The simple diagram of Figure 12–3 will show how the determinants were thought to be allocated to each cell by the mitotic division. Continuing cell division results in a continuing segregation of determinants. Finally, each cell will contain a specific determinant, which will be responsible for its differentiation into a specific type of cell.

Roux's Test of the Mosaic Theory. Roux sought to test this hypothesis in one of the first experiments ever performed on an embryo. If the hypothesis is true, one of the cells of the two-cell stage should contain the determinants for the left side of the body, and the other cell the determinants for the right side of the body. Now, if one of the cells is killed, the embryo should have the determinants for only one side of the body. It should develop into a half-embryo. Roux performed this experiment by killing one of the two cells with a hot needle. The uninjured cell cleaved as though it were still part of the entire embryo.
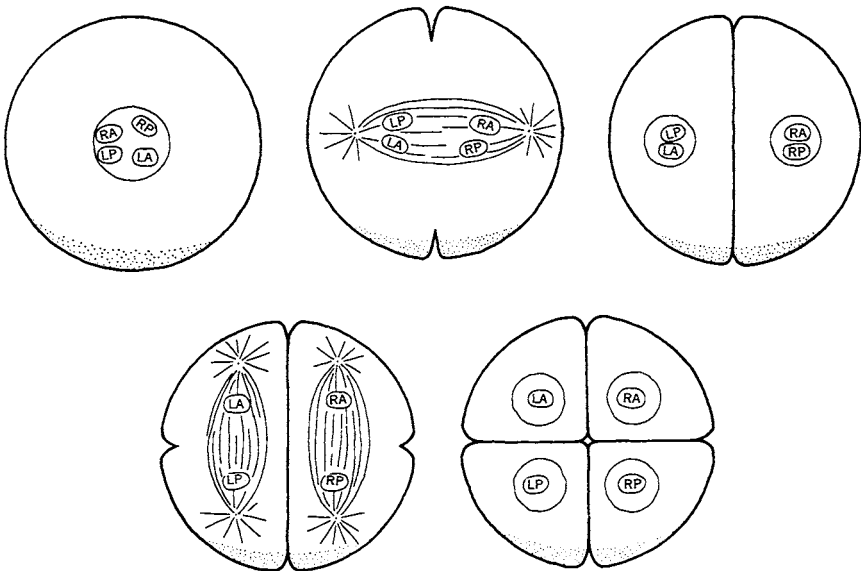
12–3 Roux's theory of the segregation of determinants during cleavage. *LA* is the determinant for the left anterior quadrant of the embryo. *RA, LP,* and *RP* are determinants for the other quadrants. Gray crescent area stippled.
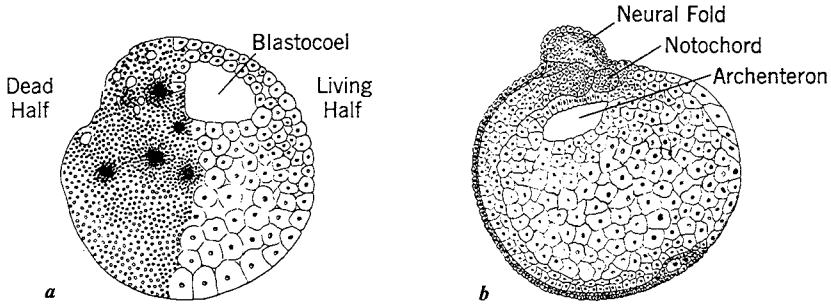
12–4 Half-embryos obtained after killing one cell of the 2-cell stage. *a* is a blastula with the dead cell adjacent to the living half-embryo. *b* is an early neurula. The dead cell has been cast off (from Roux, 1888. *Virchow's Archiv.* 114:113).

It formed a half-blastula (Fig. 12–4), underwent an abnormal gastrulation, and produced a structure that was interpreted as a half-embryo. A single neural fold was formed, and the mesoderm was present only on the uninjured side.

Roux's experiment seemed to be a dramatic proof of his theory of the segregation of determinants during cleavage. The uninjured cell formed only that part of the embryo it would have formed in an uninjured embryo. Roux interpreted this to mean that each cell was capable of forming only the structures it would in normal development. One could imagine the embryo to be a mosaic of cells, each cell capable of producing only a specific part of the adult body.

Mosaic Development in Other Embryos. Other investigators, experimenting with embryos of many kinds of animals, obtained results that seemed to confirm Roux's hypothesis. In some annelid worms it was found that cleavage is so precise that it is possible to trace the lineages of single cells. For example, it was observed that one cell, which forms at the sixth cleavage, gives rise to all of the mesoderm. In some species of tunicates (marine animals related to the vertebrates) the eggs have a pronounced pigment pattern. This enabled the observer to trace the various regions during development, in much the same way as Vogt did many years later in his experimentally stained amphibian embryos. It was found that each cell of the early embryo gives rise to specific structures in later embryo.

Embryos of annelid worms, mollusks, tunicates, and several other groups were found to behave in this manner. They were spoken of as having *mosaic development*. The embryo was regarded as an association of independent cells each developing along a predestined path to form a specific part of the adult.

The Reinterpretation of Roux's Results. The novelty of Roux's theory and its experimental verification convinced many embryologists that an understanding of differentiation was at hand. It was not long, however, before complications and exceptions were noticed.

It was found that the results Roux obtained after killing one cell of the two-cell stage embryo were due largely to the presence of the dead cell. Whether for mechanical or other reasons, the dead cell seemed to prevent the living cell from rounding up and producing a whole embryo. If one of the cells was removed by sucking it out with a pipette, the remaining one produced an entire embryo, which differed from the normal only in being smaller. In another experiment a fine thread was tied around the embryo at the two-cell stage and tightened until the two cells were separated. Each of the cells produced a normal embryo. One could conclude from these experiments that there was no evidence of a segregation of determinants as postulated in Roux's theory.

Regulative Development. The early embryo of the frog is not the mosaic of independent parts as Roux had believed. Instead, its development is more like that of the sea urchin. In the sea urchin it was found that the cells of the two-cell stage could be separated and each cell would give rise to a normal embryo. The same was true at the four-cell stage. In contrast to the mosaic embryos, in which each cell could form only the part it would in normal development, the sea-urchin embryos were said to be *regulative*. Isolated cells of the regulative eggs could adjust to the new situation and produce a whole embryo.

The conclusion was reached that there are two main patterns of development, mosaic and regulative. This was interesting but it shed no light on the fundamental causes of embryonic differentiation. Roux's hypothesis was shown to be inadequate and many years were to pass before there was a useful substitute.

THE ORGANIZER THEORY

The next major advance in the study of differentiation was made by the German embryologist Hans Spemann (1869–1941) and others in experiments on the differentiation of the amphibian nervous system. Their studies led to the organizer theory, which is the most important embryological concept proposed during the first half of the twentieth century.

Formation of the Neural Tube in the Amphibian Embryo. If we examine the cells of an amphibian embryo in the late blastula stage we find that they are essentially the same in appearance throughout the entire embryo. There is a gradient of increasing cell size extending from the

animal to the vegetal pole, and the concentration of yolk granules in the individual cells is subject to variation, but beyond this there is little to suggest the widely divergent destinies that will befall the cells of different regions. The conversion of the single-celled zygote into a many-celled blastula is brought about by cleavage, with little or no visible differentiation of the cells: the cells just get smaller. During gastrulation the cells become rearranged and the three germ layers can be distinguished, but even at this time there is little difference among the ectoderm, mesoderm, and endoderm cells. Subsequently the slow process of cellular differentiation results in various visibly different cell types, such as muscle, gland, and nerve, that make up the tissues and organs of the embryo.

We have previously learned that the nervous system is one of the first organ systems to make its appearance. Observations of the living embryo give us considerable information about its formation. At the end of gastrulation the embryo becomes flattened on the dorsal side. This flattened area is the neural plate. Next the neural folds appear as ridges along the periphery of the neural plate (Fig. 10–17). These folds move toward the mid-line and fuse along their crests (Figs. 10–18, 10–19). In this manner the neural plate is converted into a tube lying beneath the now continuous ectoderm in the dorsal part of the embryo (Fig. 10–21).

Observations of the living embryo could be supplemented by the study of sections prepared by the usual histological techniques. From these we could obtain information on the changes occurring within the embryo. We would find that by the time the neural plate is formed, gastrulation movements have brought a sheet of mesodermal cells into position beneath the neural plate (Figs. 11–6 to 11–8). Somewhat later these mesodermal cells will form the notochord and myotomes.

Repeated observations would show that the neural plate and tube are always formed in the same part of the embryo. In normal development, the ectoderm cells situated on the side of the embryo above the blastopore and their descendants produce these structures (Fig. 11–4). The appearance of the neural plate in a constant position suggests that the cells that form it differ in some way from other ectodermal cells. They alone develop into the neural plate, while the remaining ectoderm cells produce the epidermal covering of the body. It is possible to trace the positions of the presumptive neural plate and the presumptive epidermis cells back to the early gastrula, as was done by Vogt (Chapter 11). In all probability, we could even trace the presumptive regions back to the early cleavage stages.

Hypotheses of Neural Tube Formation. What is different about the portion of the ectoderm that will form the neural tube? Why does it

and no other part of the ectoderm form this structure? Observations of a normally developing embryo cannot answer such questions. We can only attack the problem by experimentation. Yet what experiment can we perform? First we must formulate a question that is precise enough for us to seek a definite answer. We already know that the group of cells that occupies the presumptive neural tube region of the early gastrula will, in later stages, form the neural plate and, still later, the neural tube. Two alternative hypotheses to explain this phenomenon could be suggested:

> *Hypothesis 1.* The presumptive neural tube cells of an early gastrula possess an inherent capacity to form neural tissue. That is, they have within themselves all that is necessary to differentiate into a neural tube.

> *Hypothesis 2.* The presumptive neural tube cells of an early gastrula do not possess an inherent capacity to form neural tissue. Influences from outside the persumptive neural tube area are necessary for differentiation of a neural tube.

Tests of the Hypotheses. These hypotheses are formulated in such a manner that they can be tested. Let us begin with the first hypothesis. If the presumptive neural tube cells possess within themselves all that is necessary for neural tube differentiation, we can make the following deduction:

> The presumptive neural tube cells should be able to differentiate into a neural tube if they are separated from the remainder of the embryo.

This separation can be accomplished in several ways.

Experiment 1. Separation of the presumptive ectoderm from the remainder of the embryo in exogastrulation. This experiment was performed by Johannes Holtfreter (born 1901). He removed the membranes from an early gastrula, oriented it with the animal hemisphere down, and let it develop in a solution with a salt concentration higher than in pond water. Under these conditions gastrulation movements were quite abnormal. The presumptive ectoderm cells did not move down over the vegetal hemisphere, but tended to pull away from the remainder of the embryo. The result was a dumbbell-shaped embryo known as an *exogastrula*. In extreme cases the presumptive ectoderm cells formed a ball connected by only a thin strand of cells with the presumptive endoderm and mesoderm (Fig. 12–5).

Further development of the two parts was very different. The cells of the presumptive endoderm and mesoderm were able to differentiate into a heart, muscles, parts of the alimentary canal and other organs
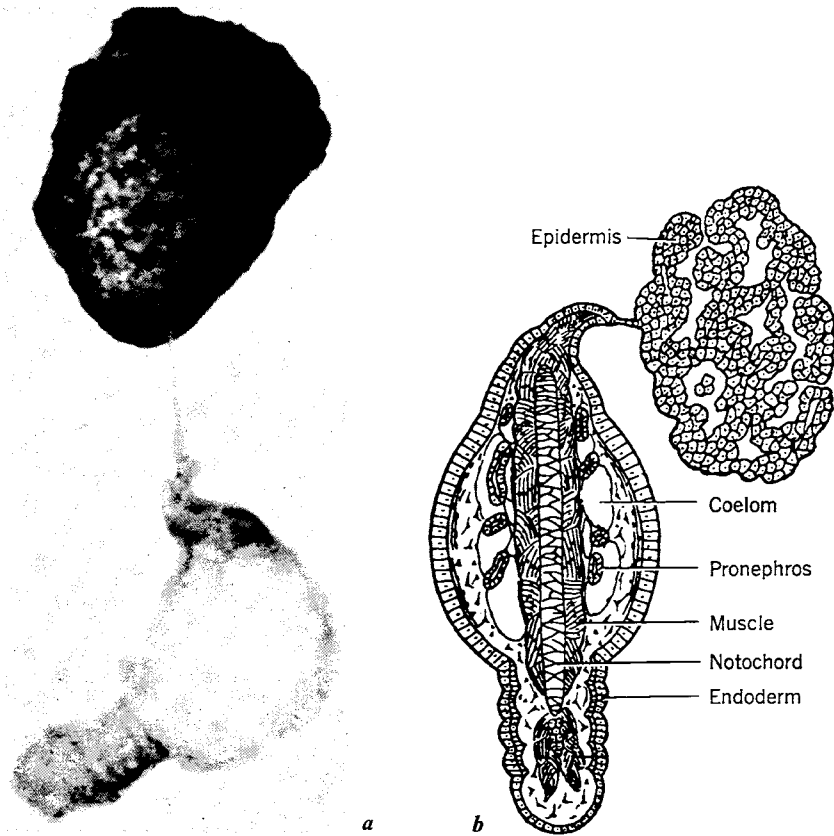
12–5 Holtfreter's exogastrulation experiment. *a* is an exogastrula and *b* is a diagram of the parts (from Holtfreter, 1933. *Biologischen Zentralblatt,* 53:404).

normally formed from these two layers. In marked contrast, the presumptive ectoderm cells remained undifferentiated. No trace of a nervous system was found. This indicates that the presumptive neural tube cells do not possess an inherent capacity to form a neural tube. Hypothesis 1 is false. We must not be too quick to accept this conclusion, however. Perhaps the manipulation of the embryo injured the presumptive neural tube cells in such a way as to prevent them from differentiating into neural tissue. If so, the experiment is not a valid refutation of the hypothesis. This explanation is improbable, however, since the presumptive endoderm and mesoderm showed a considerable amount of differentiation after being subjected to the same experimental conditions.

Experiment 2. Explanation of presumptive neural tube cells. This experiment was also performed by Holtfreter, and several other investigators. Pieces of the blastocoel roof of an early gastrula can be cut off and cultured in dilute salt solutions (Fig. 12–6). They remain alive for many days. This experimental technique is known as *explantation* and the pieces as *explants.* Explants from the presumptive neural tube and presumptive epidermis areas of an early gastrula fail to differentiate into neural tissue. They produce nothing more than a simple epidermal type of cell.

Results of Experiment 2 likewise suggest that Hypothesis 1 is false. We can only hope that neither the cutting nor the culture conditions injured the explant in some manner that would prevent the cells from revealing their full potential. Such a possibility can partially be ruled out, since explants from some other parts of the embryo are able to differentiate.

If this experiment is repeated at the end of gastrulation the results are different (Fig. 12–6). Explants of presumptive epidermis form only epidermis but explants of presumptive neural tube cells differentiate
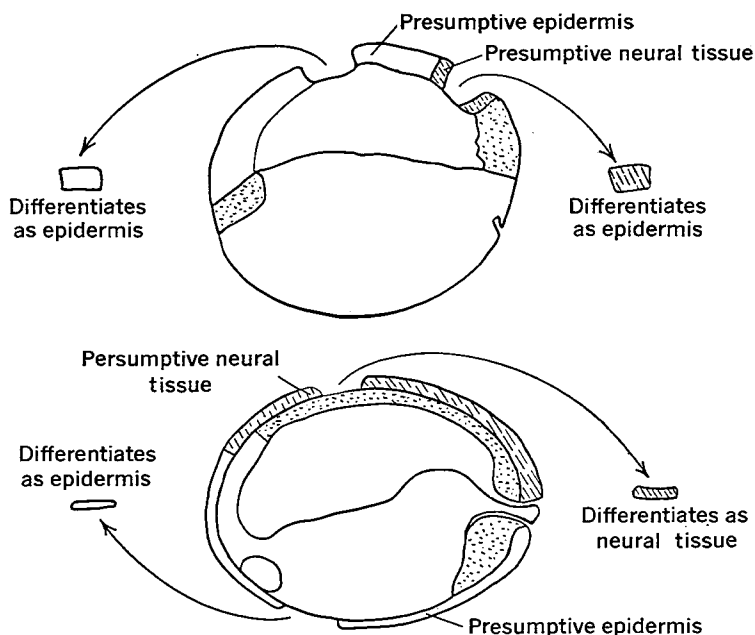
12–6 Explantation of presumptive epidermis and presumptive neural tissue in early (above) and late (below) gastrulae. (Refer to figs. 11–5 and 11–8 for full labels.)

into neural tissue. Some important change has occurred in the presumptive neural tube cells during the interval between the beginning and end of gastrulation.

The two experiments have given similar answers. If the presumptive neural tube cells are removed from an early gastrula, either by exogastrulation or explanation, no neural tissue is formed. If these results can be accepted, and it seems probable that they can, the presumptive neural tube cells in an *early gastrula* do not possess an inherent ability to form neural tissue. Thus, Hypothesis 1 is false.

Experiment 2 did show, however, that the presumptive neural tube cells of the *late gastrula* possess an ability to differentiate into neural tissue if explanted. Some change must occur in the interval between the early gastrula and late gastrula stages. This change does not occur during the development of the ectoderm in exogastrulae or in explants. Thus, it is likely that an influence from some non-ectodermal part of the embryo is responsible for this change in the presumptive neural tube cells. This is our second hypothesis, which was stated thus: 'The presumptive neural plate cells of an early gastrula do not possess an inherent capacity to form neural tissue. Influences from outside the presumptive neural plate area are necessary for differentiation.' One deduction that we might make from this second hypothesis is:

> The neural tube should form in the same position, relative to the non-ectodermal parts of the embryo, no matter how the presumptive ectoderm is oriented.

This can be tested as follows:

Experiment 3. Rotation of the animal hemisphere of an early gastrula. This experiment was performed by Spemann. It will be recalled that the presumptive neural tube cells occupy that portion of the animal hemisphere nearest to the dorsal lip (Fig. 11–4). The presumptive epidermis cells are on the opposite side of the embryo. If the upper portion of an early gastrula is cut off, rotated 180°, and put back, the cells will come into new relations with the remainder of the embryo (Fig. 12–7). The presumptive epidermis will now be closer to the dorsal lip and the presumptive neural tube will be on the far side of the embryo.

The development of the embryo on which the operation was per formed continues as though nothing has happened. The neural folds are formed in the normal relation to the blastopore. *This means that the neural folds of this experimental embryo are formed largely from presumptive epidermis, and that the epidermis is derived almost entirely from the presumptive neural tube cells.*

The results of Experiment 3 indicate that Hypothesis 2 may be cor-
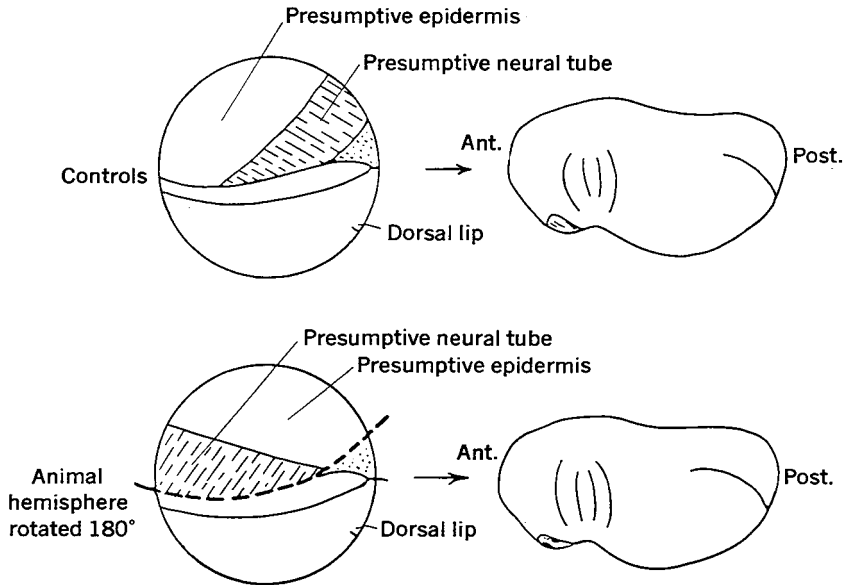
12–7 Rotation of the animal hemisphere. (Refer to fig. 11–4 for full labels.)

rect. The differentiation of the ectoderm appears to be greatly influenced by the ventral part of the embryo. Furthermore, the constant relation with the blastopore suggested to Spemann that the cells invaginated at the dorsal lip might be the stimulus for neural differentiation. In normal development these cells form the roof of the archenteron, which is immediately beneath the cells that will form the neural tube. If we regard this position as significant, we might restate the hypothesis more precisely: 'The presumptive neural plate cells of an early gastrula do not possess an inherent capacity to form neural tissue. Differentiation into neural tissue is the result of stimulation by the roof of the archenteron.' If this hypothesis is true, we might make the following deduction:

> If the doral lip cells are removed from one embryo and grafted onto another, and if they are able to invaginate under the ectodermal cells in this new position, we would expect these ectodermal cells to form neural tissue.

This deduction can be tested by an extremely delicate micro-surgical experiment.

Experiment 4. Transplantation of the dorsal lip. This experiment, reported by Spemann and Hilda Mangold in 1924, is one of the classics

of embryology. The embryos of two species of salamanders were used. In one species the embryos were nearly white and in the other they were brownish. A small piece of tissue was removed from the dorsal lip region of one embryo (Fig. 12–8). This piece of tissue was then transplanted to an early gastrula of the other species in a position 180° from the host's dorsal lip. (The embryo from which the dorsal lip was removed is called the *donor*. The embryo to which the transplant is made is called the *host*.) Since the host and donor tissues were of different colors they could be distinguished.

Spemann and Mangold's operation did not seem to affect the process of gastrulation in the host. Invagination occurred at the host's blastopore. Of greater significance was the fact that invagination occurred where the transplanted dorsal lip was placed. The donor cells invaginated through this secondary blastopore and produced a tiny archenteron. At the time the host's neural folds were forming, neural folds also appeared above the region where the donor tissue had invaginated. These neural folds were composed of host cells.

The transplanted dorsal lip had exerted a profound influence. Host cells, which in the course of normal development would have produced epidermis, were changed in such a way that they formed neural folds. Later these neural folds closed. When the embryos were examined in sections it was foun d that the transplanted dorsal lip had stimulated the formation not only of a nerve tube but other structures as well. In some cases almost an entire normal embryo arose in the region where the transplant was made.

Interpretation of the Results. Spemann and Mangold, recognizing that the dorsal lip was of great importance in the formation of embryonic structure, called it the *organizer*. This organizer was postulated to act on other cells to alter their course of development. This action is spoken of as *induction*.

This experiment, and many others, has shown that the neural tube of a normal embryo is formed under the influence of the organizer. At the beginning of gastrulation, the organizer region consists of cells above the dorsal lip, in the area corresponding roughly to the presumptive notochord of Vogt's map (Fig. 11–4). This region invaginates to form the roof of the archenteron. The roof of the archenteron then induces the overlying ectoderm cells to form a neural tube.

We now have the theoretical basis to interpret the results of the experiments on explanting pieces of presumptive neural tube cells. When these cells are explanted at the early gastrula stage they form epidermis but no neural tubes. When the presumptive neural tube cells are explanted at the end of gastrulation they are capable of forming neural

DONOR   HOST

Dorsal
lip

a

Secondary   Primary   Secondary   Secondary
embryo   embryo   embryo   embryo

b   *c*

Neural tube ⎫
Myotome   ⎪
Notochord   ⎬ Primary
Archenteron ⎪ embryo
Pronephros   ⎭

Archenteron ⎫
Pronephros ⎪
Notochord   ⎬ Secondary
Myotome   ⎪ embryo
Neural tube ⎭

d
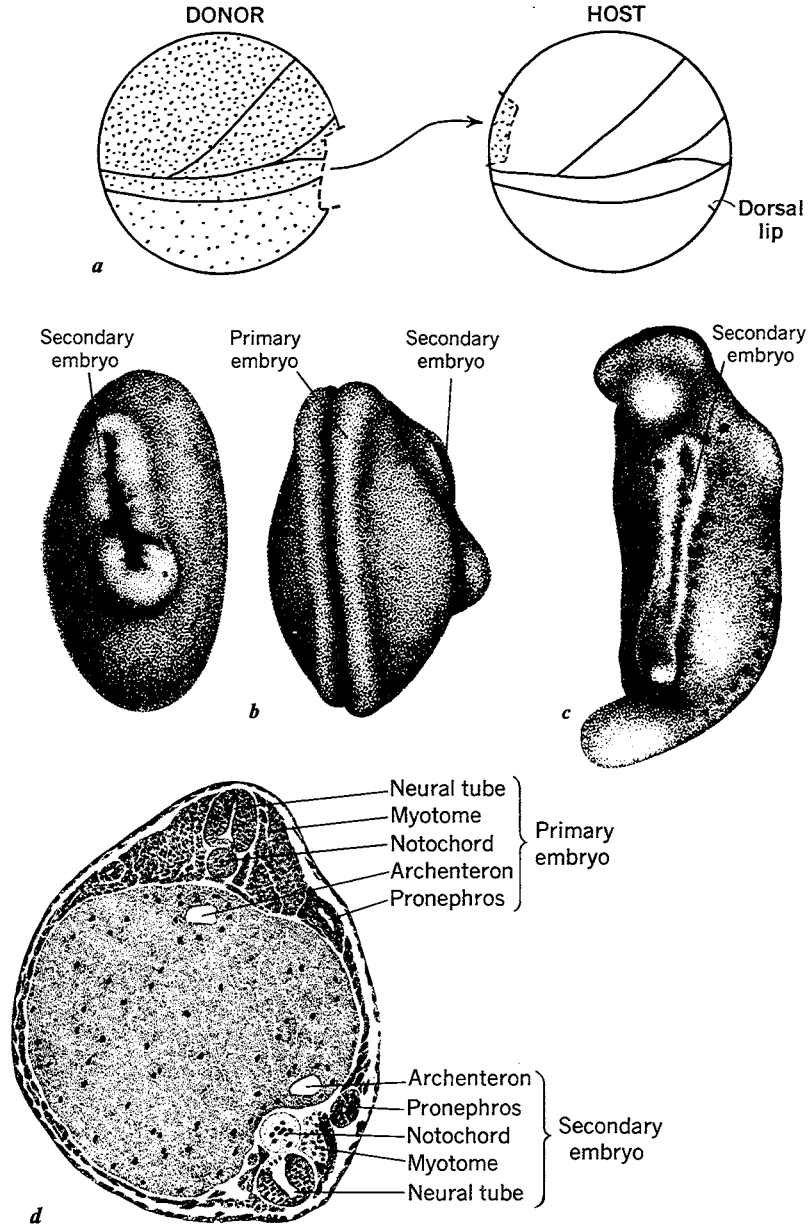
12–8 Dorsal Lip Transplantation experiment of Spemann and Mangold. *a* is a diagram of the operation. *b* is a lateral and dorsal view of a neurula with the secondary embryo. *c* is an older stage. *d* is a cross section showing the structures of the primary and the secondary embryos. (*b, c,* and *d* modified from Spemann and Mangold, *Archiv für Mikroskopische Anatomie und Entwicklungsmechanik,* 100:599. 1924.)

tubes. The explanted early-gastrula presumptive neural tube cells did not form neural tubes because the organizer had not acted upon them. At the end of gastrulation, on the other hand, the organizer area would be in the archenteron roof and the overlying presumptive neural cells would have been induced.

The early-gastrula presumptive neural tube cells are said to be *undetermined* with respect to their ability to form a neural tube. Once the organizer has acted upon them they are *determined*. Said in another way, determination results from induction. After determination has occurred differentiation is possible. Both induction and determination are invisible biochemical events. They are tested by explantation and other experimental techniques. If a piece of tissue is explanted in a suitable medium and it does not differentiate in a specific way, it is said to be undetermined. If it differentiates after explantation, this shows it was determined at the time it was removed from the embryo.

These various experiments have enabled us to choose between the two hypotheses concerning the formation of a neural tube, namely, whether (1) the presumptive neural tube cells of an early gastrula have an inherent ability to form a neural tube, or whether (2) these cells must be acted on by other parts of the embryo in order for them to differentiate in their specific manner. All the evidence given indicates that the second hypothesis is correct. At the beginning of gastrulation, the presumptive neural tube cells do not possess an inherent ability to form a neural tube. The only inherent ability they possess is to form simple epidermal cells. The ability to form a neural tube results from the induction of the presumptive neural tube cells by the organizer in the archenteron roof. At the beginning of gastrulation the entire presumptive ectoderm, whether it is in the presumptive neural tube or presumptive epidermis region, is identical in developmental capabilities. One portion forms a neural tube because the organizer acts on it; the remainder forms epidermis because the organizer does not come in contact with it.

It was soon found that there is not just one organizer but many. Organizers are known for the mouth, heart, lens, otic vesicle, pronephros, and for other structures as well. We shall consider one example of these *secondary organizers,* so called because they are produced in structures which have been formed under the influence of the primary organizer of the archenteron roof.

Formation of the Lens. Shortly after closure of the neural folds, paired outgrowth, the optic cups, appear in the ventro-lateral portion of the brain. They grow outward until they reach the epidermis. The epidermal

cells adjacent to the optic cups then differentiate into the lenses (Fig. 11–13). Vogt showed that the cells that will form the optic cup and the cells that will form the lens occupy very different regions of the early gastrula (Fig. 11–4). The optic cup area is in the presumptive neural plate, while the lens area is in the presumptive epidermis. The two areas are brought into close association by the complex movements of gastrulation and neural tube formation. Experiments have shown that, in some species at least, the optic cup contains the inductor which is the stimulus for the formation of the lens.

Let us experiment with an embryo in which the optic cups are beginning to form by making a slit in the epidermis over the brain. The optic cup on one side can be cut off and the epidermis put back in place. The cut tissues heal in a matter of minutes, and the embryo appears to suffer no injury as a result of the operation. We then allow the embryo to develop. The optic cup on the unoperated side grows out toward the epidermis and the epidermis produces a lens. On the operated side there is no regeneration of an optic cup and *no lens is formed by the presumptive lens area of the epidermis*. Thus, in the absence of an optic cup, lens differentiation does not occur. This result suggests that the optic cup may be acting as an organizer for the lens.

Another type of experiment substantiates this hypothesis: Let us remove the optic cup as it is forming, make a slit in the epidermis of the flank region of this same embryo, and push the optic cup into this slit. This slit will heal and the optic cup will then be adjacent to epidermis that would normally form only the outer layer of skin. Under the influence of the optic cup, however, the fate of this epidermis is changed—it differentiates into a lens. The embryo then has a structurally normal eye in the flank. (It is non-functional, however, since the proper nerve connections with the brain are not made.)

Obviously, these results are similar to the archenteron roof-ectoderm relationships. In the present case the optic cup is an organizer. It induces lens formation in any ectoderm of the right age with which it comes into contact. In normal development, this is the ectoderm of the head but, as experiments have demonstrated, other ectoderm will suffice.

The Nature of the Organizer. Following the discovery of the neural tube organizer, many embryologists sought to learn as much as they could about it.

The location of early gastrula cells that were capable of induction was found to be restricted roughly to the presumptive notochord and adjacent mesoderm. Transplants of living cells from other parts of the early gastrula did not induce neural structures.

The most encouraging discovery was the ability of dead organizer tissue to induce. Pieces of the dorsal lip, or of the archenteron roof, could be killed by heat or chemical means and still stimulate the formation of neural tissue in undertermined ectoderm. This suggested that the organizer was a chemical substance, and its stability after heat or chemical treatment was a sign that it might be extractable in a pure form.

This early optimism was soon shattered by discoveries that were difficult to interpret. The organizer was found to be much more widely distributed than the early experiments on living tissue had indicated. Parts of the gastrula which would not induce neural tissue when alive were found to induce after being killed. Even more perplexing was the finding that adult tissues, such as liver and kidney, could induce embryonic structures. In addition, some organic compounds appear to induce.

Some investigators have attempted to purify the organizer, but the results so far obtained have not been very convincing. Some experiments suggest that the primary organizer is nucleic acid.

Further advances in our knowledge of the nature of the organizer are awaiting new ideas and new techniques.

The Reacting Tissue. In our discussion of induction we have emphasized the organizer. This may have given the impression that the reacting tissue is passively molded by the organizer. This is not the case. The ability of tissue to respond to organizers is limited in a number of ways. Some of these will be mentioned.

We have seen earlier that any portion of the presumptive ectoderm can respond to the archenteron-roof organizer by producing a neural tube. The period during which this response is possible is very short. At or about the stage when the neural folds close, the presumptive epidermis will no longer respond to the archenteron-roof organizer. The ectoderm also has a specific period during which it can respond to the optic-cup organizer and produce a lens.

The importance of the reacting tissue can be shown in experiments involving tissue of two different species. The mouth regions of a frog larva and a salamander larva differ considerably. The frog larval mouth is bordered by prominent black, horny 'jaws' and rows of tiny 'teeth.' These jaws and teeth are ectodermal structures that have no relation to the jaws and teeth of the adult. The salamander larva lacks both the ectodermal jaws and teeth. The anterior portion of the archenteron of both frog and salamander induces the mouth region. An interesting experiment can be performed by interchanging frog and salamander ectoderm in the region where the mouth will form. The salamander

Secretion from induced ——— mucus glands

12–9 Induction of mucus glands in frog ectoderm transplanted to the ventral side of the head of a salamander embryo. Mucus glands were induced in the frog tissue and mucus is being secreted by them.

embryo will then have its mouth region covered with frog ectoderm. The frog embryo will have its mouth region covered with salamander ectoderm. Which type of mouth will form in the two cases?

The results of such experiments are clear-cut. The frog tissue on the salamander embryo is induced by the salamander mouth organizer to form a mouth. The mouth it forms, however, is of the frog type. In the same way, the salamander ectoderm on the frog embryo produces a salamander mouth. The tissue always responds in accordance with its specific genetic constitution.

A somewhat similar situation is encountered with two other structures in the head region of frog and salamander embryos. The frog has a pair of mucus glands on the ventral side near the mouth (Figs. 10–23, 10–25, and 11–14). These are ectodermal structures induced by the underlying tissue. Salamander embryos lack these mucus glands. Instead, they have a pair of balancers (Fig. 12–9), which are also induced by the underlying tissues. If presumptive epidermis of a frog embryo is transplanted to the region behind the mouth in a salamander embryo, the transplant forms mucus glands. The embryo shown in Figure 12–9 is the result of an operation of this sort. The stringy material, which appears below the host's balancer, is mucus being secreted by the induced mucus glands.

This case is of interest, since it shows that the salamander embryo can induce structures which are not a part of its own morphology. One is left with the impression that organizers are general stimuli, and that the end result of their action is modulated by the genetic limitations of the reacting tissue.

RECONSIDERATIONS AND CONCLUSIONS

The organizer concept has shed some light on the old problem of mosaic and regulative eggs. In many cases, and perhaps in most, the change

from an undertermined state to a determined state is the result of an influence external to the cells being determined. In other words, something of the nature of organizer action is involved. The main difference between mosaic and regulative eggs is thought to be the time at which these external influences cause determination. In the mosaic eggs this occurs when the egg is being formed in the ovary. The developing embryo is, therefore, mosaic from the start. In regulative eggs, on the other hand, determination occurs during development.

Modern work in genetics and embryology has put the old controversy of preformation and epigenesis in a new light. Somewhat earlier, we saw that something 'preformed' must be transmitted from adult to embryo, otherwise the frog embryo might form a toad, a fish, or an elephant. Observations on developing embryos ruled out the transmission of preformed adult structures. Genetics and embryology have demonstrated that the preformed entities which are transmitted are the genes and an organized cytoplasm. A frog embryo becomes a frog because it receives the genes and cytoplasm that control the development of a frog body. The hereditary basis of development is preformed in the structure of the gametes; the appearance of adult parts is epigenetic.

This brief survey has revealed some of the factors responsible for differentiation but embryology has not reached the point where we can say that we know in detail why an embryonic cell differentiates in a specific way. We know some of the answers and when we know more we should be able to answer a question of vital interest: 'Why do normal cells sometimes change into cancerous cells?' This also is differentiation.

Further progress in embryology will depend upon more information concerning the manner in which genes act. In the next chapter we shall explore some of the ways in which the recent data of genetics can be applied to embryological problems.

Suggested Readings

Chapter 9 in the *Readings* contains an article on mosaic development by E.B. Wilson and one on the organizer by Hans Spemann. There are also additional references.

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13 Developmental Control of Genetic Systems

The differentiation of embryonic cells must have as its basis the differentiation of the cell's genotypes. This statement must come as a surprise when one recalls a fundamental principle of genetics: mitotic cell division produces daughter cells with genetic systems identical with those of the parent cell. While this principle is probably true, the supporting data, listed below, are not absolutely convincing.

1. The data of cytology show that the chromosomes of daughter cells are identical in number, structure, and staining characteristics with those of the parent cell.
2. There are many nuclear divisions between the zygote and the adult of a species (about 35 in the frog, for example). In all the nuclear divisions of those cells in the lineage of ova or sperm, genetic integrity is maintained. We are sure of this because the genetic systems of ova and sperm can be tested by uniting them and studying the phenotype of the new individual. Only in exceptional circumstances, can we test as rigorously the genetic systems of differentiated somatic cells.
3. In many instances at least some of the somatic cells possess the same genotype as was present in the zygote. Thus, small parts of a Hydra or of a planarian worm can regenerate an entire individual. Some of the cells, therefore, must have the complete genetic information of the species.

Let us consider these facts in relation to the conclusions of Chapter 8. The data for the genetic control of protein synthesis are convincing. The DNA code is transmitted by the messenger RNA which directs the

synthesis of specific proteins. One message from DNA leads to the synthesis of hemoglobin; another message leads to the synthesis of insulin.

This explanation of the control of protein synthesis is probably correct but some exceedingly difficult questions remain unanswered. In man and other vertebrates, hemoglobin is synthesized only in the cells that are about to become red blood cells. Similarly, insulin is synthesized only by the islet cells of the pancreas. Thus, so far as synthetic abilities are concerned, the red blood cells are differentiated one way and the islet cells another way. The differentiation of these two cell types begins in the early embryo: red blood cells arise from the mesoderm and islet cells from the endoderm. As the embryo develops, the two types of cells become increasingly different morphologically and finally differ in the specific proteins that they synthesize.

How can one explain the origin of these two cell types? One hypothesis might be:

> genes for hemoglobin synthesis are present in red blood cells but are absent from islet cells; genes for insulin synthesis are present in islet cells but are absent from red blood cells.

Such a hypothesis holds that there is a genetic difference between blood cells and islet cells—a difference reflected in their microscopic appearance and in their unique proteins.

This hypothesis is clearly at variance with the data discussed in the first paragraph, which suggest there is an identical genetic system in all of an individual's cells. There are two main ways of resolving the dilemma.

First, we could assume that the data suggesting the genetic identity of all differentiated cells are inadequate and that the blood cells and islet cells are genetically different. Possibly only those cells that will form ova or sperm maintain intact the entire genetic system of the individual. Each type of somatic cell can then be thought of as genetically different from all other types. Evidence of the genetic nature of somatic cells is nearly always indirect, since normally only the genetics of germ cells can be tested adequately. Some evidence, such as regeneration in Hydra and planarian worms, suggests that at least some somatic cells have the full genetic system of the individual. Other evidence, such as the differing synthetic abilities of blood cells and islet cells, suggests that somatic cells may be genetically different.

Second, we could maintain that all cells, somatic and germ, have the same genetic systems, but that not all genes are active in all cells or at all times. With this hypothesis, we would assume that islet cells possess genes responsible for both hemoglobin synthesis and insulin synthesis, but that only those concerned with insulin synthesis are active. The genes

controlling hemoglobin synthesis would be inhibited in these cells. Furthermore, we might think of this inhibition as being either irreversible or reversible. If irreversible, it would be impossible for the insulin-synthesizing cells of the islets to produce hemoglobin under any experimental conditions. If, on the other hand, the inhibition could be removed, hemoglobin might be produced.

There are many data that bear on these possibilities, but not enough for us to reach unequivocal conclusions. Some of these will now be considered.

Tests of Genetic Identity of Somatic Cells. Since the days of Roux, every student of development has been interested in the nature of the genetic system of somatic cells. Spemann had been able to show that a single nucleus from a salamander embryo in the 16-cell stage, plus some cytoplasm, was able to develop into an entire embryo. Technical problems prevented his testing the developmental potentialities of older nuclei.

In 1952 Robert Briggs and Thomas J.King (then of the Institute for Cancer Research in Philadelphia) perfected a method for testing the nuclei of blastula and even older embryonic stages (Fig. 13–1). Basically
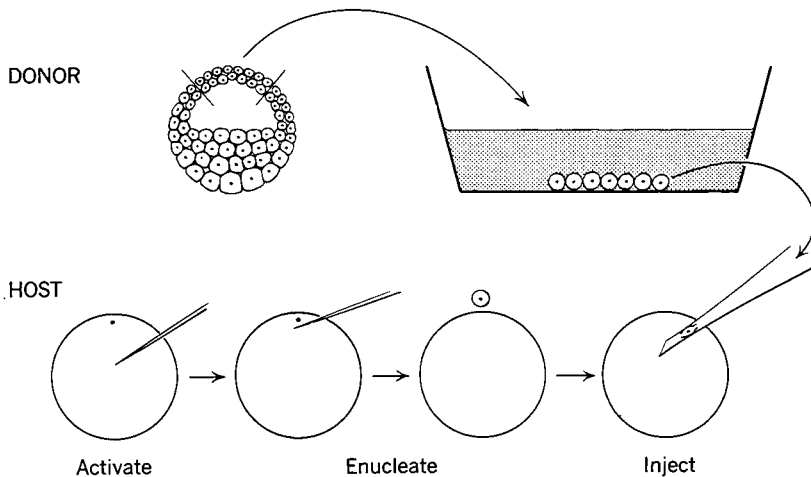
13–1 The Briggs and King method of transferring nuclei. A piece of an older donor embryo, in this case the roof of the blastocoel of a blastula, is removed and placed in a solution that causes the cells to fall free of one another. One cell is drawn into a micropipette and then injected into the host embryo. The host was previously prepared as follows: first, it was activated by being pricked with a glass needle; second, the egg nucleus was removed by flicking it out with a glass needle.

their method consists of transferring a nucleus from older embryos to an unfertilized ovum from which the egg nucleus has been removed. The nucleus to be transferred is obtained as follows. In their first experiments, Briggs and King used embryos in the middle blastula stage. A portion of the blastocoel roof was cut off and a single cell from its underside drawn into a micropipette. The pipette has a bore smaller than the diameter of the cell. Thus the cell membrane is broken as it enters the pipette but the nucleus remains intact. The broken cell with its intact nucleus is then injected into the enucleated ovum. The injected ovum may divide and form an embryo.

The experiment is difficult for both the experimenter and the embryo. Not infrequently the latter is injured, and either dies or develops abnormally. Other embryos, however, develop normally, and from these one can conclude that the transferred nucleus possesses all the information required for normal development. The injected nucleus of such an embryo would seem to be genetically identical to the zygote nucleus. Nuclei from cells of various parts of the blastula were tested in this manner. Briggs and King could find no evidence of any nuclear differentiation at this early stage.

Subsequently, John Gurdon of Oxford University was able to obtain normal embryos when the injected nucleus came from a larva, in which the cells were fully differentiated, or even from nuclei of adult cells. These discoveries gave added support to the hypothesis that all of an individual's nuclei are genetically identical. This made it all the more difficult to understand how embryonic differentation can come about.

One has to accept these facts:

1. The experiments of Briggs and King and of Gurdon seem to demonstrate that the genes of all cells in an individual are identical.
2. Since different sorts of cells produce different sorts of proteins, their genes must be functionally different.

Non-nuclear Control of Gene Action. A hypothesis is available that unites these apparently contradictory facts. One can assume that the same genes are present in all cells but that not all function in the same way. Differentiation could be a consequence of differential activation or suppression of genes. The basic mechanisms could be those discovered in microorganisms, as summarized in Figure 8–9. Recall that whether or not a gene in *E. coli* is active seems to depend on the biochemical environment in the cell.

There is a large amount of embryological data to suggest that factors external to the genes will determine which ones are active and which ones are not —and hence these external factors will control the direction

of differentiation. Some of the evidence that supports this hypothesis will now be cited.

Most geneticists of the first third of the twentieth century probably looked upon genes as controlling the cell's activities in ways quite uninfluenced by the cell as a whole. To be sure, genes required the substance of the cytoplasm for their work but this substance was something to be molded by the genes, not something to mold them. The data of genetics were most readily interpreted in this manner. Such an interpretation, however, was quite unacceptable to experimental embryologists. They were impressed by the importance of cytoplasmic materials, especially in mosaic eggs.

One of the most dramatic examples was discovered in the mollusk, Dentalium, by E.B.Wilson, whom we met as a cytologist many chapters back. The pattern of cleavage in Dentalium is precise and each cell has its fixed role in development. The first event that one notices after fertilization is the formation of a protuberance near the vegetal pole (Fig. 13–2), called the *polar lobe*. (Similar s tructures are found in many invertebrates but neither their function nor significance has ever been fully determined.) The plane of first cleavage goes to one side of the first polar lobe and, when cleavage is complete, the polar lobe flows into one of the two cells. The result is a smaller cell, known as *AB,* and a larger one with the contents of the polar lobe, known as *CD*. Shortly before second cleavage, the *CD* cell forms a second polar lobe. Its contents flow back into one of the cells, known as *D* at the end of the second cleavage. Third cleavage divides the embryo into four slightly smaller animal cells, known as *1a, 1b, 1c,* and *1d,* and four slightly larger vegetal cells known as *1A, 1B, 1C,* and *1D*.
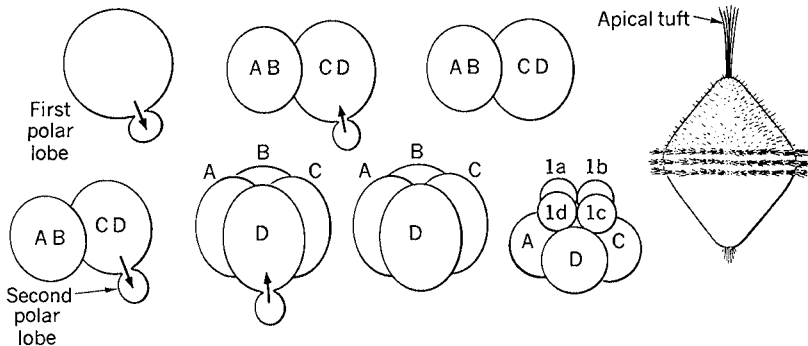
13–2 Dentalium. The pattern of cleavage in the early embryo and the trochophore larva.

The cleavages continue in a very exact way and after a day a trochophore larva is formed. This is a spindle-shaped little creature with a tuft of long flagella at the apical end and a band of cilia, the prototroch, around its middle.

Wilson found that it is possible to separate the cells of the early embryo and he did so in the hope of finding the role that each plays in the formation of the trochophore larva.

The first thing he did was to cut off the first polar lobe. Apart from the fact that the second polar lobe failed to form, early development seemed to be normal. The larva, however, was a disaster. It lacked an apical tuft and the entire region posterior to the prototroch. Clearly the first polar lobe is essential for the formation of a normal trochophore.

After first cleavage occurred, he isolated the *AB* and *CD* cells. The *AB* cell failed to form a second polar lobe but otherwise it cleaved as though it was still part of a whole embryo (i.e. mosaic development). It formed a larva that lacked both apical tuft and posttrochal region. The *CD* did form a second polar lobe and cleaved as though part of the whole. It produced a larva with both the apical tuft and the posttrochal region (an unusually large one, in fact).

Thus, something necessary for the formation of the apical tuft and the posttrochal region is localized in the first polar lobe and then passes into the *CD* cell.

Wilson removed the second polar lobe and observed that the larva still lacked the posttrochal region but almost always had an apical tuft.

When the individual cells of the four-cell stage were isolated, *A, B,* and *C* each produced a larva lacking the apical tuft and the posttrochal region. *D* alone produced a larva with both.

Finally after the third cleavage had occurred, he isolated the four cells of the animal hemisphere: *1a, 1b, 1c,* and *1d.* All produced swimming larvae. None had a posttrochal region and *1d* alone had an apical tuft.

Putting these data together, Wilson concluded that the substances in the egg that are necessary if the posttrochal region is to develop are originally in the vegetal hemisphere of the uncleaved egg. Subsequently they are localized in the first polar lobe, *CD* cell, second polar lobe, and *D* cell. Similarly, the materials necessary for the apical tuft are first in the vegetal hemisphere and then in the first polar lobe, *CD* cell, *D* cell, and then in the *1d* cell.

The polar lobes do not contain a nucleus so the substances responsible for the apical tuft and the posttrochal region must be cytoplasmic. Without these substances the genes of Dentalium are unable to make either

an apical tuft or the posttrochal region. When these substances are present, they can.

If the model for gene action that was proposed for *E. coli* (Fig. 8–9), holds here as well, possibly the observations on Dentalium can be interpreted as follows. The genes that are involved in the formation of the apical tuft are normally 'turned off.' That is, their regulator proteins are combined with the operator sites and prevent the synthesis of mRNA. The substances that were in the first polar lobe and finally in the *1d* cell are able to combine with the regulator proteins and prevent them from attaching to the operator sites. With no repression, the genes can make the mRNAs that are responsible for the formation of the apical tuft.

This hypothesis is consistent with the thinking of embryologists, who fail to see how a genetic system, identical in all cells, alone provides for cellular differentiation. They conceive, instead, that external conditions or cytoplasmic substances interact with a uniform cellular genetic system to provide for differentiation. Though the genetic system specifies what a cell may do, nongenetic phenomena influence what it actually does. This point of view, which once would have been reasonable to an embryologist but not to a geneticist, now seems reasonable to both.

Now another example will be given.

Cytodifferentiation in Fucus. Cellular differentiation in the seaweed Fucus (commonly called rockweed) is present at the first possible opportunity, that is, by the two-cell stage. At first the zygote is spherical (Fig. 13–3). Before first cleavage begins, a protuberance appears on one side, giving the zygote roughly the shape of a snowshoe. The polarity thus developed is the basis of further differentiation.

First cleavage cuts across the long axis of the zygote, producing two cells of different shapes—only one having the protuberance. The developmental fates of the two cells are entirely different. The cell with the protuberance divides repeatedly to give rise to the rhizoid, which attaches the Fucus to the rocks. The other cell gives rise to the thallus, which is the leaf-like part of the plant. According to D.M.Whitaker, who made these observations on Fucus, 'when the point of origin of the rhizoid protuberance is determined, the polarity and whole developmental pattern of the embryo is determined.'

The factors responsible for the formation of the protuberance, therefore, are of fundamental importance in determining the differentiation of cells in Fucus. Whittaker found that seemingly minor environmental differences could determine the point of outgrowth of the protuberance. If the zygotes are in a group, the protuberances are directed inward
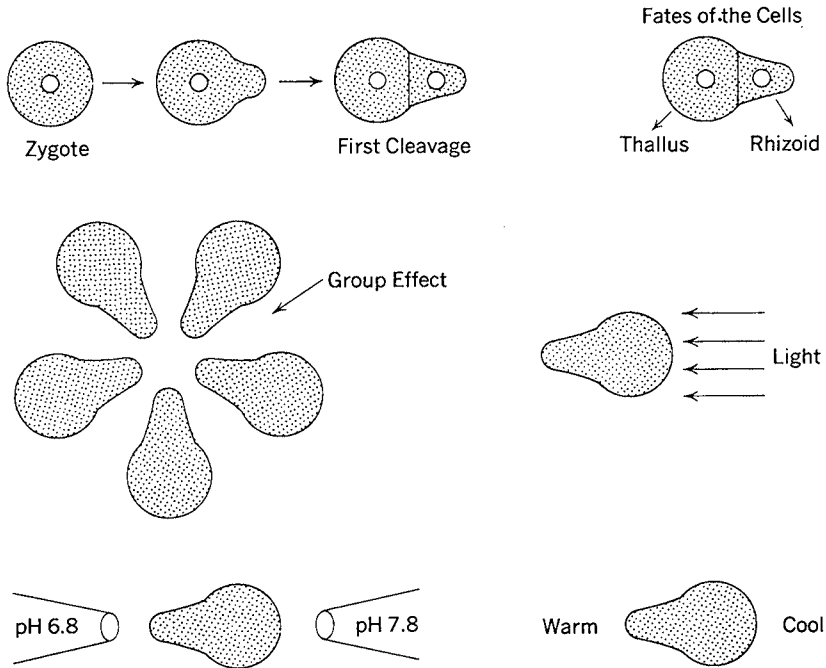
13–3 Early development of Fucus and some of the factors that influence the formation of the protuberance.

(Fig. 13–3). If the zygote is placed between two pipettes, one with seawater agar at pH 7.8 and the other at pH 6.4, the protuberance appears on the side with the lower pH. If the zygotes are exposed to white light, the protuberance appears on the dark side. If the zygotes are kept in a temperature gradient, the protuberance appears on the warmer side.

Thus an initial stimulus, which has nothing to do with the nucleus, controls the beginning of a series of events that is of profound importance in cellular differentiation. The protuberance begins when the embryo contains a single nucleus. The first divisions occurs some hours after the protuberance has been induced, and in a plane related to the protuberance itself. Recall also that, once the protuberance has formed, the basic polarity of the developing embryo has been determined. What the nucleus will do later, therefore, is the result of an initial environmental stimulus.

There is much evidence from many other organisms suggesting that the basic polarity of an egg, hence of the embryo itself, is determined

by conditions external to the maturing egg cell. In these important events, the egg's nucleus seems to play no determining role. In many mosaic embryos the pattern of development is closely correlated with the distribution, during cleavage, of visibly different cytoplasmic regions.

The establishment of some polarity or regional differences in an embryo is an exceedingly important factor in development. Consider the result if this were not the case: a nucleus dividing by mitosis in an unorganized cytoplasm would produce only a group of similar cells, certainly not a differentiated embryo. There must be some initial induction of a difference, some defining of polarity. Once this has been brought about, one can suggest models for the manner in which cellular differentiation can proceed, even with nuclei that are at first functionally identical.

Cellular differentiation may be thought of as an interaction between nuclei, which are genetically identical, and different cytoplasmic regions. In many cases these cytoplasmic regions are formed under the control of genes that acted much earlier, usually when the ova were being formed. In other cases, such as Fucus, the environment may be important in inducing cytoplasmic differences. Possibly the substances that give the cytoplasm its regional specificity interact with the substances (Fig. 8–9) produced by the regulator genes. Whatever the mechanism may be, it seems probable that there is a functional nuclear differentiation concomitant with the differentiation of the cell as a whole. A nucleus of an islet cell does not develop the way it does because of some innate specificity. Instead it *is developed*— developed in a specific way because of the cytoplasm in which it happens to lie.

A generation ago few embryologists or geneticists would have predicted that a synthesis of their fields would be made possible by studies on the bacterium *Escherichia coli*. But this microscopic creature, with no embryology of its own, has shown a way. Now it is difficult to distinguish between a geneticist and an embryologist, as they advance their science beyond what each might independently achieve.

Suggested Readings

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