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FURTHER STUDIES ON THE MODIFICATION OF THE GERM-CELLS IN MAMMALS: THE EFFECT OF ALCOHOL ON TREATED GUINEA-PIGS AND THEIR DESCENDANTS

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NINE FIGURES

CONTENTS

1. INTRODUCTION

The present contribution presents the results obtained during the sixth and seventh years of an experiment on the modification of mammalian germ cells by the treatment of parental generations with alcohol. A number of new facts are added to our previous findings, and the data now permit a more thorough analysis. Treating the results obtained during these two years separately may be looked upon as taking a cross-section of the entire experiment. And when this isolated portion of the investigation is compared with the previous studies, it supplies a further most important control for the experiment as a whole.

The earlier reports of this investigation (Stockard, '12, '13, and '14; Stockard and Papanicolaou, '16) were made after the first two years, three years, and five years of its progress. These reports showed, in what seems to us a definite way, that the germ cells in either the male or female mammal may be changed or affected by a chemical treatment administered to the body of the individual. The progeny derived from such chemically treated animals showed more or less marked deviations from the normal in many definitely measurable qualities, such as their mortality records, structural appearance, nervous reactions, and ability to reproduce. The treatment also affected in the male, the crucial germ-cell test for mammals, their ability to beget offspring when mated with normal females.

In general it may be stated that the offspring produced when treated males were paired with normal females were inferior in several respects as compared with other offspring from the same normal mothers bred to control males of exactly the same original stock. Further, when the male offspring from treated fathers

were mated with normal females, the individuals resulting from such matings were as a group decidedly inferior to the young produced by normal females when mated with control males. This group inferiority was not only present in the grandchildren, or F_2 generation, but also in the F_3 generation descended from alcoholized great-grandparents.

Fortunately, since these experiments were first reported, several similar studies by other investigators using the methods here employed have been conducted on other mammals and birds. Our results have been corroborated, though the response to the treatment has in some instances been thought to differ from that shown by the guinea-pigs. Useful and suggestive interpretations of the results have been advanced, yet certain points of view are presented with which we are not always able to completely agree. The bearing of these studies on the present results will be discussed in a section beyond.

In particular we are indebted to Pearl ('17) for his recent characteristically clear and exact analysis of the influence of alcohol fumes on domestic fowls and their progeny. This study has suggested to us the importance of a considerable amount of data contained in the card-catalogue records of our animals which had not been fully valued in the previous discussions of the experiments. In the present report we have followed several of Pearl's ideas in more completely separating the qualities to be contrasted between the alcoholic and control lines.

As might be expected, various objections have been advanced from time to time regarding the cause and explanations of the results which we have reported on the effects of alcohol in the guinea-pig. In all cases, however, the objections have been raised either by persons entirely unfamiliar with these animals and their breeding qualities or by others who have not been sufficiently interested or careful to read the descriptions of the animals and breeding methods used. It has been suggested on certain occasions that the defects and degenerate conditions which have been reported in our alcoholic lines were probably present in the original stock on which the experiment was conducted. Such a remark in the face of the experimental control

which has been fully described scarcely warrants discussion, yet
we should like to state in the beginning for the benefit of the
casual critic who may not wander through the following pages
the real nature of the original control.

A group of forty animals, eleven males and twenty-nine fe-
males, was obtained from a reliable breeder in the early fal of
1910. These animals were all under one year old and strong and
vigorous in appearance; most of the females were pregnant.
All the females were kept until they had produced a normal
litter of young. Their production was what would ordinarily be
obtained from healthy guinea-pigs; all of the young were nor-
mal in appearance and about 80 per cent of them survived under
the by no means perfect system of care then employed.

Three males and six females, after the test matings, were then
taken for alcohol treatment. The choice was entirely random,
there being no evident marks of superiority or inferiority in any
of them as compared with the other animals retained as normal
control. One of the three males selected for treatment lived to
be more than seven years old, and the others were all healthy,
strong animals that lived long and bred vigorously. These
treated males were mated with alcoholic females and with nor-
mal females. The same normal females were mated at different
times with normal males and such offspring were considered
control. From the beginning of the experiments it may be said
that the same normal female often serves as part of the experi-
ment, being mated to alcoholic males and again as the control.
The same is true of normal males; they are frequently mated
successively with alcoholic females and normal females. From
this original stock the normal animals, both males and females,
have invariably given rise to average normal offspring when
paired with normal mates, while, on the other hand, the treated
animals being part of the same breed, have in the quality of their
offspring shown a decidedly inferior condition even when paired
with normal mates.

After the experiment had been in progress for eighteen months,
in March, 1912, a new stock of animals of an entirely different
source from the first lot was introduced. Again, after testing

their breeding ability by one normal mating, certain of this lot were taken for alcohol treatment, and these animals were bred both separately and with the original lot. Yet the records of the alcoholic and normal individuals were again different

Finally, in October, 1915, when the experiment was five years old, we obtained four new stocks of guinea-pigs from different dealers and introduced them into the experiments in various ways along with our now pedigreed lines from the old stock. The records of these new animals as well as our old lines known for three or more generations regarding inbreeding and other conditions are to be considered in the present paper. These experiments bring out additional facts in the study, and we believe they supply an unquestionable control on the previous results. In other words, this may be taken as a new study considering the conditions of 1,170 guinea-pigs born from various alcoholic lines as well as from normal control animals. About 600 of the animals are born of alcoholic lines with no inbreeding in any case back through their great-grandparents. About 300 of them are from alcoholic lines and at the same time somewhat inbred; these are for all considerations treated separately from the straight alcoholic lines. The control animals with which the alcoholics are compared are of the same blood lines as the alcoholics and are also not inbred.

2. QUALITY OF THE EXPERIMENTED AND CONTROL ANIMALS

a. Selection of animals

As briefly mentioned above, the control and the first treated individuals are derived from exactly the same original stock. During the progress of the experiment other animals have been subjected to the treatment, and these in many cases are of known pedigree for several generations in our colony. In all cases only vigorous animals are used for the treatment and they are invariably tested by being mated at least once before the treatment is commenced. This precaution is undoubtedly of much importance, equally as important as knowing the blood lines, in selecting normal breeders. These test matings are

further strengthened by the fact that the same normal males
are mated with alcoholic females and with normal females, and
normal females are mated with alcoholic males and again with
normal males as a control, etc.   In this way the experimental and
control animals are actually in some cases the same individuals
and in all cases they are constantly being bred together.   There
is no question that the animals treated with alcohol and the
control are equally general or random samples of the popula-
tion.   Yet there is a marked contrast between the records of
their offspring and descendants.

b. Inbreeding

The alcoholic lines which we shall analyze in detail in the
following considerations are practically devoid of inbreeding.
Almost all of these animals are known in our colony for three or
more parental generations, and we mean in stating that they are
not inbred that a given individual in their ancestry never ap-
pears more than once back through the great-grandparent gen-
eration.   In the first table to be considered the straight alcoholic
lines may be compared with other lines that are not only alco-
holic, but also inbred, usually to a slight degree, and it is seen
that inbreeding in either the alcoholic or the control to a lim-
ited degree gives no indication of any significantly injurious
effects.

In our former report ('16) there were shown to be more in-
jurious effects in the alcoholic inbred lines than in the non-
inbred.   This difference has now disappeared on account of the
fact that the animals in the former table were more closely in-
bred and were earlier generations than the bulk of those in the
present consideration.   The degree of inbreeding in the inbred
lines is now much reduced as compared with the earlier table,
and the records have improved.   This difference between the
earlier and the present results indicates that inbreeding in these
alcoholic lines may be easily carried to a degree which will make
the injurious effects more marked.   We have avoided even the
slightest approach to such a degree of inbreeding in the straight

alcoholic lines. The pedigrees of a great majority of these alcoholic animals could readily be given to cover several generations, but it does not seem advisable to enter into this detail, since there is no possible chance that any differences which might exist between the normal and alcoholic lines are due to different degrees of inbreeding among the individuals of the two groups. And further, in all the groups it is entirely out of the question that any difference between the records of the control and the records of the alcoholic may be due to the control having been by chance originally good breeders and the alcoholics originally bad. The control animals are in almost all cases either sisters, brothers, parents or other blood relations of the treated animals.

c. The number of animals alcoholized

Recognizing the great variability in the breeding results from the different individuals in a group of higher animals, such as mammals, it has been deemed entirely essential to make our experiment on a considerable number of males and females. The mating records of two normal male guinea-pigs are frequently quite different even though paired with the same females. It is also highly probable that different individuals will differ in their susceptibilities and responses to the treatment, so that the records of two or three males might easily prove confusing even though all might exhibit some effects of the experimental treatment. Thus the following twenty-eight males have been treated with alcohol, and a number of matings from each of these and their descendants have supplied the breeding records. The first three are from the original 1910 stock Nos. 4 ♂, 5 ♂, and 6 ♂, and the remaining twenty-five are animals bred and reared in the colony or from the newly introduced stocks: Nos. 43 ♂, 45 ♂, 70 ♂, 72 ♂, 80 ♂, 81 ♂, 678 ♂, 887 ♂, 913 ♂, $\frac{AN}{A}$ 129 ♂, 157 ♂, 168 ♂. 183 ♂, 302 ♂, 353 ♂, 365 ♂, 574 ♂ $\frac{AA}{A}$ 493 ♂. $\frac{(NAp)\,(AA)}{A}$ 771 ♂, $\frac{\frac{AN}{A}N}{A}$ 889 ♂, 1091 ♂, 1134 ♂, 1153 ♂, 1326 ♂ and 1327 ♂.

In the case of the females an attempt has also been made to lessen the error caused by individual differences in breeding capacity and in responses to the treatment by using a number of animals. Thirty-four individuals have been treated in all. Many of these females were bred for a number of times as control before being subjected to the fume treatment, after which they are placed of course among the alcoholics. Their earlier breeding records are therefore part of the control data and their subsequent records part of the data included for the alcoholic lines. The same thing is true of a number of the males mentioned above. In none of these cases can it be objected that the animals had become too old for normal vigorous breeding while being used in the alcoholic lines. We have constantly guarded against breeding the alcoholic animals after there is any question as to age affecting their breeding capacities when compared with the normal breeding cycle of these guinea-pigs. The treatment of the large majority of the animals is begun when they are less than one year old, and they have a vigorous breeding span of at least four years. The individual females which have been subjected to the alcohol fumes are the following: The first six are from the original 1910 stock, Nos. 8 ♀, 9 ♀, 10 ♀, 11 ♀, 12 ♀, and 34 ♀; the following twenty-eight are animals reared in the colony or from the newly introduced stocks: Nos. 55 ♀, 57 ♀, 59 ♀, 60 ♀, 61 ♀, 62 ♀ 64 ♀, 65 ♀ 66 ♀, 88 ♀ 90 ♀, 117 ♀, 158 ♀, 161 ♀, 654 ♀, 847 ♀, 865 ♀, 946 ♀, $\dfrac{NA}{A}$ 122 ♀, 200 ♀

228 ♀, 397 ♀, 1139 ♀, $N\dfrac{\frac{NA}{A}}{A}$ 796 ♀, 1002 ♀, 1105 ♀, $N\dfrac{N\frac{NA}{A}}{\frac{A}{A}}$

1468 ♀ and 1469 ♀.

There are no contrasts between the histories and capacities of the experimented and control animals that can be fairly accounted for as due to differences in either their origins, blood lines, or relationships. As far as experiment and control with biological material may be practically useful, any differences which may exist between the records of the alcoholic guinea-

pigs and the normal control lines are due to the treatment administered to the alcoholic lines. We further believe that if the differences which do exist between the alcoholics and control are so slight that the crudest mathematical calculations are insufficient to indicate their presence, the experiment has then produced no d·   of biological interest or importance since conducted on aniual material of such complexity as a group of mammals. This statement is made with no intention or presumption to question the real importance and value of modern biometrical methods, but is only what we believe should apply to this particular experiment.

3. EXPERIMENTAL METHOD AND THE CARE OF ANIMALS

Throughout these experiments alcohol has been administered to the guinea-pigs by a method of inhalation which was devised in the beginning. The animals to be treated are placed in fume tanks fully described and illustrated in an earlier communication (Stockard, '12) and absorbent cotton soaked with commercial 95 per cent ethyl alcohol is placed on the floor of the tank beneath a wire screen on which the animals stand. The fumes of evaporating alcohol very soon saturate the atmosphere of the tanks and the guinea-pigs introduced into this saturated atmosphere are allowed to remain until they show distinct signs of intoxication. During the earlier years of the experiment they remained for one hour each day in such tanks, but during the past twelve months we have increased the treatment to two hours per day for the males and three hours for the females.

This longer treatment is much better in that the animal, of course, gets a larger dose and its tissues may become more quickly influenced by the treatment. The animals may remain until they are completely intoxicated, in which case they are unable to walk, and therefore lie in a typical drunken stupor, or they may be affected to such an extent that they attempt to walk and in so doing stagger and fall in a manner characteristic of the drunken state. The amount of treatment here employed, however, does not produce complete intoxication.

It would be perfectly possible with an elaborate system of measurements to determine exactly the quantity of alcohol fumes

each individual receives per day, per month, or per year, but, as we have pointed out before, such knowledge would be of no advantage either to us or to others in estimating the results of these experiments. No two individuals would be affected to exactly the same degree by the same dose, and as is the case with man the later influences of the treatment no doubt differ in different individuals. There is also no particular interest here in the amount of alcohol used, since our primary problem is whether or not an active chemical substance may be given in sufficient amounts to the parent mammal to produce effects upon its offspring or descendants by modifying its germ-cells, or in the case of the pregnant female by acting through the mother on the developing embryo.

We have thus employed, as stated in our previous reports, a simple physiological index of the amount of treatment, giving enough each day to perceptibly influence or intoxicate the animals, but not enough to produce a complete drunken stupor.

Animals may remain for very long times in these treatment tanks when alcohol fumes are not present without in any way suffering for want of breathing space. This method has many advantages so far as the general health of the individual animal is concerned over drinking alcohol into the stomach, as will be discussed in the following section.

The only object in choosing alcohol as the treating agent is on account of the fact that considerable knowledge exists as to its physiological actions on certain animal tissues and it is known to be an active organic substance that might produce effects. It had further been used by one of us (Stockard, '10) in producing various developmental abnormalities in fish embryos which could be treated directly with diluted alcohol, and the general nature of the effects on these embryos had been studied. A final advantage in using alcohol in such experiments is the ease with which it may be administered to the animals by the inhalation method which we have described.

Caging and care of animals. All of the guinea-pigs, both the experimental and the control animals, are kept in the same type wooden cages. These are group cages, each containing twenty

compartments one foot high by one foot wide by two feet long. Each compartment is sufficient to fully accommodate one female with her litter of young or three adult animals. In all of the cages some of the compartments are occupied by the alcoholic animals and others by the control so that the cage accommodations for the two classes are identical. The cages are thoroughly cleaned, the floor sprinkled with sawdust and fresh hay put in daily. In addition to the hay, which is eaten with relish, the animals are fed every day with fresh carrots and several times per week oats are given with occasional cabbage or kale. It is also important for their perfect health, though not necessary for their existence, that guinea-pigs be given fresh water every day during the warmer months and several times per week during the winter. This is frequently neglected in keeping these animals since it is commonly thought that they get a sufficient amount of water from the green foods. At the present stage of this experiment, along with several other problems now being studied, a stock of over 500 animals is constantly kept on hand. One reliable keeper devotes his entire time to cleaning the cages and feeding. He in no case discriminates in his treatment of different animals and from the cage numbers is unable to know all of the alcoholic line animals or the controls.

From the beginning of this experiment, in making the matings a male is placed in a compartment with one female during her heat period (Stockard and Papanicolaou, '17); in this way there is no opportunity for preferential or choice matings. A male might discriminate in his behavior between an alcoholic and a normal female if in a compartment with the two, as Pearl believes his roosters have done when placed in a pen with both normal and alcoholic hens. After the male has remained in the pen for one month, the female is carefully examined and at this time with some practice the investigator may feel the small embryos in the horns of the uterus. The male is removed and the female remains alone in the compartment. A list of all pregnant animals, both alcoholic and control, is kept and their compartments are examined both morning and late afternoon of each day in order to detect an abortion should it occur, since the

female may devour the early aborted young. In addition to this, each pregnant female is reëxamined once or twice and the number of fetuses in the right and left horns of the uterus recorded each time on her catalogue record.

By this method it has been found that a number of females may often absorb their embryos, either one or all, and so give birth to a smaller litter than originally began development or to none at all. The absorption of individual embryos seems so far as we have detected not to interfere with the development of the remaining ones. These examinations of pregnant females have been repeatedly controlled by opening the animals and observing the contents of the uterus, and the examinations in all cases have been very accurate. This thorough watch over the females has furnished us much more exact data as to prenatal deaths, early absorptions, etc., than were contained in our former reports.

The entire care of the animals has been much improved during the past two years. Our records for monsters and other weakened conditions are, therefore, somewhat reduced; yet the same marked contrast between alcoholic and control is present even though the weakened alcoholic lines have no doubt profited more by the improved methods of care and feeding than have the healthier controls. The defects are also the same in type as those formerly observed, though not so marked in degree.

4. THE INFLUENCE OF ALCOHOL INHALATION ON THE INDIVIDUAL

The immediate effects on guinea-pigs of inhaling alcohol are somewhat similar to those observed after drinking it. As stated above, the animals after some time become unable to walk without staggering as a result of loss of muscular coördination and finally reach, with a long treatment, a state of complete alcoholic stupor.

The presence of alcohol in the blood of the guinea-pig after the inhalation treatment is readily detected by even simple chemical tests, as we have frequently pointed out. Other investigators also find that alcohol is easily introduced into the

general system of birds and several mammals by this method. Pearl ('16 b) definitely recognizes the fact that alcohol is readily taken into the system by the inhalation method, but makes the following statement regarding the effect: "It is true that it is practically impossible to induce by the inhalation method in animals habituated to alcohol that state of muscular incoördination which is usually, but by no means always, the most striking objective symptom of the condition of being drunk." Our observations on guinea-pigs show them to respond very differently in this respect from the fowls used by Pearl. In the case of guinea-pigs habituated to alcohol, it is very easy by the inhalation method to induce a state of muscular incoördination due to the drunken state and finally a complete anaesthesia, the muscles being entirely relaxed and the animal unable to move. It may be that fowls are peculiar in their reaction to alcohol and it may also be extremely difficult to administer to them a highly effective dose without fatal results. Such an idea is suggested by the fact that Pearl does not get the gross symptoms of intoxication by leaving his fowls in the tanks for one hour, yet they ' accumulate a fatally toxic dose of alcohol by staying in the same tank under the same conditions for from twenty minutes to half an hour longer." Guinea-pigs do not at all react in this manner after an hour or two in the tank they may show signs of intoxication by becoming groggy, with their muscles generally relaxed so that when lifted their bodies are almost entirely limp. Yet they have not consumed anything near the fatal dose, since they may remain in the same tank under the same conditions for even two or three hours longer before becoming completely intoxicated so as to be unable to move; and in order to inhale a fatal dose they must remain still longer, at least six or seven hours

We have treated only one fowl, a white leghorn cock, in our tanks. This bird responded much as the guinea-pigs do showing decided muscular incoördination, staggering and frequently almost falling as it walked. He was also able to withstand a long treatment and never, though treated several times, did he show any tendency to suddenly accumulate a fatally toxic dose as Pearl found his fowls to do.

*a. Contrast between the immediate effects of alcohol taken by
inhalation and by stomach*

An important point to keep in mind when considering these
animals intoxicated by the inhalation method is that on being
removed from the tanks they use up the alcohol in their systems
very rapidly and also begin to throw off alcohol by respiration.
The intoxication is, therefore, of short duration so that the
animal may be fairly well recovered within half an hour or perhaps
only a few minutes, depending upon the amount of the treatment.
In other words, this is an acute short intoxication closely com-
parable to an ether anaesthesia from which the animal readily
recovers when the fumes are no longer inhaled, but which during
the inhalation may give a complete intoxication. On the other
hand, a drunken condition resulting from taking alcohol into the
stomach is of much greater duration since the gradual absorption
of the alcohol continues for a longer time before the system
begins to burn it up or throw it off to such a degree that the
amount present begins to be continuously reduced, permitting
the animal to slowly recover from the drunken state. A guinea-
pig receiving a dose of about 25 cc. of 15 per cent alcohol into
its stomach will be decidedly intoxicated within fifteen or twenty
minutes, and the extent of intoxication will increase until the
animal becomes unable to walk or stand and lies in a drunken
stupor. Such a condition may persist for six or seven hours or
longer, and the body temperature may be lowered from one to
even four degrees Fahrenheit.

It seems to us, therefore, that the chief difference between
inhaling alcohol and drinking it into the stomach is that in the
first case the action of the substance on the animal system is of
shorter duration, lasting but little longer than the length of the
sojourn in the fume tanks—a short acute effect—while alcohol
in the stomach is gradually and continuously absorbed for a
considerable length of time so that the animal's tissues are acted
upon for hours after receiving the dose. Another very serious
phase of the stomach alcohol, aside from the typical intoxication
effects, is its tendency to derange the animal's powers of diges-

tion and thus to cause very injurious results.   The inhalation method is accompanied by no such complications.

We have now considerable data bearing on this problem and are conducting an experiment to determine the quality of the effects on the animal body and the progeny produced when dilute alcohol is taken into the stomach of guinea-pigs for long periods of time.   The results of this study are to be compared with the data from the fume-treated animals.

b. *The vigorous condition of the animal after daily inhalation of alcohol for long periods*

A number of the guinea-pigs have now been treated with alcohol fumes almost to a state of intoxication six days per week for from five to six years.   Few guinea-pigs in captivity live so long a time.   There were two males treated for over six years, one of which lived to be more than seven years old.   So far as we know, this is the longest life reported for a guinea-pig.   The treatment was continued with these very old animals but they were not used for breeding.   In no case when the treatment was begun on an animal over three months old could any injurious effects on its general welfare or length of life be discovered.   We have called attention to these facts in our previous publications.

There are certain direct injuries resulting from the inhalation of ethyl-alcohol fumes during the early stages of the treatment. The mucosa of the respiratory tract is considerably irritated during the first few months and secretes freely while the animals are in the tanks, causing a watery flow from the nostrils and mouth.   The membranes become more resistant as the treatment goes on and later little effect can be noticed.   This irritation has never given rise to any noticeable inconvenience to the animals.   The surface of the eye is also greatly irritated during the first few months, causing an abundant secretion from the lachrymal glands while in the fume tanks, and finally resulting in many instances in an opacity of the cornea.   In some cases this opacity disappears after a few weeks and the animal is again able to see, yet some of the animals treated for several years have remained entirely blind.

A number of the treated animals have died and many others have been killed at various times during the progress of the experiment. Their organs and tissues have been carefully examined at autopsy and later studied microscopically. All tissues have appeared practically normal and none of the various well-recognized pathological conditions occurring in human alcoholism have been discovered. Tissues from animals treated as long as three years have been carefully studied, and the heart, stomach, liver, lungs, kidney, and other organs present no noticeable conditions that might not be found in normal individuals. Alcoholized animals are usually fat, but no fatty accumulation has been noted in the parenchyma of any organ.

Several males and females have been semicastrated during the experiment, and the ovaries and testes have been found to be in a generally healthy condition. It has seemed, however, that the ovaries of treated animals as well as all animals of the alcoholic lines show an unusual tendency to become cystic as compared with the ovaries of normal individuals. We have not, however, made sufficient comparisons to give the foregoing statement any greater weight than a mere supposition.

The general condition of all animals under the fume treatment is particularly good, and, as stated above, they continue to grow if the treatment is begun on individuals before they have attained full size, and all become fat and vigorous, taking plenty of food, living long, and behaving in a typically normal way.

The accompanying illustrations of five treated animals photographed along with control individuals show their perfectly normal appearance. In figure 1 is seen two male guinea-pigs and from the photograph as well as in life it would be impossible for any one to detect signs of physical inferiority on the part of one or the other. Yet the animal on the right, No. 80 ♂, was four days less than 5 years old when the photograph was made and had inhaled alcohol over one hour per day, a sufficient dose to give signs of intoxication, for six days per week, during four years, two months and five days. During the last seven months he had inhaled alcohol fumes two hours per day. He is perfectly well and alert, as the photograph clearly shows. His

companion on the left is a normal animal, No. 150 ♂, being 4 years and 3 months old when photographed. The sober existence of this male has not given him any advantage in appearance over the old alcoholic; both are very good males, each weighing almost 900 grams when photographed. This is well above the weight of the ordinary adult guinea-pig.

Figures 2 and 3 show again on the left the same normal animal, No. 150 ♂, in order that the reader may obtain a more definite impression of the uniformly good condition of the three alcoholic males. The alcoholic male No. 72 on the right in figure 2 was 5 years, 1 month and 10 days old when photographed,
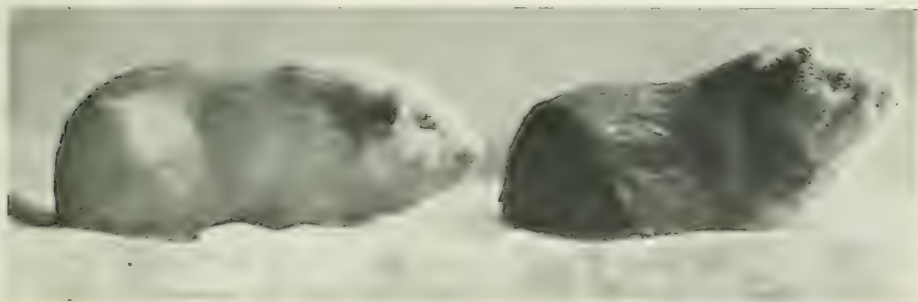
Fig. 1   The animal on the left is a normal male, No. 150, over four years old. The one on the right, No. 80 ♂, is almost five years old and had been treated with the fumes of alcohol six times per week for four years and two months, yet is seen to be in a vigorous condition.

weighing over 900 grams. He had been treated with alcohol fumes one hour per day until the last seven months, when he was treated two hours per day for six days per week. The entire duration of his treatment when photographed was four years, two months and five days.

Figure 3 shows on the right alcoholic male No. 70. This animal was 5 years, 1 month and 11 days old when photographed, and weighed 885 grams when 5 years old. He had received the same amount of alcohol treatment as the other two. The three were bred and reared in our colony and are above the average male guinea-pig in size and vigor. They have been good breeders as young normal specimens, as well as during

their alcoholic careers, but there has been a decided difference in the quality of their offspring during the two periods.

Figures 4 and 5 show two female alcoholics photographed along with the same black male No. 116. In figure 4 the alcoholic is an albino, No. 65♀. She was introduced into the experiment with the second stock from a new source in March, 1912. This female had been treated with alcohol fumes for two years, seven months and seventeen days when photographed. During the first two years of the treatment she inhaled one hour per day for six days per week and during the remaining seven months was treated for three hours per day, until fairly well intoxicated each time. The normal male No. 116 was 4 years, 8½
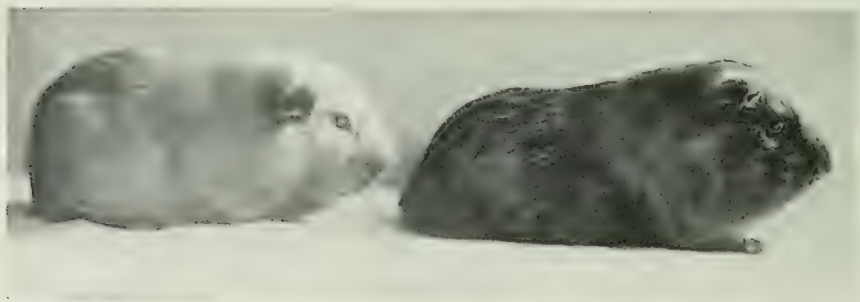
Fig. 2 The animal on the left is the same control individual, No. 150 ♂. The one on the right is an alcoholic male, No. 72, which was more than five years old and had been treated with alcohol fumes for four years and two months.

months old when photographed. The female No. 65 gave normal young before her treatment began, but now produces offspring with very poor records.

The female No. 158 is shown on the left in figure 5. This animal was produced in our colony from normal parentage and was 4 years and 3 months old when photographed. She had been treated for fourteen months one hour per day and for three hours per day during the last seven months. She is a large vigorous female. These photographs illustrate to some extent the fact that the treated animals themselves are little changed or injured so far as their normal appearance goes, and should there be inferior qualities in their offspring these cannot

be attributed to a condition of general depression in the parents, but more clearly to a peculiar action of the strange chemical material in the blood upon the glands of reproduction or the germ cells of the males and females.

In his study of the influence of alcohol inhalation on the domestic fowl, Pearl has found the treated individuals to respond in a way closely similar to our treated guinea-pigs. He has fortunately reported his results in much more thorough detail than we, yet the facts contained are practically the same for the two groups of animals. The mortality records of treated fowls show an advantage over similar records from untreated
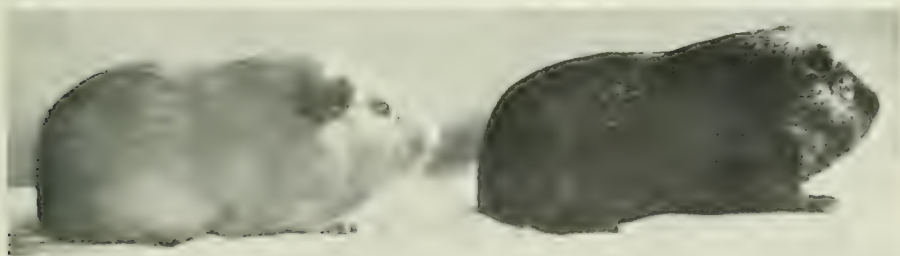
Fig. 3   Two male guinea-pigs.   One on the left the normal animal, No. 150, more than four years old.   On the right, No. 70 ♂, more than five years old and had been treated with alcohol fumes for four years and two months.

control. Our card catalogue contains the record of every death that has occurred among the guinea-pigs since the beginning of the experiments, and we may state in a general way that the mortality statistics for the treated animals is certainly as good and perhaps slightly better than those of the control.

Pearl has very naturally considered these findings in connection with the "widespread popular opinion that life-insurance statistics have 'proved' that even the most moderate use of alcohol definitely and measurably shortens human life." On careful investigation of the statistics Pearl finds them to be entirely unconvincing and to be based on biological evidence insufficient to prove anything. This is exactly in line with our own experi-

ence in studying the literature of any phase of human alcoholism. We have studied very thoroughly the literature relating to the influence of alcoholism in men and women on their progeny and, including the study of Elderton and Pearson, find it to suffer from the defects which Pearl points out in the longevity studies. Some of these contributions we shall discuss beyond, but none give any exact statement as to the amount of alcohol consumed or the length of time during which it had been consumed or any definite information as to other conditions or the general behavior of the individuals considered. The data are usually collected by persons entirely untrained and incapable of

Fig. 4 On the left normal male No. 116, almost five years old, and on the right an alcoholic female, No. 65, more than five years old that had been treated with alcohol fumes for about two and one-half years.

accumulating biological evidence. These extremely inexact records are often subjected to very careful and exact mathematical analysis which tends to give a scientific aspect to the consideration, but in no way improves the quality of the incorrect data used. Unfortunately, this renders it difficult to make comparisons between the responses of human alcoholics and those of selected animals used in well-regulated experiments.

Yet aside from the above, even should the data relating to the influence of alcohol on human longevity justify a comparison with experimental results, we feel that such a comparison could not properly be made with either Pearl's observations on the effect of alcohol on the mortality record of fowls or ours on the life record of alcoholized guinea-pigs, since in both experiments

the animals have been treated by inhalation of alcohol fumes, while human alcoholics have taken the substance into the stomach.

The difference between the effects on the treated individual of the two methods of administering alcohol cannot be too strongly urged. By the inhalation method the individual experiences only the stimulating or with further dosage the intoxicating and anaesthetizing effects of alcohol. As far as we have detected there are no injurious secondary effects on the individual's welfare resulting from habitual inhalation of ethyl-alcohol fumes. The results are very different, however, when the guinea-pigs drink daily doses of 15 per cent ethyl alcohol.

Fig. 5   The same male, No. 116, is shown on the right and an alcoholic female, No. 158, is on the left. She was more than four years old and had been treated with alcohol for over two and one-half years, yet she is in no way injured in appearance.

Only a few animals and a short time are sufficient to demonstrate the fact. A number of animals were given alcohol into the stomach at the beginning of these experiments and their digestion and metabolism were so deranged by the treatment that we were forced to devise and adopt the inhalation method as a more likely means of conducting an experiment of long duration.

It has been shown by us for guinea-pigs, and Pearl has demonstrated with fowls, the prosperous manner in which animals withstand the inhalation of alcohol vapor.

We may now give briefly the effects on three guinea-pigs of drinking daily doses of alcohol for only three weeks. The animals, Nos. 173 ♀, 1098 ♂, and 1184 ♂, at the beginning of the

experiment weighed respectively, 822, 635, and 527 grams, the female being old and the two males young growing specimens.

They were each given about 20 cc. of 15 per cent ethyl alcohol in tap water daily, except that once each week they were given almost 30 cc., which was a completely intoxicating dose. The 20-cc. dose causes all of them to be groggy for a few hours after drinking it; the effect increases for an hour or so and then gradually wears off. There is only slight if any change in rectal temperature. The animals seem fully recovered on the following day and have a normal appetite, but do not eat so ravenously as do untreated individuals.

When 30 cc. of 15 per cent alcohol is given in three 10-cc. doses at fifteen-minute intervals the animal is badly intoxicated and unable to walk within fifteen or twenty minutes after the last dose. The hind legs are particularly uncertain, the animal often tumbling over almost on its back, kicking frantically and having great difficulty in righting itself. Should its mouth come in contact with food the guinea-pig will chew in a peculiar manner, seeming in all reactions to be typically drunk. After one and a half or two hours the animal lies on its side with its trunk muscles often undergoing spasmodic contractions several times per minute, if taken up or made to move it struggles and falls panting in the drunken condition. By this time the body temperature may have fallen as much as 2 degrees below the pretreatment record. After three hours it is still unable to stand or walk and is breathing heavily with a temperature as much as $2\frac{1}{2}$ degrees Fahrenheit below normal. After four hours the condition is about the same and so for several hours longer until it gradually begins to recover and by the following morning it is fully recovered, but shows in its appearance the effects of the experience of the previous day.

When animals are given five partial and one complete intoxication by stomach alcohol per week they begin after a few days to regurgitate some of the stomach contents on receiving the first swallow or so of alcohol, but after this they take the dose without further disturbance, though they resist taking it more and more each time. Their desire for food is somewhat reduced as the treatment is continued.

After the first week No. 173, the old female that should have weighed the same or gained in weight under normal conditions, had lost 50 grams, or 6 per cent of her total weight. The two young males should have gained, No. 1098 gained 17 grams, only 2.6 per cent of his weight, while No. 1184 lost 4 grams or practically stood still. Their weight records for the indicated intervals are as follows:

	173 ♀	1038 ♂	1184 ♂
	grams	grams	grams
May 7	822	635	527
May 14	772	652	523
May 21	740	656	477
May 28	759	659	469
June 2	735	637	483
June 11	775	621	475

The alcohol was taken from May 7 to 28, and during that time the first guinea-pig lost 63 grams, or 7.6 per cent of its original weight. Of the two young males one gained 24 grams, or 3.7 per cent of his weight, while the other lost 58 grams, or 11 per cent of his original weight. During the next two weeks after the treatment stopped the male that had gained 24 grams lost 38 grams, so that at this time each animal weighed less than when it began to take alcohol.

This may have been a rather strong dose, but allowing for that, it was readily recognized that these animals were suffering from the treatment, while other guinea-pigs inhaling alcohol for three hours per day until groggy showed no injured appearance. Animals taking alcohol into the stomach suffer mainly on account of the injurious effects on their digestion. Alcohol acts on the gastric mucosa in such a way that the individual is placed at a disadvantage in handling its food and the ill effects observed are more largely due to this derangement of digestion than to the toxic action of alcohol on the animal system. Alcohol in the stomach makes the case complex, while we believe that inhaling alcohol gives effects simply due to the chemical action of alcohol itself on the tissues. For these reasons we do not believe that comparisons are easily made between the conditions of animals

that have inhaled alcohol fumes and the conditions of other animals that have taken alcohol into the stomach, since the latter individuals may be reacting more to a deranged digestion than to alcoholic intoxication. Therefore, there is objection to making comparisons between the mortality records of animals treated with alcohol by the inhalation method and the reports on the effect of alcoholism in man.

Yet, on the other hand, it may be possible that the influence of alcohol on the germ cells of an animal is the same whether the alcohol reaches the reproductive glands by being inhaled into the lungs or swallowed into the stomach. Such a position is not inconsistent with the discussion above if we take into account the possible, though unknown, effects of the deranged metabolism of the parent on the germ cells.

5. A GENERAL COMPARISON OF THE PROGENY FROM ALCOHOLIC LINES WITH THOSE FROM NORMAL LINES

The consideration above has brought out the fact that the inhalation of alcohol fumes sufficient to produce partial intoxication six times per week for long periods does not cause any easily recognized disadvantages in the general bodily condition or powers of existence of guinea-pigs. Pearl's experiments demonstrate the same fact in connection with the domestic fowl. This is, o course, leaving out of account the irritating effects of the fumes on the surface of the eye which may result in bl ndness, although even this is no handicap to either feeding or eproduction under cage conditions. I, then the genera body tissues are not sufficiently injured to cause an easily noticeable change in their powers of function, why should the germ cells be particularly susceptible to the treatment? The germ cells within the body of a mammal are undifferentiated generalized cells with no known function except to exist and await their time to develop. The soma or body, in respect to the germ cells, is simply a culture medium in which they live. The nourishment necessary for their existence is delivered to them by the body fluids. Any strange chemical substance which may find its way into the body fluids will reach the germ cells, and should this substance be sufficiently active and injurious in its effects

the germ cells may be so modified as to render them incapable of normal development. This might easily occur without differentiated somatic tissues being sufficiently damaged to greatly impair their usual functions. In other cases, and probably as a rule, the somatic tissues are also injured by any offensive substance present in sufficient quantities to modify the germ cells, and there are many reasons for believing this to be the result in several chronic human infections.

One must not infer from these statements that the germ cells are readily injured by poisons taken into the system; indeed, they seem on the contrary to be protected to a remarkable degree against such effects, and for this reason it is difficult to obtain a substance which may be used in experimental studies on the modification of the germ cells.

Should the germ cells be modified through the action of any substance, the point of particular importance is that all cells arising from such a modified germ will be similarly modified, since they are merely products of its division, and thus the soma and germ cells of the resulting individual will deviate from the normal in proportion to the degree of the primary modification of the cells from which it arose. Provided the change is one of such a nature that the cell or its parts are unable to recuperate, for example, if their specific chemical or physical make up be altered, then not only will the generation resulting from the originally modified germ cells be affected, but all future generations arising from this modified germ plasm will likewise be affected.

It seems also highly probable that should such results occur, the modifications to be observed in the somatic generations will be of a generalized nature affecting the organism in various ways so as to render its development less vigorous, its chance of survival less certain, and its ability to behave in a normal fashion more or less hampered. In certain cases the animal might really show no evident signs of its altered character. It seems to us, on the other hand, that only through the very rarest chance, one in possibly thousands, would any of the small number of definite characters under observation happen to be modified by their response to the treatment. The inheritance of coat-color,

for instance, may not be affected, although the germ plasm might
be so seriously altered as to give rise to the most extremely ab-
normal individuals. The same would apply to the very few other
characters in mammals the inheritance of which have been
studied from the Mendelian standpoint.

Finally, then, the fact that the soma seems little injured by
the alcoholic inhalations is in no way an index of what may be
expected from the development of the germ cells of guinea-pigs
which have been under habitual treatment.

Arlitt and Wells have very recently reported that the admin-
istration of alcohol in the food of male white rats for two or
more months results almost constantly in the appearance of
marked degenerative alterations in the testicles although other
organs were apparently uninjured. They find that these changes
affect the steps of spermatogenesis in inverse order to their
occurrence, so that for some time before sterility and complete
aspermia result, the animal is producing spermatozoa with all
possible degrees of abnormality. The probable relation of such
phenomena to the production of defective offspring is obvious.

A general survey of the progeny from the normal and alcoholic
lines as a whole will first be undertaken and is based on the data
presented in table 1. In this table the animals are arranged in
four groups, the first column containing the records of those
produced by normal control matings without inbreeding, the
third column records of normal animals somewhat inbred, while
the second column gives similar records for animals produced in
the alcoholic lines without inbreeding, and the fourth-column
animals are not only alcoholic, but also somewhat inbred. The
table contains in all records of 1170 animals, from our catalogue
numbers 613 to 1909 except 126 animals that could not properly
be included such as 39 new stock adults, 22 killed for different
purposes during early embryonic life, 31 derived from mothers
with only one ovary, and others too heterogeneous in origin, as
those from ancestors treated during pregnancy, etc., to be cer-
tainly placed. They represent, as stated above, the animals
produced during the sixth and seventh years of the experi-
ment and none from the earlier years.

The figures of the first horizontal space may be used to indicate

TABLE I

MORTALITY AND QUALITY RECORDS OF THE PROGENY FROM NORMAL AND ALCOHOLIC LINES

	Normal lines	Alcoholic lines	Normal inbred	Alcoholic inbred
Total number	1 2 3 4 5 10 46 90 72 15 24.03% 37.33% Average litter 2.77 Mat fail 4 (4.54%) 233	1 2 3 4 5 38 162 279 100 15 33.61% 19.32% Average litter 2.41 Mat fail 56 (13.04%) 594	1 2 3 4 5 0 8 21 12 0 19.75% 29.26% Average litter 2.92 Mat fail 2 (12.5%) 41	1 2 3 4 5 13 80 144 40 25 30.79% 21.52% Average litter 2.60 Mat fail 16 (13.12%) 302
Lived over 3 months	1 2 3 4 5 10 39 75 45 12 100% 84.78% 83.33% 62.5% 80%	1 2 3 4 5 31 132 170 48 2 81.57% 81.47% 60.93% 48% 13.33%	1 2 3 4 5 0 8 16 8 0 100% 76.20% 66.66%	1 2 3 4 5 13 53 98 13 7 100% 66.24% 68.05% 32.50% 28%
Absorbed, Premature, Stillborn	1 2 3 4 5 0 4 7 16 0 (51.92%)	1 2 3 4 5 4 19 79 34 12 (70.14%)	1 2 3 4 5 0 0 2 4 0 (66.66%)	1 2 3 4 5 0 19 27 15 16 (65.25%)
Died within 3 months	1 2 3 4 5 0 3 8 11 3 (48.08%)	1 2 3 4 5 3 11 30 18 1 (29.85%)	1 2 3 4 5 0 0 3 0 0 (33.33%)	1 2 3 4 5 0 8 19 12 2 (34.74%)
Total dead	1 2 3 4 5 0 7 15 27 3 15.21% 16.66% 37.5% 20% (22.31%) = 100	1 2 3 4 5 7 30 109 52 13 18.42% 18.51% 39.06% 52% 86.66% (36.52%) N 18.79% = 189	1 2 3 4 5 0 0 5 4 0 23.80% 33.33% (21.95%) N 22.46% = 98	1 2 3 4 5 0 27 46 27 18 33.75% 31.94% 67.5% 72% (39.07%) N 18.59% = 210
Defective	1 2 3 4 5 0 0 0 0 0	1 2 3 4 5 0 1 12 2 0 0.61% 4.30% 2.0% 2.52%	1 2 3 4 5 0 0 0 0 0	1 2 3 4 5 0 1 8 1 0 1.25% 5.55% 2.5% (3.31%)
Oversized (more than 500 gr. when 3 months old)	1 2 3 4 5 3 5 5 0 0 30% 10.86% 5.53% (5.58%)	1 2 3 4 5 3 11 3 0 0 7.89% 6.79% 1.07% (2.86%)	1 2 3 4 5 0 0 0 0 0	1 2 3 4 5 2 3 3 0 0 13.58% 3.75% 2.08% (2.64%)
Undersized (less than 300 gr. when 3 months old)	1 2 3 4 5 0 1 0 0 0 2.17% (0.42%)	1 2 3 4 5 0 2 2 4 0 1.23% 0.71% 4.0% (1.34%)	1 2 3 4 5 0 0 1 0 0 4.76% (2.43%)	1 2 3 4 5 1 3 2 3 2 7.69% 3.75% 1.38% 7.5% 8.0% (3.64%)

the productivity of the different lines. The numbers 1 to 5 indicate the number of young, one, two, three, four, or five produced by a female in a single litter. Litters of five individuals are the largest that have occurred from this strain of guinea-pigs. The average number of young in a litter from the normal lines is 2.77, and of the 233 animals included in this column 24.03 per cent of them were born in litters of one or two young. About 39 per cent were born in litters of three, while 37.33 per cent of the animals were members of large litters of four and five individuals. There were only a few normal inbred animals, as shown in the third column, but their general occurrence in the different-size litters was about as in the straight normal lines, half of the animals were born in litters of three, and almost 30 per cent in larger litters, and only about 20 per cent in litters smaller than three. The average litter happens to be in the small number of inbred animals a little higher than in non-inbred stock.

The arrangement of the young in large and small litters in the alcoholic and alcoholic inbred lines is almost exactly the reverse of what we have just seen for the normal. Again, a little less than half of the animals occur in litters of three. But over 30 per cent of the individuals are from litters of only one or two, while about 20 per cent are born in litters of four or five. Stated in other words, in the normal lines one and one-half times as many individuals are born in litters of four or five as in litters of one or two, while in the alcoholic lines one and one-half times as many are born in litters of one or two as in litters of four or five.

The explanation of this, we believe, is as follows: About half of the pregnancies in this stock of guinea-pigs should result in litters of three, as is found to be the case in all of the lines of table 1. All litters of less than three young are due in the first place to a low productivity on the part of the female as is probably indicated by the production of more than one-fifth of the normal young in such litters. In the second place, small litters are frequently due, particularly in the alcoholic lines, to the death and absorption in utero or early abortion of one or more members of an originally large litter. The absorption in utero of such embryos, often of rather large size, may occur in a nor-

mal guinea-pig, yet such a phenomenon is not very common, although in the alcoholic lines it is frequently observed. We shall consider this process below, the only point of interest here being its effect on the size of the litter.

The exactly reversed percentages of individuals born in large and small litters in the normal and alcoholic lines, as shown by the table, may indicate that one-third of the animals in alcoholic lines that are born in litters of one or two were originally in litters of three, four or five. For example, the normal lines have in all well over 12 per cent more animals born in litters of four or five than in litters of one or two, and the alcoholic lines have over 12 per cent more in litters of one or two than in litters of four or five, and this 12 per cent probably has been thrown from the larger into the smaller litters on account of early abortions and absorptions which occur in the former. The too frequent occurrence of small litters is undoubtedly indicative of not alone an actually low productivity, but a very early prenatal mortality.

Another occurrence also partly due to an early prenatal mortality is the failure of a mating to produce a result. No doubt in rare cases fertile guinea-pigs may be mated during the heat period of the female, as these have been, without a following conception. In the normal lines four out of eighty-eight matings, or 4.54 per cent, failed, giving negative results, while in the alcoholic lines three times as many matings failed, and very probably this excess represents those cases in which not only a part of the litter is lost through an early prenatal mortality, but the entire litter is destroyed. Of course, some cases of actually infertile matings are also represented.

By this 'early prenatal mortality' is meant the absorption or loss of an embryo before it is of sufficient size to be detected on carefully feeling the uterus through the body wall of the mother. With experience an embryo eight or ten days old may be detected by an external examination of the uterus. Through our routine examination of the females after being with the males for one month, any embryo lost after this time will have been discovered and is definitely recorded in the third horizontal space of the table. If absorption or early abortion of one or more embryos in a litter may actually be observed to occur after as

much as ten days of development, there must certainly be a pre-
natal mortality of some extent previous to this time.   Experi-
ments with the eggs of lower forms which develop outside of the
mother, permitting direct observation, speak for the great pre-
ponderance of an early embryonic mortality, many such eggs
dying during the cleavage and gastrular stages when subjected
to even slightly unfavorable conditions.   We have some direct
evidence on 'early prenatal mortality' in female guinea-pigs
which have been examined by operation after repeated 'mating
failures.'   The ovaries of some such animals contain corpora
lutea of pregnancy indicating that an embryo had been present
shortly before the examination.

Pearl records that the eggs from alcoholized fowls are to a
high degree infertile.   This he believes is due to many of the
germ cells as such having been killed by the treatment.   By
infertile, Pearl means, of course, that no fertilization or zygote
formation took place, yet it is extremely difficult in all cases to
detect whether the early stages of development may not have
occurred and been followed by death and degeneration.   The
death may have occurred during the cleavage or gastrular stages
while the egg was yet in the uterus of the hen and many of
the 'infertile eggs' might really be classed among the early pre-
natal mortalities.   We make these suggestions merely as pos-
sibilities which to us are somewhat tempting, since if there was
actually an early prenatal mortality in some of these 'infertile
eggs' it would bring the effects of the alcohol treatment on the
fowls and mammals still closer together.   It is only through our
recent analysis of the size of litters and mating failures, along
with careful examination of the pregnant females, that we have
become aware of the sometimes frequent very early embryonic
death.

The second horizontal space shows the number of young from
the several lines that reached maturity, or lived over three
months.   Here again the size of the litter is an important factor.
It may be stated generally that the power of survival of a guinea-
pig varies inversely with the size of the litter in which it is
born.   We shall see beyond that this is also true of their birth
weight, growth rate, and certain other qualities so that in mak-

ing comparisons between young guinea-pigs it is important to know whether the individuals concerned occurred in litters of equal size.

In the normal lines all individuals born singly survived, and, as the seventh space shows, 30 per cent of them were unusually large or over size when three months old. Normal animals born in litters of two or three survive in about 84 per cent of the cases and are often of large size. The members of litters of four survive in only 62.5 per cent of the cases and are not generally vigorous animals. The records show that 80 per cent of the young in litters of five survived, but this is very unusual and is due probably to the small number involved, and possibly to a slight extent to the extreme care with which the pregnant females with the larger number of young were handled. This extreme care, however, only saved 13.33 per cent from the same number of alcoholic-line young born five in a litter.

The second column indicates that over 81 per cent of alcoholic animals born in litters of one or two are capable of survival. Such a record is almost as good as the control, showing how very strong the members of small litters are and indicates again that an early individual selection may have played some part, since no doubt there has been a prenatal mortality among the weaker individuals which originally existed in some of these litters. This is emphasized further by the fact that the members of litters of three survive in only 60.93 per cent of the cases. Here the prenatal mortality has not played so severe a rôle and many weaker individuals are born. The power of survival of animals born three in a litter from the control is about 23 per cent better than from the alcoholic lines. Only 48 per cent of the alcoholic-line individuals from litters of four were able to live three months. Recognizing the small numbers involved, only 13.33 per cent of the alcoholic guinea-pigs born in litters of five were viable. It thus appears that when the alcoholic animals produce large litters the quality of the young is very poor, whereas their small litters contain animals with good survival records. There is little doubt that this apparent difference in quality is in part due to a prenatal selection which, in the case of the small litters, has eliminated most of the weaker individuals and left only the

stronger to be born. In addition to this, it must also be recognized that the ability of the female to properly nourish the members of the large litters is somewhat overtaxed. Three or less than three embryos are very well nourished by normal mothers. It must be recognized here that the inferior records of the alcoholic lines are not alone produced by alcoholic mothers, but come also from alcoholic fathers as following tables will show.

The survival records of the normal inbred lines are about the same as those from the straight control, and are almost equally superior to the alcoholic lines.

The alcoholic inbred animals have a survival record closely similar to the straight alcoholic lines, and again decidedly inferior to either the normal or normal inbred lines.

The fifth horizontal space contains the mortality records which are the reverse of the survival records just considered. However, we have given here not only the actual mortality in litters of different sizes, but have corrected the total mortality record on the basis of the occurrence of large and small litters and their mortality in the different lines as compared with the control. We have also expressed the mortality in numerical proportion in the several lines, taking the control as 100. The total mortality in the normal lines is 22.31 per cent. This is a very good record, since it not only includes the postnatal mortality, but all exact prenatal mortality as well. We mean by exact prenatal mortality those cases of absorption in utero and premature abortion which were actually observed, and not those calculated on the basis of size of litter, mating failures, etc., as was discussed in connection with the productivity of the different lines.

The total mortality of the normal inbred is 21.95 per cent, or almost the same actually as well as when corrected for litter sizes as the straight normal lines.

The total mortality of the alcoholic lines without inbreeding was 35.52 per cent, or almost 1.6 times greater than the mortality of the control. But this does not fully represent the real difference between the two lines unless it be corrected on the basis of the mortality record for the different-size litters in the alcoholic and the normal. The mortality is much higher among

the members of the large-size litters than among those in the small litters, and the large litters are 1.7 times more frequent in the control than in the alcoholic lines.

The mortality is corrected on the basis of the normal records as follows: The rate for the normal animals born one in a litter is zero; two in a litter, 15.21 per cent; three in a litter, 16.66 per cent; four in litter, 37.5 per cent, and five in litter, 20 per cent. On this basis what should be the number of alcoholic animals dying in the several different-size litters? The numbers should be zero instead of 7 for one in litter animals; 24.64 instead of 30 for two in litter animals; 46.48 instead of 109 for three in litter animals; 37.5 instead of 52 for individuals born four in litter, and 3 instead of 13 for five in litter. These numbers give a total of 111.62, which divided by the number of alcoholic animals, 594, shows a mortality percentage of 18.79. On the basis of the control mortality for the different-size litters, this is what the mortality should have been in the alcoholic lines, yet instead of 18.79 per cent it was actually 35.52 per cent, or almost double the normal rate. Again to express the corrected mortality in the alcoholic lines in terms of the control as 100, we find that for every 100 of the control animals that die 189 from the alcoholic lines die.

The last column shows the 302 alcoholic inbred animals to present a still worse record. The actual mortality here is 39.07 per cent, or one and three-fourths times higher than in the control. Here again correcting as in the preceding cases, the mortality on the basis of the control record in the different-size litters, it should normally be 18.59 per cent, but instead the mortality is 2.1 times greater than this among these alcoholic inbred animals. In other words, for every 100 control animals that die 210 alcoholic inbred individuals succumb. While the normal inbred animals, although their numbers are small, present a slightly better record than the straight control, 98 of these dying to 100 of the control.

In the third and fourth horizontal spaces of the table the total mortality is divided into the prenatal and postnatal deaths. The proportion of prenatal to postnatal death in the different lines presents peculiar arrangements that will be seen to exist, not only

in this, but in several of the tables to follow. The prenatal records include embryos that die and are absorbed in utero, never passing to the outside, other embryos and fetuses which die and are passed out or born prematurely, and finally full-term young which die shortly before birth and are, therefore, still born or born dead. The postnatal deaths include all animals dying before reaching three months of age, at which time guinea-pigs are about mature.

In the control lines 51.92 per cent of the total mortality occurred before birth or was prenatal, while 48.08 per cent of the deaths occurred after birth. Considering the numbers involved, it therefore may be said that the pre- and postnatal mortalities are about equal in the straight control lines. There is no evidence here of a particular tendency on the part of the young animals to succumb at any given or critical stage in their development.

The numbers contained in the normal inbred column are certainly too small to be considered.

In both the alcoholic and the alcoholic inbred lines where the numbers involved are considerable (the records showing 329 deaths among 896 animals), the prenatal mortalities are double the postnatal deaths. The alcoholic column shows 70.14 per cent of the total mortality to occur before birth, while only 29.85 per cent of the individuals that died were lost after birth. The last column gives for the alcoholic inbred animals 65.25 per cent of the total mortality as prenatal and only 34.74 per cent as postnatal. This consistent arrangement in the two columns indicates a tendency on the part of the weak and subnormal individuals of the alcoholic lines to succumb during early stages of their development. Such an interpretation is exactly in accord with and is substantiated by the high early prenatal mortality which exists in these lines as indicated by the size of their litters and frequent mating failures when compared with the control.

A mortality arrangement of this kind accords with what is known of almost all weak or diseased stocks —there is a very high loss during the early stages of development, as well as during

later embryonic or uterine life.   Furthermore, many individuals die very soon after birth, while those that happen to survive the periods shortly following birth are often capable of an almost or quite normal existence.

The mortality in the control is low, but half of this, or a high proportion, occurs after birth.   The mortality in the alcoholic lines is high, but only a low proportion, about one-third of this, occurs after birth.   It may be added further that the young alcoholics which die after birth in the majority of cases die within a few days, while the control young that die after birth are more likely to be scattered along over a number of days or weeks.

It is thus seen that in both the alcoholic and alcoholic inbred lines there is a decided tendency for the developing embryos and young to succumb during the early periods of their development. This would suggest that these affected individuals were often incapable of passing through the early critical stages of uterine life.   But if they were sufficiently fit to survive these periods, their chance for existence was good, so that their postnatal mortality, although actually higher than the control, was proportionally much lower.   Thus we have a somewhat rigid individual selection taking place during the stages of uterine life, so that the sum total of the individuals at a given stage is of a better average quality than during any previous stage and vice versa.   Therefore, as is clearly shown beyond, those animals of the alcoholic lines which live to become mature and prove to be fertile are a strictly selected few and in each generation the proportion of strong to weak individuals through this selection constantly tends to increase.

The sixth horizontal space shows a complete absence of defective individuals in either the normal or normal inbred groups. It may be stated here that during the entire seven years of this experiment not one grossly defective or deformed individual has appeared in the nonalcoholic or control lines.   This is a rather remarkable record for any group of animals, and it speaks strongly for the perfection of the original stocks from which both the control and the alcoholic lines have been derived.

In the alcoholic lines about $2\frac{1}{2}$ per cent of the individuals were grossly defective. By defective is meant those specimens which show deformities, such as one abnormally small eye, cataract or opaque lenses, deformed limbs, paralysis of the limbs, gross tremors which make the animal incapable of locomotion or proper feeding, etc. There are slightly more defectives in the alcoholic inbred groups, 3.31 per cent in all.

The next line records the over-size or unusually large animals, those weighing more than 500 grams when three months old. Among the control 30 per cent of the individuals born singly or one in a litter grew to be unusually large specimens. More than 10 per cent of those in litters of two were also unusually large, and 5.53 per cent of the three in litter animals are included in this class. None of those born in litters of four or five were able to attain such a size. Of the total control animals over five and one-half per cent were of this large size, while only about half as many from the alcoholic and alcoholic inbred lines attained such a distinction, yet in both treated groups there were over two and one-half per cent of large specimens.

The last line of the table shows the occurrence of unusually small animals, those weighing less than 300 grams when three months old. Among 233 control animals only one such individual appears, 0.42 per cent. The alcoholic lines contain more than three times as many of these as the control, but still very few, only 1.34 per cent. The numbers in the normal inbred column are too small for consideration. Among the 302 alcoholic inbred animals there were eleven under-size specimens, or 3.64 per cent. This is almost three times as high a percentage as occurred in the alcoholic lines and over eight times as high as is recorded for the control animals.

Comparing the present results with those of our earlier papers, particularly with the similar table 2 ('16), it will be noticed that the numbers involved are almost twice as great and the records of the animals considered are decidedly better than were formerly shown. This improvment in the quality of all lines is due to several factors. In the first place, the breeding methods have been decidedly improved since studying the oestrous cycle of

the females and determining the exact time of the 'heat periods' (Stockard and Papanicolaou, '17). This has enabled us to pair the animals at the most favorable periods and thus to obtain far better and more exact mating records than was possible on the basis of the previous conceptions of the guinea-pig's sexual behavior. Secondly, the housing, care, and feeding of the animals are decidedly better during the last three years than during previous times, and on this account the mortality in all lines has been reduced, but as might be expected, the weaker alcoholic lines have profited more by this improved condition than have the control animals. For example, the mortality record of the control has been lowered only a little more than 3 per cent, while in the alcoholic lines it has been lowered a much as 18 per cent. This improvement in the alcoholic lines is also partly due to the existence of more late-generation animals with many normal ancestors. Thus, although the lowered mortality record of the alcoholic may not be entirely due to the better living conditions, yet it serves as a striking illustration of the difference in response to the change on the part of the control animals and the alcoholics. The previous somewhat unfavorable state did not greatly impair the powers of existence of the control animals, but it did evidently eliminate some of the weaker alcoholic individuals that might have survived under more ideal arrangements.

It must be recognized, in the third place, that for the alcoholic inbred animals the degree of inbreeding among the later generations here included is less intense than was the case with earlier generations in the former reports. And for this reason the previous rather decided differences which were shown between the straight alcoholic group and the alcoholic inbred animals have almost, though not entirely disappeared.

Lastly, the fourth point of difference to be borne in mind in comparing the earlier and present records is that there are now more late-generation alcoholic descendants with less affected material in their total germ-cell complex than was true of the animals in the former tables, which as a group were composed of generations closer to the direct alcohol treatment. For ex-

ample, an animal derived from a directly alcoholized father and a normal mother could be said to contain half affected and half normal germ plasm, whereas another in whose pedigree the only alcoholic individual was an alcoholized grandfather, would undoubtedly contain a smaller amount of affected stuff.

Finally, then, in the light of the facts involved, the general table presents an impression closely similar to that derived from the previous records of these experiments, but it adds data of much importance for a clearer understanding of the problems concerned.

The improvement in the present records over the former ones might suggest that should the methods of breeding and caring for the animals reach perfection, the differences between the alcoholic lines and the control might be entirely erased. This would be possible if the improvement was due alone to method, but such a suggestion ignores the fact that the improvement is more largely due to the presence of late-generation animals with only a small amount of alcoholic germ plasm in their ancestry and a large number of normal progenitors. The analysis of the following table 2, in which the several generations are treated separately, will fully substantiate the validity of the foregoing statement.

Before considering this table, however, we may discuss briefly the phenomenon of absorption of embryos in utero and our methods of examining pregnant females in order to fully record the fate of all embryos that begin to develop. A knowledge of this prenatal mortality is involved not only in the table just studied, but in several of those that follow.

6. ABSORPTION OF EMBRYOS IN UTERO AND ABORTIONS OF PARTS OF LETTERS: METHODS OF DETECTING THESE PROCESSES

After having observed the course of pregnancy and the size of the litters produced in a large number of cases, we became convinced that many of the small litters delivered at full term were only partial litters. Particularly in the alcoholic lines it became evident that abortions of one or two members of a litter might

occur without hindering the further development to term of the remaining members. It was also recognized as is known even for the human female that embryos might be absorbed in utero. In the guinea-pig we have found that the absorption of one or more embryos in utero, as is true of partial abortion, may not interfere with the further normal development and birth of the remaining members of such litters.

When it was realized that these absorptions and abortions of parts of litters were taking place, the necessity arose of definitely detecting each case in order to make the prenatal mortality records approach correctness. A systematic examination was, therefore, begun of every female after being with a male for one month up to within a week or ten days of delivery.

The female to be examined is allowed to stand on a flat surface and the investigator with both hands presses the ventral abdominal wall so as to feel with the fingers the horns of the uterus against the dorsal abdominal wall. With considerable practice the small embryos and placentae may be definitely counted within one or both horns of the uterus. The number of embryos and their position in the two horns of the uterus are noted on the record card of the female. After this initial examination she is reexamined once or twice during the pregnancy and each time the number and position of the embryos with the date of examination are recorded. The number of young finally born helps to show how nearly correct the examinations have been.

The records now contain several hundred such examinations and show that absorption of embryos may take place not only during early stages, but after the fetuses have attained considerable size. The difference between absorption and partial abortion may usually be recognized by the fact that the embryo being absorbed may exist for some time as a small lump in the uterus, while the aborted embryo disappears from the uterus and leaves no palpable remains. There are exceptional cases in which the uterus is unusually swollen or congested after the abortion and these on being felt would still seem to contain a partial embryo. The cages of the pregnant females are exam-

ined every morning and afternoon and aborted embryos or placentae are generally located, yet instances do occur of early abortion possibly during the night in which no trace of the aborted material is found, since the female very quickly attempts to eat the aborted products.

Fig. 6   On the right a normal 19-mm. embryo taken from the right horn of the uterus of an alcoholic female.   The left horn of the uterus contained the degenerating mass shown on the left which was attached to a small placenta and represents an embryo in the process of being absorbed in utero.   The mother had an alcoholized father.

During the two years which supply the data for the present study the females have been very carefully and consistently examined throughout their pregnancies, and the records of absorbed and premature or aborted young are very accurate for all

periods after the embryos are of sufficient size to be detected by this method of external examination. To convey some idea of how accurately one may detect a structure by palpation through the abdominal wall of the guinea-pig, it may be stated that a slightly cystic ovary has frequently been diagnosed by such an examination.

A normally developed embryo 19 mm. crown rump length is shown in figure 6 and near it is seen an amorphous embryonic mass 2 mm. in longest diameter which represents the other member of the litter. The two were in different horns of the uterus. The placenta of the normal embryo was of the usual size, while the one associated with the arrested specimen was only about one-half as large. The entire mass of the smaller ovum in the uterus was about that of a ten-day specimen, while the normal individual was a typical twenty-day specimen. This case was detected by external examination and was merely opened in order to use the embryos for illustrating the phenomenon. In the explanation of the figure the ancestry of the embryos is given.

The intrauterine absorption of embryos, as stated above and indicated in table 1, may occur in normal guinea-pigs. A. W. Meyer ('17) has very recently described the histological conditions found in partially absorbed embryos which he had obtained during a study of the prenatal growth of the guinea-pig. There is considerable data from our study to indicate that this absorption of embryos is somewhat more frequent in the alcoholic than in the normal lines.

7. A COMPARISON OF THE QUALITIES IN THE DIFFERENT GENERATIONS OF THE ALCOHOLIC LINES AS THEY BECOME FURTHER REMOVED FROM THE GENERATION DIRECTLY TREATED

It has been mentioned in discussing the improvement of the records in table 1, as compared with our previous reports, that this advantage is partly due to the larger number of late-generation animals at present included. We may now analyze the alcoholic lines for a comparison of the qualities of the early and

late generations, F_1 to F_4, on the basis of their productivity and mortality records. Such an analysis is of particular importance to test in the first place whether the effects of the alcohol treatment on the germ cells are permanent, altering their qualities in inheritance, and in the second place whether an increasing amount of normal germ plasm acquired with each generation may tend to offset the original alcoholic effect by dilution.

Table 2 contains the data from the non-inbred alcoholic lines divided into different generations. The first vertical column gives the records for 233 control young as a standard of comparison. These are the same records shown in the normal column of table 1, except that in the present table we have included in the first horizontal line under each group the average birth weight of the litters produced. This is termed the average litter weight and is recorded in grams. For the normal stock this average productivity is 197.12 grams; that is, the average weight of all the litters at birth was this amount. The average litter weight is in a way associated with the average litter size, since a litter containing several young though each individual may not be so large, will probably weight more than a litter of fewer or of one young. Thus a group having a higher average litter than another group will also probably have a higher average litter weight, though this is not necessarily the case, as will be seen on comparing the several columns of the table.

The second column contains the alcoholic line animals. This again is the same 594 alcoholic animals shown in the second column of table 1 and is given here for comparison with the four following groups, each of which is a certain portion of this total column. The average productivity for the alcoholic animals is 170 grams, or 37 grams less than the control, and when corrected on the basis of the average litter size, it is 5.6 grams less than it should be according to the normal standard.

The third column gives the records of 186 animals with one or both parents treated with alcohol, the F_1 generation. Thirty-three of these animals also had a slight alcoholic history in their ancestry, and thus the entire group are not pure F_1 alcoholics. The proportion of large and small litters in this column is about

TABLE II

A COMPARISON OF THE RECORDS OF ANIMALS OCCURRING IN THE DIFFERENT GENERATIONS OF THE ALCOHOLIC STOCK

	Normal stock	Alcoholic animals of all generations	Alcoholic animals with treated parents F_1	Alcoholic animals with treated grand parents, great-grand parents and great-great-grand parents great-grand parents F_{2-3-4}	Alcoholic animals with treated great-grand parents and great-great-grand parents great-great-grand parents F_{3-4}	Alcoholic animals with treated great-great-great-grandparents F_4
Total number	1 2 3 4 5 10 46 90 72 15 24.03% 37.33% Average litter 2.77 Average litter wt 197.12 gr Mat. fail. 4(a 54%) 233	1 2 3 4 5 38 162 279 100 15 35.61% 19.32% Average litter 2.47 Average litter wt 170 c9 Mat. fail 36(13.04%) 594	1 2 3 4 5 9 54 90 28 5 34.61% 17.24% Average litter 2.51 Average litter wt 165.51gr Mat. fail 11(12.94%) 186	1 2 3 4 5 28 106 192 72 10 32.84% 20.09% Average litter 2.47 Average litter wt 172.21% Mat. fail 25 (13.15%) 468	1 2 3 4 5 10 26 66 40 5 24.47% 30.61% Average litter 2.62 Average litter wt 176.76gr Mat. fail. 9(13.84%) 147	1 2 3 4 5 2 4 21 8 0 17.11% 22.85% Average litter 3.01 Average litter wt 193.53gr Mat. fail 1(7.14%) 34
Lived over 5 months	1 2 3 4 5 10 39 75 45 12 100% 84.78% 83.33% 62.5% 80% 77.68%	1 2 3 4 5 31 132 170 48 2 81.57% 81.48% 60.93% 48% 13.33% 64.47%	1 2 3 4 5 8 44 48 6 0 88.88% 81.48% 53.33% 21.42% 56.98%	1 2 3 4 5 22 86 123 42 2 18.57% 81.13% 64.06% 58.33% 20% 67.10%	1 2 3 4 5 9 18 48 25 2 90% 69.23% 72.72% 62.5% 40% 69.38%	1 2 3 16 8 0 100% 75% 76.29% 100% 82.35%
Absorbed, Premature, Stillborn	1 2 3 4 5 0 4 7 16 0 51.92%	1 2 3 4 5 4 19 79 34 12 70.14%	1 2 3 4 5 0 6 32 13 5 70.0%	1 2 3 4 5 4 14 47 21 7 61.92%	1 2 3 4 5 1 7 18 12 2 77.77%	1 2 3 4 5 0 0 2 0 0 33.33%
Died within 5 months	1 2 3 4 5 0 3 8 11 3 48.08%	1 2 3 4 5 3 11 30 18 1 29.85%	1 2 3 4 5 1 4 10 9 0 30.0%	1 2 3 4 5 1 2 6 22 9 30.08%	1 2 3 4 5 1 0 22.22%	1 2 3 4 5 1 0 66.66%
Total dead	1 2 3 4 5 0 7 15 27 3 15.21% 16.6% 31.5% 20% (22.31%) = 100	1 2 3 4 5 7 30 109 52 13 18.42% 18.51% 39.06% 52% 86.66% (35.52%) N 18.79% = 189	1 2 3 4 5 1 10 42 22 5 11.11% 18.51% 46.66% 89% 33% 100% (43.01%) N 19.65% = 230	1 2 3 4 5 6 20 69 30 8 21.42% 18.86% 35.93% 41.6% 80% (32.59%) N 18.89% = 172	1 2 3 4 5 1 8 18 15 3 10% 30.76% 27.27% 37.5% 60% (30.61%) N 21.02% = 145	1 2 3 4 5 1 0 25.29% (17.14%) N 20.25% = 34

the same as in the total alcoholic column, 34.6 per cent of the animals were born in litters of less than three and only 17.74 per cent in litters of more than three. The normal record as pointed out before is just about the reverse of this. The average-size litter in which the F_1 animals occur is 2.51, which is slightly larger than for the total alcoholic column, but the average weight of these litters is less than for the entire alcoholic lines, being 165 against 170 grams. As compared with the control the average productivity of this column is 32 grams low, and when corrected on the basis of the average-litter size, the litters are then more than 13 grams less than the control standard. The mating failures are about the average alcoholic result, 12.94 per cent.

The mortality record of the F_1 animals is not so good as for the entire alcoholic group, only 56.98 per cent of them living longer than three months as against 64.47 per cent. The total mortality is 43.01 per cent, and when this is corrected on the basis of the normal mortality for the various-size litters in which the individuals occurred, we find that the F_1 mortality is almost 2.3 times the control record, or 230 against 100. The corrected mortality here as compared with the entire alcoholic group is 230 against 189, or 41 points higher.

The proportion of prenatal to postnatal mortality corresponds closely to that of the entire alcoholic group and contrasts with the control in the same way as discussed in considering table 1.

Finally, then, the F_1 group of animals from either one or both treated parents, are inferior to the alcoholic group as a whole in having a higher mortality record and in occurring in litters of a lower average weight although of equal average size.

The fourth column contains the records of animals more than one generation distant from the alcohol treatment; that is, those having treated grandparents, great-grandparents, or great-great-grandparents, or combinations of these, F_2, F_3, and F_4 generations. All of the alcoholic animals from column 2 are included in this column, except the third column of F_1 animals; there are thus 408 individuals.

The distribution of the animals in large and small litters is closely the same as in the two preceding columns, over 30 per cent being in litters of less than three and 20.09 per cent in litters larger than three. The average-size litter and the average litter weight are just about what is found for the total alcoholic group and somewhat better than for the F_1 group. The percentage of surviving animals is a little better than the total alcoholic group and considerably better than the F_1 group. The prenatal and postnatal mortality proportions follow the typical arrangement for the alcoholic lines, the prenatal being about two and one-third times higher than the postnatal. The total mortality among these animals is about 10 per cent lower than for the F_1 group and slightly below the record of the total alcoholic lines. When the mortality is corrected in terms of the normal mortality for the different-size litters and stated on the basis of 100 for the control stock, it becomes 172 as against 230 for the F_1 column and 189 for the all generations alcoholic column.

The fifth column records 147 animals still further removed from the treated generation; these had treated great-grandparents or great-great-grandparents or both, the F_3 and F_4 generations. Some of these animals may have had only one or two alcoholic ancestors out of eight or sixteen; therefore, the proportion of modified to normal germ plasm is often very small.

The arrangement in large and small litters differs from the other alcoholic groups and approaches that shown by the normal lines very closely, there being a higher percentage born in large litters than in small. The average-size litter is larger than in the three preceding columns, although still well below the control. The average litter weight is low when compared with the normal lines and only about the same as in the three preceding columns when taken in connection with the average size of the litters. When corrected for the average size, the weight of the litter falls more than 10 grams below the control record. The mating failures still show the high percentage of the alcoholic lines, being over three times as many as in the control.

A greater percentage of individuals survived than in any of the preceding groups except the control.

The proportion of prenatal to postnatal mortality shows the arrangement characteristic of the alcoholic groups. As a matter of fact, the prenatal mortality is really unusually high, and this is probably due to the high percentage of large litters, as among these the prenatal mortality is most frequent. It is as though the animals of this group had produced almost as high a proportion of large litters as the control animals and still they were not sufficiently good quality as compared with the control to keep down the prenatal mortality in these high litters.

The total mortality when corrected on the normal rate for the litter sizes and expressed on the basis of 100 for the control becomes 145. This is a decided improvement over the other alcoholic groups, although poor in the light of the control.

From a survey of this column it may be concluded that animals as far as three generations removed from the direct alcohol treatment are still differentiated as a group from the control in regard to the weight of the litters in which they are born, the tendency of the matings to result in failure, the high proportion of prenatal mortality over postnatal, and the total mortality which is one and one-half times higher than the normal. All of these differences exist in spite of the fact that more and more normal germ plasm has been introduced during each generation until some of these animals may have had as many as six or seven normal great-grandparents against one or two treated or alcoholic great-grandparents, though the average of course had somewhat more treated ancestry than this.

One of the F_3 individuals, descended from treated great-grandparents, is shown in figure 7. The animal on the left was a non-inbred female, No. 803, with six of its eight great-grandparents treated with alcohol and only two, on the paternal side, were normal. Its great-grandparents may be written thus: A indicating alcoholic and N normal, the ♀ on the left, in the formulae: [(AxA) (AxA)] [(NxA) (AxN)]. The animal on the right is an ordinary normal guinea-pig born on the same day as the small degenerate specimen which weighed only one-third

Fig. 7   On the left a non-inbred female, No. 803, with six of its eight great-grandparents treated with alcohol and only two on the paternal side not treated. She was small and degenerate and lived only one day.   On the right is shown a normal animal born on the same day, the two being photographed on one plate.

Fig. 8   Two F_3 guinea-pigs born in the same litter from a normal father and a mother derived from four alcoholized grandparents.   The albino female, No. 955, on the left weighed at birth 90 grams, the small defective male on the right weighed only 38 grams and died within two days; the sister is still alive.

as much and lived only one day. Although some young from control parents do die shortly after birth, they are not so unusually small nor degenerate in appearance as the defective young of the alcoholic lines.

Another even more striking example of the small defective animals appearing in the F_3 generation is shown by the photograph, figure 8. The two individuals in this picture were born in the same litter. Their mother was a black and red animal from four alcoholized grandparents and their father was a normal albino male, [(AxA) (AxA)] [N]. The F_3 animal on the left, No. 955, is an albino female weighing at birth 90 grams. She is thus an unusually large animal to be a member of a litter of three and is of the type of the normal albino father. Her small degenerate brother on the right weighed only 38 grams at birth, had a severe tremor which rendered him incapable of normal progressive movements, and he lived only two days. His degeneracy and black and red color are both qualities for which he was indebted to his alcoholic mother. A marked discrepancy in either size or condition between two members of the same litter at birth is entirely lacking among our control lines. It is rarely so decided as this case illustrates, yet very frequent in the alcoholic lines and particularly in the F_2 and F_3 generations. A number of illustrations of this type could be continued to show that the quality of the later generations from alcoholized ancestors is decidedly subnormal.

Such conditions as the above occur not only in spite of the introduction of normal germ plasm which tends to overshadow the alcohol effect, but also in spite of a rather harsh individual selection which is at work tending to improve the stock with each generation. Almost all of the badly defective individuals in the alcoholic lines are lost early in their career, as is shown by the high prenatal mortality; other less defective ones die soon after birth, such as those pictured above, and only the best live to become fertile adults. It is thus found that even this selected group mated with many normal individuals still possesses enough of the modified germ plasm which resulted from the early alcohol treatment to cause their offspring to be inferior to

the control animals in a number of important qualities that render them less capable of survival.

These two factors, the constant introduction of more normal germ plasm and the elimination of all the weaker alcoholic individuals so that only the stronger reproduce, may finally in late generations so purify the alcoholic lines as to cause them to attain a condition equally as good as the normal.

The sixth and last column of table 2 may illustrate such a condition, though it contains the records from only a few animals. These animals are descended from one or more treated great-great-grandparents, the F_1 generation. They are four generations removed from the alcoholic treatment.

The average-size litter is almost as large as in the control, and on the basis of its size it is actually heavier than the control average. It may be said from the evidence shown that the productivity here is equally as high as in the control.

A higher percentage of individuals survived than among the control, and even though the mortality figures are small there was certainly no tendency toward a high prenatal mortality. On the contrary, there was scarcely any prenatal mortality, so that the record in no way resembles that of the alcoholic lines. On the basis of 100 for normal stock mortality, the mortality here corrected for litter size is only 84, or 16 per cent better than the normal. It is actually in the table 5 per cent lower than the control.

After having considered the last column, the F_4 animals with their very good record, it should be recognized that these same animals are included with the F_2 and F_3 individuals in the fourth and fifth columns. Their presence in these columns, particularly in the fifth, has tended to incline the records toward the normal. One must realize, therefore, that the F_2 and F_3 animals if considered alone would present even stronger alcoholic records than are indicated in the fourth and fifth columns.

The table shows that the nearer to the direct alcohol treatment an animal is produced, the more inferior in quality it will be as a result of the high amount of modified germ plasm contained in the germ-cell complex from which it arises. Therefore, the records

of F_1 individuals are worse than the records of the sum of all alcoholic generations, as is seen on comparing the third column with the second. The later generations being further and further removed from the treatment and having less and less modified germ plasm on account of the constant introduction of normal stock are progressively improved until finally the F_4 generation has its modified germ plasm diluted to such a degree that its record is on par with the control.

The ancestors of these late-generation animals were also successively selected from the least affected of the alcoholic stock, being those animals capable of survival and reproduction, while the most highly affected died or were sterile and incapable of reproduction. We assume the probability that the more nearly normal animals with stronger bodies also carry germ cells that are less affected than those in the more degenerate individuals. Most of the grossly defective individuals which reach maturity are sterile as evidence in this direction. Thus individual selection being in this case a selection of germ plasm as well as soma, helps materially to improve the quality of the later generations.

8. A COMPARISON OF ANIMALS FROM DIRECTLY TREATED FATHERS AND FATHERS OF ALCOHOLIC STOCK WITH ANIMALS FROM DIRECTLY TREATED MOTHERS AND MOTHERS OF ALCOHOLIC STOCK AND WITH OTHERS FROM BOTH PARENTS OF ALCOHOLIC STOCK

Are the general conditions induced by directly treating the father with alcohol the same as those resulting from treating the mother, and are they equal in extent? Do fathers of alcoholic ancestry beget offspring of better or worse quality than offspring produced by mothers of similar alcoholic ancestry? Or are the effects of the alcohol treatment on the germ cells, which is expressed through several generations, carried with equal degree by both the alcoholic father and the alcoholic mother? We shall attempt in this and the following section to supply data which may serve to partially, at least, satisfy these queries as well as furnish an analysis of several other more detailed propositions.

TABLE III

AN ANALYSIS OF THE EFFECTS ON THE PROGENY WHEN ONLY FATHER IS ALCOHOLIC, MOTHER ALCOHOLIC OR BOTH PARENTS ALCOHOLIC

	Alcoholic animals with treated parents (first generation)			Alcoholic animals with parents of alcoholic descent, but not directly treated (other generations, except first) 408			All animals of alcoholic descent (all generations, first included) 594		
	Only father treated	Only mother treated	Both parents treated	Only father alcoholic	Only mother alcoholic	Both parents alcoholic	Only father alcoholic	Only mother alcoholic	Both parents alcoholic
Total number	1 2 3 4 5 / 6 18 24 12 0 / 40% 20% / Average litter 2·30 / Aver. product 110·58 / Mat. fail. 81 (33·51%) = 60	1 2 3 4 5 / 1 18 51 16 0 / 20·65% 17·39% / Average litter 2·78 / Aver. product 171·57 / Mat. fail 2·15 (11%) = 92	1 2 3 4 5 / 1 0 0 0 0 = 1	1 2 3 4 5 / 6 16 12 20 5 / 37·28% 49·37% / Average litter 2·45 / Aver. product 110·95 / Mat. fail. 61·20 = 59	1 2 3 4 5 / 7 38 109 21 5 / 24·7% 15·93% / Average litter 2·63 / Aver. product 176·05 / Mat fail 14·06 (9·3%) = 182	1 2 3 4 5 / 15 52 72 29 0 / 40·1% 16·16% / Average litter 2·31 / Aver. product 168·82 / Mat. fail 6 (7·64%) = 167	1 2 3 4 5 / 12 36 36 32 5 / 39·6% 30·5% / Average litter 2·41 / Aver. product 171·09 / Mat. fail 140 (53%) = 121	1 2 3 4 5 / 8 56 165 40 5 / 21·35% 16·43% / Average litter 2·69 / Aver. product 175·11 / Mat. fail 16 (13·3%) = 206	1 2 3 4 5 / 18 70 78 22 5 / 44·22% / Average litter 2·28 / Aver. product 163·76 / Mat fail 6 (6·45%) = 199
Lived over 3 months	1 2 3 4 5 / 6 16 17 1 0 (66·66%)	1 2 3 4 5 / 1 14 25 5 0 (48·91%)	1 2 3 4 5 / 1 0 0 0 0	1 2 3 4 5 / 4 12 11 10 0 (62·71%)	1 2 3 4 5 / 6 30 73 11 2 (67·03%)	1 2 3 4 5 / 12 44 39 21 0 (69·46%)	1 2 3 4 5 / 10 30 28 11 0 (65·28%)	1 2 3 4 5 / 7 44 98 16 2 (60·04%)	1 2 3 4 5 / 14 58 44 21 0
Absorbed, Premature, Stillborn	1 2 3 4 5 / 0 1 6 5 0 (60%)	1 2 3 4 5 / 0 2 15 8 0 (14·46%)	1 2 3 4 5 / 0 0 0 0 0	1 2 3 4 5 / 2 4 1 5 5 (77·27%)	1 2 3 4 5 / 1 5 25 12 2 (15%)	1 2 3 4 5 / 1 5 21 4 0 (60·78%)	1 2 3 4 5 / 2 5 7 10 5 (63·04%)	1 2 3 4 5 / 1 7 50 20 2 (71·76%)	1 2 3 4 5 / 1 7 31 4
Died within 3 months	1 2 3 4 5 / 0 1 1 6 0 (40%)	1 2 3 4 5 / 0 2 3 3 0 (25·53%)	1 2 3 4 5 / 0 0 0 0 0	1 2 3 4 5 / 0 2 0 5 0 (22·72%)	1 2 3 4 5 / 0 3 10 1 1 (25%)	1 2 3 4 5 / 1 2 3 3 0 (39·21%)	1 2 3 4 5 / 0 1 1 11 0 (30·95%)	1 2 3 4 5 / 0 5 17 4 1 (25·23%)	1 2 3 4 5 / 3 5 12 3 0 (31·04%)
Total dead	1 2 3 4 5 / 0 2 7 11 0 (33·33%) N = 118 = 118	1 2 3 4 5 / 0 4 32 11 0 (51·03%) N = 281 = 281	1 2 3 4 5 / 0 0 0 0 0	1 2 3 4 5 / 2 4 1 10 5 (37·27%) N = 170 = 170	1 2 3 4 5 / 1 9 35 13 3 (33·96%) N = 177 = 177	1 2 3 4 5 / 3 8 33 7 0 (39·21%) = 167	1 2 3 4 5 / 2 6 8 21 5 (34·71%) N = 171 = 171	1 2 3 4 5 / 1 12 67 24 3 (39·01%) N = 206 = 206	1 2 3 4 5 / 4 12 34 7 5 (31·16%) N = 176 = 176

Table 3 is an arrangement of the records of animals on the basis of paternal and maternal alcoholism. The first group of animals are those from parents treated directly and having no other alcoholic history. Thus the total 153 differs from the total 186 animals with treated parents in the third column of table 2, since in thirty-three cases the former group had not only treated parents, but also treated ancestors. The second group contains all the animals with parents of alcoholic descent, but not directly treated, the total number is 408; these are the same animals that compose the fourth column of table 2. The third or last group contains all of the 594 non-inbred animals of the alcoholic lines.

The individuals in each of the three groups are separated into three classes. The classes of the first group are those with only father treated, those with only mother treated, and those with both parents treated. In the second group the young are classified as those from only father alcoholic, which means the father was descended from treated ancestors which may have been either treated males or females. In other words, this is not the record of a pure alcoholic male line, but merely the alcoholic effects, if any, that reach the recorded individual through an alcoholic father regardless of the origin of his alcoholism. The second column of this group shows the records of animals from alcoholic mothers. Here again the mother's alcoholism may be due to treatment of any of her ancestors, male or female. It is not a purely female alcoholic line, but a maternal alcoholic line. The third column of the second group shows records of animals from parents both of which were alcoholic.

In the entire second group the alcoholism of the parents is ancestral, not being due to direct treatment, while in the third group the alcoholism is either direct, ancestral, or both. The third group is, therefore, an arrangement of all the animals from alcoholic lines for a comparison of the influences of maternal and paternal alcoholism.

In the first column of table 3 it is seen that when the father only is treated the results contrast decidedly with the control.

There is a high percentage of small litters and a low percentage of large litters, thus giving next to the lowest average litter contained in all the records, only 2.30 against 2.77 for the normal. The average litter weight is very low on account of the small average litter size. When this is corrected for the proportion of weight to number of individuals in the control litters these small litters from the treated fathers weigh more for their size than do the control, being over 6 grams heavier. This is not an actual advantage since the majority of young born in small litters of one and two are larger than those born in high litters of four or five. The percentage of mating failures is unusually high, 23.52 per cent against only 4.54 per cent in the control. All of these facts would seem to indicate that the treatment of the fathers had evidently lowered their productivity or fertility, causing them to fail to sire offspring in almost one-quarter of the matings and to beget unusually small litters in the other three-quarters of the cases. There must have also been a high 'early prenatal mortality' in view of the remarkably great percentage of small litters and high percentage of mating failures. We must necessarily divide the mortality into prenatal and postnatal, and the prenatal again into 'early prenatal,' as indicated by the small average size litter and high number of mating failures, and 'late prenatal' based on the exact observations of absorptions, abortions, and still births.

Two-thirds of the offspring from treated fathers survived against over three-fourths from the control. The prenatal mortality is a larger proportion of the total than in the normal. The total mortality when corrected to the normal rate for the different-size litters in which the animals were born is 178 in terms of the control as 100. This is only slightly below the mortality rate of 189 for the entire non-inbred alcoholic group.

When the mother alone was treated the records of the offspring differ considerably from the above. The percentage of small litters is only slightly higher than the percentage of large litters, and the average-size litter, 2.78, is as large as the normal. There are very few mating failures, in this regard again

almost a normal record. The productivity of these treated mothers is high and the size of the litters would indicate a very low 'early prenatal mortality.' Here, however, their good records stop.

Although the litters contained as many individuals as the control litters, their average weight was 26 grams below the normal. The large litters from treated mothers actually weighed only as much as the very small litters from treated fathers; therefore, the individual members of the litters from treated mothers were unusually small animals. The 'late prenatal mortality' was proportionately very high—three times the postnatal. Thus many of the young died in utero or were still-born, and those that were born alive were small specimens. The total mortality was 51.08 per cent, corrected for the litter sizes, and expressed in terms of the control as 100 it becomes 281—the highest mortality on record.

We see from the table that treating the mother with alcohol does not appreciably affect her productivity, but greatly depreciates the quality of offspring to which she gives rise. While in the case of the alcoholic father the productivity is greatly reduced, and although the quality of offspring which he begets does not compare favorably with the control, it is considerably superior to that from the treated mother. In the treated mother the alcohol may act not alone on the ova or germ cells, but on the developing embryo as well, while in the father it acts, of course, on the germ cells alone. Does the difference between the qualities of the offspring from these two cases represent the action of the treatment on the developing young in utero? Further, does the reduced productivity on the part of the treated male indicate that the spermatozoön or male germ cells are more sensitive to the treatment than the egg? The remaining columns of this and the following table may throw some light on these questions.

During the period of the experiments now under consideration practically no matings between treated males and females have been made, as the third column of this group shows.

The next group in table 3 are animals derived from parents of alcoholic descent which had not themselves been treated. These are the same 408 animals recorded in the fourth column of table 2. The first class in this group are animals obtained from fathers of alcoholic ancestry and normal mothers; the second class are from mothers of alcoholic ancestry and normal fathers, and the third class are animals produced by two alcoholic parents. As mentioned above, the alcoholic father or mother may owe their condition to either male or female or to both male and female ancestors. These are not purely male or female alcoholic lines such as will be found in the next table.

A comparison of these three columns with the normal records shows clearly the alcohol effects, though not so strongly expressed as when the father or mother is directly treated. The father and mother columns of this group differ very little from one another, which is in marked contrast to the striking differences when the fathers and mothers are directly treated, as seen in columns 1 and 2. In the present columns all of the modified conditions are due to an injury of the germ cells in the treated ancestral generations. This is equally as true of the alcoholic-mother column as of the alcoholic father. For example, the mortality records in the alcoholic father and mother columns are about the same, while there is a remarkable discrepancy between the mortality records of young from treated fathers and treated mothers in the first two columns. The extremely high mortality, largely late prenatal, among the offspring of directly treated females is to some extent due to the direct action of the alcohol upon the early developing embryo in utero. If this action could be eliminated the treated father and mother columns of the first group might become as nearly similar as the alcoholic father and mother columns of the second group. The individuals in the latter two columns are on an average about the same distance removed from the ancestral alcohol treatment, and, therefore, the records would be little affected by a correction on the basis of the generations treated.

When both parents are from alcoholic ancestry the productivity is considerably lowered as shown in the third column by

the high percentage of small litters and low percentage of large litters and consequently the very low average litter of 2.31. This is likely due to the male partner in the combination, as the preceding columns would suggest. The average litter weight, however, is high, so that the individual members of the litter are as heavy as the normal; this, again, may be due to the male influence as expressed in the high early prenatal mortality.

The mortality records, though markedly inferior to the normal, show an advantage over the two previous columns. There is probably a high 'early prenatal mortality' as indicated by the low average litter, but the 'late prenatal mortality' is lower than in any of the foregoing columns except that of the treated fathers, where again the litter was very small and the probable 'early prenatal mortality' high. This close association between the small litters and the low late prenatal mortality makes it seem all the more probable that the litter size is associated with an 'early prenatal mortality' that occurs so near the beginning of development that it cannot be directly observed. On the other hand, this result could be interpreted as due to a lowered fertility. If this were brought about through an elimination of the weaker germ cells we might except also the associated low late prenatal and postnatal mortality, and would have a condition in exact accord with Pearl's interpretation of the results on fowls. We should be glad to accept such an explanation, but for the considerable amount of evidence in our records which points towards a high 'early prenatal mortality' rather more than infertility as the underlying cause of the small litters and low late mortality. It must also be remembered that the infertility among the fowls was found in the females as well as the males, while here it would be confined to the males only.

The slight advantages which appear in favor of the records from both parents alcoholic as compared with records from alcoholic mothers or fathers are due largely to the distance from the treatment of the generations concerned. In the majority of cases the generations are more remote in the both-parent column than in either the father or mother column, and on the basis of

the evidence shown in table 2 this may readily explain the apparent advantages.

The last three columns show the results of the first two groups combined and in addition contain a few records from mixed cases that could not be properly included in any of the previous classes; for example, animals with one parent of alcoholic ancestry and the other parent directly treated, etc.

Here again there is considerable contrast between the alcoholic-father and the alcoholic-mother columns, these differences being due to the influence on the totals of the F_1 records from the treated-father and treated-mother columns of the first group. The productivity when only the father is alcoholic is low, the litters being small and over 21 per cent of the matings result in failure. It may be inferred that there was a rather high 'early prenatal mortality.' The average litter weight, however, was about as good as normal. The late prenatal and postnatal mortality records are better than those from the alcoholic mothers.

The average-size litters from the alcoholic mothers was rather large and the mating failures were much less frequent than from the alcoholic fathers, indicating a lower probable 'early prenatal mortality.' The average litter weight was lower than from alcoholic fathers, taking into account the size of the litters in the two classes. The total mortality from alcoholic mothers was high and the proportion of late prenatal to postnatal was excessive. It is thus seen that a high prenatal mortality is followed by a low postnatal death rate, and this is in accord with our assumption that a high 'early prenatal mortality' will be followed by not only a low postnatal, but also a low late prenatal mortality. In other words, the more thorough the elimination of defective embryos and fetuses the greater the probability of survival for the selected few that remains to be born.

The last column with both parents alcoholic has a mortality record as good as the alcoholic-father column and better than the alcoholic-mother, but this is only apparent and not real. The column contains only one individual from directly treated parents, and consequently the alcoholic treatment was applied

on the average to more remote generations than was the case in the two single alcoholic-parent columns.

In spite of the generations concerned, there is a higher per cent of small litters and a lower per cent of large litters here than in any other class in the entire table. Consequently there is also the lowest average litter. In so extreme a case there was no doubt a high early prenatal mortality. The average litter weight is actually low, but allowing for the small-size litter the average birth weight of the individuals is about as much as the control, again indicating that an individual selection has occurred through an elimination of the weaker embryos during the early developmental stages.

The extremely small-size litter and the high 'early prenatal mortality' may also in addition to the generations concerned explain to some extent the relatively low total mortality and especially the lower rate of late prenatal mortality as compared with postnatal.

The questions involved in the present section may be still further analyzed by rearranging the data on the basis of only male ancestors treated or only female ancestors treated instead of only father alcoholic and only mother alcoholic. Table 4 presenting this arrangement will be reviewed in the following section, after which several points of interest may be better discussed.

9. A COMPARISON OF LINES FROM ONLY MALE ANCESTORS ALCOHOLIC WITH LINES FROM ONLY FEMALE ANCESTORS ALCOHOLIC AND WITH THOSE FROM BOTH MALE AND FEMALE ANCESTORS ALCOHOLIC

The records tabulated on the basis of male or female ancestors treated supplement the arrangements in table 3, where the groups are classed for only father or mother alcoholic. In table 3 the alcoholic father may owe his alcoholism to the treatment of any of his ancestors, either male or female or both. The alcoholic effects, if any, are there due to the paternal ancestry. The same applies to the groups with only mother alcoholic.

In table 4, on the other hand, the groups with only male ancestors treated owe their modified conditions, if such exist, entirely to the effects of the treatment on male animals, though the individual being considered may have inherited this alcoholic effect through its mother. Thus animals in the columns with only male ancestors treated were not necessarily derived from alcoholic fathers, but may have been produced by alcoholic mothers which, however, owe their alcoholic condition to one or more treated male ancestors. The table permits a comparison of the action of the treatment on the male germ cells and the transmission of the effects with the action of the alcohol treatment on the female germ cells and the effects transmitted to the different generations. While the last table permitted a comparison of the animals derived from males of alcoholic stock with others derived from females of alcoholic stock. The two tables serve to analyze very completely the problem of the parts played by the sexes in the acquisition and transmission of the effects of the alcohol treatments.

The three columns in the first group of table 4 are the same as those of the first group of table 3, being the records of F_1 animals derived from treated fathers, which have only one male ancestor treated according to the table 4 arrangement, and F_1 animals derived from treated mothers or from only the one female ancestor treated. This group was discussed in reviewing the third table. The points of chief interest in the present connection are the decidedly inferior conditions of the offspring from the treated females as compared with those from the treated males, in so far as their measured mortality records and birth weights per litter are concerned. On the other hand, the records from treated males suffer as regards the 'early prenatal mortality' indicated by the small average-size litter and the high percentage of mating failures, while the records of the treated females in regard to these conditions are equally as good as those of the control animals.

The next three columns of table 4 are highly important, since they contain the results of matings when, first, only male ancestors are treated; second, when only female ancestors are

TABLE IV

AN ANALYSIS OF THE EFFECTS ON THE PROGENY WHEN ONLY MALE ANCESTORS WERE TREATED. FEMALE ANCESTORS TREATED OR BOTH MALE & FEMALE ANCESTORS TREATED

	Alcoholic animals with treated parents (first generation)					Alcoholic animals with parents of alcoholic descent, but not directly treated (other generations, except first)				All animals of alcoholic descent (all generations, first included)			
	Only father treated	Only mother treated	Both parents treated	Only ♂ ♀ onces	Only ♀ onces	Only ♂ onces	♂ & ♀ onces	Only ♀ onces	Only ♂ & ♀ onces	Only ♀ onces	♂ & ♀ onces		
Total number	60	92	—	145	181	92	181	223	408				
Lived over 3 months													
Absorbed, Premature, Stillborn													
Died within 3 months													
Total dead								161	211				

treated, and third, when both male and female ancestors are treated with all first generation, F_1 offspring, excluded. The modified conditions shown by these records are due to an hereditary transmission of the defects and not in any case to the direct influence of the treatment on the developing animals.

The fourth column from only male ancestors when compared with the normal stock in tables 1 and 2 shows a higher 'early prenatal mortality' based on the average litter size and high number of mating failures, a lower average litter weight, a higher late prenatal mortality, and a higher total mortality. The results of these matings are, therefore, from any point of view worse than the results of normal matings. And they prove the hereditary transmission of the defects arising from the treatment of the male animals.

The same can be said for the female column, the results shown here also being worse than from the normal matings. The 'early prenatal mortality' is higher, the average litter weight, indicating the total productivity is smaller, the late prenatal and total mortality are higher, while the mating failures are about the same as in the control records. Therefore, the treatment of female individuals also induces effects that are transmitted to later generations through the germ cells.

When, however, the records of the fourth and fifth columns are compared, it is found that the treatment of male ancestors gives in every point considered more marked effects on the qualities of the descendants than the treatment of female ancestors. Among the descendants of treated males there is a higher early and late prenatal mortality, a decidedly higher total mortality, and more mating failures than among those from treated female ancestors, while the first and second columns show the opposite to prevail so far as litter weight and mortality are concerned for first-generation, F_1, animals from directly treated males and females. These inferior results, so far as late prenatal and total mortality are concerned on the part of the offspring from the directly treated female, may be interpreted as due to the direct influence of the treatment upon the young in utero. On the other hand, the improved records from the

treated-female line during later generations can probably be explained in part by the higher mortality of the offspring in the first generation, thus bringing about a greater elimination of the weaker individuals. In other words, these animals from only female ancestors treated have withstood a somewhat more severe selection during the first generation than have the offspring from only treated male ancestors.

As a final possibility it must be recognized that the superior records of the late generations descended from treated female ancestors as compared with the records of similar generations descended from treated males, may be due to a smaller influence of the alcohol treatment on the ova, or female germ cells, than on the spermatozoa.

The sixth column from treated male and female ancestors shows, in comparison with the two preceding columns, the highest 'early prenatal mortality' based on the many small-size litters. There is also the lowest average litter weight. The late prenatal mortality, total mortality, and mating failures, while higher than for the treated-female line, are lower than in the treated-male line. The complete absence of matings between directly treated animals as seen in the third column of this table makes comparisons and explanations of the results in this sixth column very difficult.

The last three columns of the table show the combined results from all generations. The column for both male and female ancestors treated shows the highest early prenatal mortality, the male treated line the highest late prenatal mortality, and the female treated line the highest postnatal mortality.

In general it may be stated after reviewing this and the foregoing table that the treatment of males produces in their descendants a high early mortality, especially early prenatal. The treatment of females produces in their descendants a high later mortality, especially late prenatal and postnatal. The treatment of male and female ancestors produces in their descendants the highest early prenatal mortality, but the lowest late prenatal and postnatal mortality.

In table 4, male and female ancestors treated does not necessarily indicate that all records in the column were derived from matings between two alcoholic parents, since both males and females may have been treated among either the ancestors of the mother or the father, but not necessarily both. For this reason the sixth and ninth columns of table 3, in which both parents were in all cases from alcoholic ancestry, show more decidedly that two alcoholic parents when mated together give the very highest early prenatal mortality, but a low late prenatal and postnatal mortality. This last conclusion is extremely interesting in connection with Pearl's results on fowls.

Pearl found that when two alcoholic fowls were mated together, the percentage of infertile eggs was higher than from any other combination, while the prenatal mortality, embryos dying in shell, and the postnatal mortality were the lowest. This is exactly what the guinea-pig records show, provided our 'early prenatal mortality' (indicated by the small litter size, the frequent mating failures, and the observed mortality occurring in utero during all later stages of development) can be considered the same as many of Pearl's 'infertile eggs.' Without intending any adverse criticism of the designation 'infertile,' we may again suggest the possibility that a certain proportion of these eggs had really begun development, but had died in the early cleavage or gastrular stages, and yet on examination, other than a minute microscopic study, they appeared as infertile or unfertilized eggs. If this were true, they could be classed in the early prenatal mortality records. Such an adjustment would serve to harmonize the fowl and guinea-pig records in another important respect.

Pearl has attributed the good qualities of the offspring from his alcoholic parents to a germinal selection which has tended to cause all weak germ cells to be completely put out of commission by the alcohol treatment and only the very best have survived to produce embryos, and these therefore show a low percentage of deaths in shell and a low postnatal mortality. A selection is also playing its rôle in the case of the guinea-pigs, but here it is not acting alone on the germ cells, but more evi-

dently on the developing individuals. The selection in our case is a continuous selection of individuals, eliminating, no doubt, certain of the least resistant germ cells, but continuing to act on the embryonic population to eliminate the most defective of these during very early developmental stages, and so on until the individuals born are a mixture of strong specimen and others only sufficiently strong to have reached birth and possibly to survive in a subnormal fashion for a shorter or longer time. This continuous, both germinal and individual, selection seems to us more to be expected than the abruptly broken germinal selection advocated by Pearl, which completely eliminates all weak germs, and therefore no weak individuals begin development. We must admit that the data from Pearl's double alcoholic matings considered alone strongly suggest only a germinal selection, but the results from our double alcoholic matings, while leaning in the same direction, still show a greater late prenatal and postnatal mortality than do the control matings, and in addition present much evidence to suggest a very high early embryonic elimination. This same early embryonic elimination may be included among the high percentage of infertile eggs resulting from the matings of two alcoholic fowls, and in the case of the fowls it may be so much more severe that the later mortality records compare favorably with the control. This again would lead us to an abrupt break after the high very early prenatal mortality and might be thought to vitiate our entire supposition, yet the guinea-pig records show almost all gradations up to the condition for the fowls.

Our results show that in the alcoholic lines the higher the early prenatal mortality and consequently the smaller the average-size litter, the lower the late prenatal and postnatal death rate, much as Pearl also finds for fowls. These findings will be still further discussed in connection with the sex ratio, table 6.

10. TREATING MALES WITH ALCOHOL FOR ONE AND TWO GENERA-
TIONS AGAINST TREATING FEMALES FOR ONE AND
TWO GENERATIONS

An experiment has now been in progress for some time in which straight male lines have been treated with alcohol for several generations in order to compare the results with those from the treatment of straight female lines for several generations. That is, the original males are treated, their sons are then directetly treated, their grandsons, great-grandsons, and so on; these we consider the straight male lines. The treated females, their directly treated daughters, granddaughters, and so on constitute the straight female lines. There are now a few third- and fourth-generation individuals, though not a sufficient number to tabulate. We shall thus for the present confine our attention to the records from the originally selected and treated males and females and the treated sons of these males and daughters of the females.

The records are arranged in table 5. In considering the table it must be stated that the original animals in this experiment have been carefully selected large strong specimens that were particularly good breeders. Such a choice has been made on account of the severity of the treatment to which the descendants are to be subjected through a number of generations. Only the best animals are likely to produce descendants sufficiently strong to be treated with alcohol and to continue to reproduce for one generation after another. The fact that such a selection is possible does not reflect on the general population, since no population is so perfect that certain individuals are not better than others. This selection probably accounts for the presence in all the groups of table 5 of some offspring of unusually large size.

The pedigrees or conditions of the animals in the different generations are expressed by the following symbols or formulae. A normal animal is represented by the letter N and one treated with alcohol by A. The symbol for the male is placed to the right of that for the female. Thus the first column NN are the normal control animals for comparison, the second column NA

TABLE V

MALES TREATED WITH ALCOHOL FOR TWO GENERATIONS COMPARED WITH FEMALES TREATED FOR TWO GENERATIONS

	NN	NA	N_NA	AN	AN_AN
Total number	1 2 3 4 5 10 46 90 72 15 24.05% 37.33% 233 Average litter 2.71 Mat. fail. 41.14%	1 2 3 4 5 39.15% 34.11% 223 Average litter 2.50 Mat. fail.	1 2 3 4 5 42.42% 12.12% Average litter 2.55 Mat. fail.	1 2 3 4 5 26.51% 21.08% Average litter 2.71 Mat. fail. 0 51	1 2 3 4 5 2.64% Average litter 2.87 Mat. fail. 2 (20%) 23
Lived over 3 months	1 2 3 4 5 10 39 75 45 12 100% 84.78% 83.33% 62.50% 80% (71.68%)	2 3 4 5 100% 94.33% (60.08%)	2 3 4 5 100% 84.33% 60% 25% (66.66%)	2 3 4 5 73.5% 50% 25% (52.65%)	2 3 4 5 50% 33.33% (34.78%)
Absorbed, Premature, Stillborn	2 3 4 5 4 7 10 (51.92%)	2 3 4 5 (65.45%)	2 3 4 5 6 3 (63.63%)	2 3 4 5 11 8 (77.77%)	2 3 4 5 13 0 (86.66%)
Died within 3 months	2 3 4 5 3 8 11 (48.08%)	2 3 4 5 0 5 (44.44%)	2 3 4 5 0 3 (36.36%)	2 3 4 5 4 1 (22.22%)	2 3 4 5 14 0 (63.33%)
Total dead	2 3 4 5 7 15 27 3 15.21% 16.66% 37.5% 20% (22.31%) N=100	2 3 4 5 16.66% 100% (39.13%) N 21.56%=181	2 3 4 5 16.66% 40% 75% (33.33%) N 17.60%=189	2 3 4 5 15 9 21.42% 50% 75% (47.36%) N 20.35%=232	2 3 4 5 50% 66.66% (65.21%) N 16.47% 345
Defective	2 3 4 5	2 3 4 5	2 3 4 5 6.66% 25% (6.06%)	2 3 4 5 7.14% 3.22% (10.52%)	2 3 4 5 4.16% (4.34%)
Oversized (more than 500g. when 3 months old)	2 3 4 5 3 5 30% 10.86% 5.55% (5.57%)	2 3 4 5 5.33% 16.66% (8.69%)	2 3 4 5 8.33% 6.66% (6.06%)	2 3 4 5 7.14% (1.75%)	2 3 4 5 4.76% (4.34%)
Undersized (less than 300g. when 3 months old)	2 3 4 5 1 0 2.17% (0.42%)	2 3 4 5	2 3 4 5	2 3 4 5	2 3 4 5

show records of offspring derived from a treated father A and a normal mother N. The third column are offspring from treated males which were also derived from treated fathers and normal mothers, $\frac{NA}{A}$, mated with normal females, N. The next straight male generation treated and paired with normal females would be expressed by $N\dfrac{N\frac{NA}{A}}{A}$, the offspring from such a combination would have had their father, a grandfather, and a great-grandfather treated with alcohol and their mother, grandmothers, and great-grandmothers all normal, and so on for later generations. Animals of these higher pedigrees will be recorded in a future communication. In the table 5 only records from treated fathers are given in column 2 and from treated father and grandfather in column 3. The fourth column shows records from normal fathers and treated mothers, AN, and the fifth column from normal fathers mated with treated females which were derived from treated mothers, $\frac{AN}{A}$ N.

The numbers in all of the columns are rather small, but in every case the records differ from the control. There is a remarkable similarity between the two treated-male groups and also between the two treated-female groups, but a striking contrast exists between the male records as a class and the female records.

In the two male columns the average litter is very small and the mating failures high. The percentage of surviving young, though well under the control record, is equally above the female records. The corrected total mortality in both columns is over 180 against 100 for the control. The proportion of late prenatal to postnatal mortality is slightly contrasted in the one treated male generation column, but more so in the two treated generations column. There are no defective animals in the NA column, but a small per cent of such are seen in the $N\frac{NA}{A}$ group.

The average litter size is high in both female columns and the mating failures lower than in the male groups. While the total mortality is extremely high, being in the two-generation treated column on the basis of litter size over twice as high as either treated-male column and about four times the control record. The proportion of late prenatal to postnatal mortality is in the first female column over three to one and in the last column over six to one. There were some defective animals in both female groups. The records of the females in this and the two preceding tables are out of accord with the records from fowls and do not fit an explanation based on a germinal selection or partial infertility. The total productivity is good and the late prenatal and the postnatal mortality are high.

It is seen at once that the records from the treated-female generations are far worse than from the treated-male generations; in fact, so much worse that we are led to conclude that the alcohol has not acted on exactly the same things in the two cases. The increased effect of the treatment in the double female column is much more evident than in the two male generation column. The results in the male columns are due only to an action of the treatment on the spermatozoa or male germ cells, while the results in the female columns are also due to the effects of the treatment on the germ cells or ova, but more largely to the effects of the alcohol on the developing embryos within the uterus of the treated mother. Provided the effects of alcohol were equal on the sperm and ova of guinea-pigs, the difference between these two sets of records would then represent the action of the treatment on the developing embryo itself.

Although the records in table 5 involve only small numbers, we are led to believe that they represent the true trend of the effects, since they harmonize so perfectly with the data of different composition yet much more complete shown in tables 3 and 4.

Here again, as in tables 3 and 4, the treated-male lines show the early prenatal mortality (based on the average litter size and frequent mating failures) to be unusually high while in the female line it is low. In the male lines the late prenatal and

total mortality is low while in the female lines the late pre-
natal mortality is extremely high and the total mortality very
great.

Finally, this table may be considered as supplying evidence of
the increased effect of higher or longer alcoholic dosage. The
double male records which have usually been derived from ani-
mals that have had longer or more treatment during the two
generations are somewhat inferior to the one generation male
treated records, and this inferiority is very much more decided
for the female groups in the case of the higher-dosed two-genera-
tion records.

11. THE SEX-RATIO IN RELATION TO PATERNAL AND MATERNAL ALCOHOLISM AND TO THE TREATMENT OF MALE AND FEMALE ANCESTORS WITH ALCOHOL

In the last group of table 3 it will be remembered that all of
the non-inbred acoholic descendants were separated into three
classes with only father alcoholic, only mother alcoholic, and
both parents alcoholic. Again, in the last group of table 4,
these 594 animals were rearranged into three classes, from only
male ancestors treated, only female ancestors treated, and both
male and female ancestors treated. The difference between
these classifications are made clear in the discussion of tables 3
and 4. If we now record the number of males and females com-
posing each of these six classes and express their sex-ratios on
the basis of the number of males to every 100 females, a most
peculiar result is obtained, and one for which it is very difficult
to give a completely satisfactory explanation.

The number of males and females and their mortality records
in each of the six classes are shown in table 6. As a standard of
comparison the 233 control animals are similarly recorded in
this table. For further comparisons a total sex-ratio and the
sex-ratios for animals born in different size litters are given be-
low the table. The total sex-ratio calculated for about 1600
animals is 109.6; that is, 109.6 males to every 100 females. Many
of these animals were from alcoholic lines, so that this sex-ratio
may not be exactly normal. Yet a further perusal of the table

TABLE VI
THE SEX-RATIOS OF THE PROGENY AFTER THE TREATMENT OF MALE AND FEMALE ANCESTORS OR FROM ALCOHORIC FATHERS AND MOTHERS

	Normal line			Only father alcoholic			Only mother alcoholic			Both parents alcoholic			Only ♂ ancestors treated			Only ♀ ancestors treated			♂ and ♀ ancestors treated		
	♂	♀	Unknown Sex	♂	♀	Unknown Sex	♂	♀	Unknown Sex	♂	♀	Unknown Sex	♂	♀	Unknown Sex	♂	♀	Unknown Sex	♂	♀	Unknown Sex
Total number	120	106	7	59	59	4	121	125	28	103	85	11	107	98	18	77	89	15	44	81	10
Sex ratio	113.20		(2.77)	101.72		(2.41)	96.80		(2.64)	121.17		(2.28)	109.18		(2.42)	86.51		(2.60)	123.48		(3.22)
Lived over 3 months	97	84	0	41	38	0	85	81	1	73	64	0	72	68	1	51	57	0	76	58	0
Total dead	23 19.16%	22 20.75%	7 100%	18 30.5%	20 34.48%	4 100%	36 29.15%	44 35.20%	27 96.42%	30 29.12%	21 24.70%	11 100%	35 32.7%	30 33.6%	17 94.44%	26 33.76%	32 35.95%	15 100%	23 23.25%	23 28.39%	10 100%
Percentage of total dead	19.91%			32.47%			32.52%			27.12%			31.10%			34.93%			25.55%		

Total mortality of ♂ 29.68%
" " " ♀ 31.71%

Total sex ratio 109.63
" " " of 1 in litter animals   113.18 (114)
" " " of 2 in " "   114.86 (578)
" " " of 3 " " "   101.74 (579)
" " " of 4 " " "   106.66 (249)

will suggest a tendency on the part of the different alcoholic lines to level the sex-ratio to normal when they are combined as a grand total, yet we are not by any means comparing the sex-ratios from the alcoholic lines with an average alcoholic ratio. The sex-ratios of 194 animals born one in a litter was 113; of 578 born two in a litter, sex-ratio 114.8; of 579 born three in a litter, sex-ratio 101.7, and of 248 animals born in litters of four, the sex-ratio was 106.6.

The first column of table 6 shows that of the 233 non-inbred control animals, 120 were males and 106 were females. The proportion of males to females is thus 113.2 to 100; that is, a sex-ratio of 113.2. The average-size litter in which these animals were born is shown in parentheses in the sex-ratio space as 2.77. The mortality record for the males was about the same as for the females, having only a very slight advantage.

The third column of animals from only mother alcoholic are also in the majority of cases individuals from only female ancestors treated, the sixth column, but not entirely so, as many of the mothers may have been alcoholic on account of a treated father or grandfather. In general, however, the third and sixth columns are rather the same in composition, the sixth being a purely female treated group while the third column is largely so but not entirely, and while not necessarily to be treated together they may be considered in connection with one another. A point of immediate notice is that the sex-ratios in both of these columns, 96.8 and 86.5, are very low.

When only the father is alcoholic, second column, or only the male ancestors are treated, fifth column, the sex-ratios are higher, 101.7 and 109.1. While if both parents are alcoholic, fourth column, or male and female ancestors are treated, seventh column, the sex-ratios are very high, 121.1 and 123.5. It must also be noticed that these differences in sex-ratios are more accentuated in the last three columns, giving the descendants from only female ancestors treated with alcohol the lowest sex-ratio in the entire table; those from only male ancestors treated a considerably higher ratio, and from both male and female ancestors treated the highest sex-ratio for all groups. A differ-

ence of 37 between the number of males to 100 females in animals from treated-female ancestors as compared with those from both male and female ancestors treated is indeed very great.

Are these differences in sex ratio a result of the direct influence of alcoholism upon sex determination or sex differentiation? Or are they indirectly brought about by a difference between the early prenatal mortality rates of the two sexes in the several groups considered? Or are these merely chance differences? There is no doubt that chance plays a large part in the make up of all sex-ratios, but to be consistent in six straight cases as the six groups show can scarcely be dismissed as a chance result.

It is very peculiar that these different sex-ratios should coincide in a direct manner with differences in the early prenatal mortalities among the several groups of table 6, and thus suggests that the explanation for the sex-ratio differences may be in part at least along the line of the second of the above propositions. After considering this probability of differences between the early prenatal mortalities of males and females, we may then discuss the further possibility of the direct effects of the treatment on the sex-ratios.

The groups having the lowest sex-ratios, female lines with ratios 96.8 and 86.5, also have, as shown in tables 3 and 4, the largest average litters, 2.69 and 2.66, or the lowest early prenatal mortality. The lines having a somewhat higher sex-ratio, male lines with ratios 101.7 and 109.1, have correspondingly somewhat higher early prenatal mortalities, as indicated by the smaller average litters, 2.41 and 2.42; while the lines having the highest sex-ratios, double lines with ratios 121.1 and 123.5, have along with these the highest early prenatal mortalities as shown by the smallest average-size litters, 2.28 and 2.37.

This is certainly a very suggestive parallelism. And if one now considers the fourth line of the table giving the total dead of each sex, in every column, with one exception, it will be seen that the female mortality is higher than the male. The exception is in the column from both parents alcoholic; here the

sex-ratio is very high and yet the late male mortality is higher than that for the females. The total mortality for the females is higher than that of the males, 31.71 per cent against 29.68 per cent. It is further shown below the table that small litters have a higher sex-ratio than large litters, the sex-ratios for litters of one and two young being respectively 113.1 and 114.8. While for litters of three and four young the sex ratios are 101.7 and 106.6. It has been pointed out before that the small litters are often due to an early prenatal mortality which has destroyed some of the original members, and since the sex-ratios of such litters are high the majority of embryos dying may have been females. We may see finally by a study of table 7 that female animals are generally smaller at birth than males in the same litter, and as their total higher mortality would indicate, they are probably also weaker.

TABLE VII

THE BIRTH WEIGHTS OF MALE AND FEMALE MEMBERS OF MIXED LITTERS

	Number of Litters	Total weight of males in grams	Total weight of females in grams	Total excess Weight of males over females	Average excess weight of males over females	Percent of excess weight of males over females
Litters of 1♂ 1♀.	105	7861	7525	336	3.20	2.18%
Litters of 2♂ 1♀	136	9513	4654 (9308)	205	0.75	1.08%
Litters of 1♂ 2♀	125	4428 (8856)	8790	66	0.26	0.37%
Litters of 2♂ 2♀	36	2325	2239.5	85.5	1.19	1.87%

Table 7 only includes mixed litters; that is, those containing both male and female members. It shows that in 105 litters of two animals of opposite sex the total birth weight of the 105 males was 7861 grams and of the 105 females only 7525 grams, or 336 grams less. The average excess weight of males over females in these litters of two was 3.2 grams, giving a percentage of excess weight of 2.18 in favor of the males.

One hundred and thirty-six litters of three, consisting of two males and one female, are recorded. The total weight of the

272 males was 9513 grams; that for the 136 females was 4654 grams. If this total weight be doubled for comparison with the total weight of the double number of males, we have 9308 grams. The males again have a total advantage, amounting here to 205 grams. The average excess weight of the males is 0.75 gram, or a 1.08 per cent excess weight of males over females.

It will be noted in this table that the average weight of the individuals is very low. This is due to the fact that a number of abortions in which the sex could be distinguished, as well as premature still-births are included. These small specimens have brought the average in some cases almost below the birth weight which permits survival. It is also noticed that the 272 males in the second line only weigh about one-quarter more than the 105 males in the first line of the table, and this is due to the fact that there were many more abortions and early premature births of litters consisting of three individuals than of two. While the males in the litters of one male and one female averaged almost 75 grams, the males in litters of two males and one female averaged less than 35 grams.

The third line of the table shows 125 litters of one male and two females. The 125 males weighed 4428 grams which may be doubled to give 8856 grams for comparison with the total weight of 8790 for the 250 females. There is a total advantage of 66 grams in favor of the males. The average excess of male weight is 0.26 gram, or 0.37 per cent over female weight.

The last case of thirty-six litters, consisting of two males and two females each, gives a total excess of 85.5 grams to the males. The average excess weight of the males over females is 1.19 grams, and the per cent of excess of males over females is 1.87.

It is thus seen that the males born in litters consisting of both sexes possess a superiority in body weight over the females in every combination. We do not attribute this constant excess in favor of the males to a sexual dimorphism in size. In a group of guinea-pigs both young and adult females are often larger in size than comparable males, and no constant size difference between the two sexes is known. It seems more probable that

this advantage in weight on the part of the males, the majority of which are of alcoholic ancestry, is in line with the lower mortality records of the males shown in the various columns of table 6. And this may further bear on the explanation of the high sex-ratios in those lines with high early mortalities or small average litters.

There is, therefore, much evidence to indicate that among alcoholic guinea-pigs the females very probably suffer a much higher early prenatal mortality than do the males, and it is shown that the female mortality is higher than that of the males at all other periods, table 6.

Before proceeding further with our theoretical explanation of the different sex-ratios in the several groups, which leads finally to a consideration of views expressed in a previous communication, still another important relationship may be pointed out between early prenatal mortality and the sex-ratio, on one hand, and the late prenatal and postnatal mortality, combined in table 6 under 'total dead,' on the other. Stated concisely, the higher the sex-ratio and the early prenatal mortality, indicated by the small average litter, the lower will be the total late prenatal and postnatal mortality, and vice versa. The columns with the highest sex-ratios, 123.58 and 121.17, and at the same time the highest early prenatal mortalities or the smallest average litters, 2.37 and 2.28, show the lowest late mortalities, 25.55 and 27.12 per cent. In the opposite way the columns with the lowest sex-ratios, 96.8 and 86.51, and the lowest early prenatal mortalities, or the largest average litters, 2.69 and 2.66, have the highest later mortalities, 34.93 and 32.52 per cent. This is in line with what was brought out during the discussion of table 4 showing that the higher the early prenatal mortality, or the smaller the average litter, the lower will be the late prenatal and postnatal mortalities.

There is one very evident objection to the foregoing explanation of the peculiar sex-ratios as being due to a differential sex mortality during the early prenatal periods. That is, among the normal stock the sex-ratio is rather high, although the early prenatal mortality is probably very low as indicated by the

large average litter. With a large average litter the sex-ratio should be very low as in the female lines. We could only avoid this difficulty by assuming that the control lines are out of the consideration, since the other sex-ratios being discussed are all shown by modified alcoholic groups among which entirely different conditions obtain from those existing in the control. Whereas there are reasons for such a position, it would seem preferable at present to admit that the case of the control is a real objection. And such an objection would serve to indicate that while a higher mortality on the part of the female embryos in the alcoholic groups might actually exist, yet it accounts only in part for the peculiar sex-ratios found. A recognition of the normal record also makes it difficult to account for the very low sex-ratios of the female lines. Here the early prenatal mortality was low on the basis of the average size litter, but if any early prenatal mortality did occur it could not have been partial to the female embryos, but must on the contrary have been confined almost totally to male embryos or else a sex-ratio could never fall 25 below the control. Is it possible that wherever a treated male is concerned, as in the male columns and the double columns of table 6, there is a high early prenatal mortality among the female embryos, and on the other hand where only a treated female is concerned there is a high early male mortality? It is difficult to believe so, and therefore differences between the early mortalities of the sexes can, on our present data, only partially explain the sex-ratios found in table 6. This leads to a final explanation which may seem highly theoretical, yet it does have a basis of fact.

In an earlier communication (Stockard and Papanicolaou, '16), we presented some evidence which seemed to indicate a possibility that the action of the alcohol treatment not only differed in its effects upon the two sexes treated, but also acted differently on the two groups of spermatozoa in the male, the so-called male-producing and female-producing sperm.

We suggested that the action of the treatment was more severe on the germ cells of the male than on those of the female; in other words, that the spermatozoa were more susceptible

than the ova.   The inferiority of the column from male ances-
tors treated as compared with that from female ancestors treated
in the second group of table 4 seems to substantiate such a
position.   The possibility exists, however, that the treatments
of the male and female ancestors may not have been equally
severe, since they have been treated in different fume tanks.
This question is now being studied.   At any rate, we believe it
is proved that the germ cells of the female are as definitely in-
jured and modified by the treatment as are the germ cells of the
male.   This is the point of importance in the present connection.

The female offspring from treated fathers were found in the
report cited to be inferior as a group to the male offspring as
regards their powers of existence and structural perfection.   The
opposite was indicated among the offspring of treated mothers,
the males being inferior to the females.   Our explanation of
these conditions was that the two classes of spermatozoa which
differ structurally also differ in the degrees of injury suffered
from the treatment.   We are further testing these suppositions
by selected matings and hope to report on them in the future.
For further details regarding the supposed differences between
the behavior of the two classes of spermatozoa, the reader is
referred to our 1916 paper.

An explanation of the sex-ratios in table 6 may now be given
along similar lines and the peculiarities found among these sex-
ratios are exactly in accord with our previous theoretical consid-
erations.   If the male guinea-pig does possess, as has been claimed
(Stevens, '11), heteromorphic spermatozoa, one class with a
small Y chromosome, the male producing, and the other class
with a larger X chromosome, the female producing, the follow-
ing may be assumed: In the treated-male lines the female-pro-
ducing spermatozoa are more decidedly affected, possibly on
account of their larger quantity of chromatin, and therefore, in
the competition to fertilize the eggs they are not so successful
as the less injured male-producing sperms.   Consequently, more
male animals are produced than female.   Or, if the female-
producing sperm are not in any or all cases actually prevented
from fertilizing eggs, nevertheless the individuals produced by

such a fertilization are inferior and more apt to die during early developmental stages, and thus a greater number of male embryos would survive and be born.

When the alcoholic mother or early female treated lines were mated with untreated normal males, the sons were inferior to the daughters. Here again, taking into consideration the two structurally different classes of spermatozoa, the normal males paired with alcoholic females contribute a smaller amount of normal chromatin to the complex producing male offspring than to that giving rise to the female offspring. The records of the males are hence inferior to those of the females. And in the present connection such males might be expected to suffer a higher early prenatal mortality and so give rise to the very low sex-ratios shown by the columns from 'only mother alcoholic' and 'only female ancestors treated.'

Such reasoning from the present data is admitted to be highly speculative; nevertheless, if the morphological differences which have been found to exist between the two classes of spermatozoa in a number of animal species have any significance, they must sooner or later be recognized as the underlying cause of such results as table 6 shows for sex-ratios in alcoholized guinea-pigs.

These ideas also account for the fact that the sex-ratios of the normal animals is out of accord with the ratios of all the treated groups on the basis of the average litter size. This discord was recognized as a possible objection to the purely differential sex mortality explanation previously discussed. In the present connection we may take the following position.

The normal group has been subjected to no injurious action which has tended to modify the expression of the sex-ratio, while in the alcoholized groups there is evidence of a deviation from the normal, in one direction or the other, depending upon the combination concerned. And this deviation is imagined to be due to a lower fertilizing ability on the part of certain spermatozoa.

There is another question to be considered in connection with the differences in response on the part of the two classes of spermatozoa; that is, the possibility of certain eggs being more sub-

ject to fertilization by either the X or Y type of spermatozoa. Even though the egg might be practically equally accessible to both types under normal conditions, a peculiarly affected egg might become much more readily fertilized by one class of sperm than the other, and almost all male offspring might result in one case and females in the second. One might feel that these are large suppositions on the basis of the minute differences between the two groups of sperm. But it may be replied that the differences are only minute from the standpoint of the minuteness of the structure considered. Corresponding differences between great things would necessarily seem much more important, but with present powers of observation only very great differences between cellular structures are visible at all.

There is evidence from a study of the control of sex-ratios in normal guinea-pigs to indicate that certain females have a very strong tendency to produce male offspring regardless of the male with which she is paired (Papanicolaou, '15). Other females have as decidedly marked tendency to produce female offspring. Such females may be said to have either a male or female tendency, while other females are in this regard indifferent, producing as many offspring of one sex as of the other. These tendencies may be explained in accord with the above discussion as due to a high affinity for one type of sperm on the part of the ova of one female, while the ova of another female are particularly susceptible to fertilization by the other class of sperm. The indifferent females are those with ova which are fertilized equally as well by one type of spermatozoa as the other. There are striking cases among the ascidians and other forms illustrating selective fertilization, and the above suggestions are by no means without foundation.

Certain male guinea-pigs are also known to have a strong tendency to beget female offspring regardless of the females with which they are paired. Other males have a high male-producing tendency and still others are more or less indifferent in their sex-determining quality. This may be readily imagined to result from a difference in the activity or fertilizing powers of the

two types of spermatozoa in certain male animals. And there is evidence to show, as cited in our previous paper, that the fertilizing power of the spermatozoa may be modified in such a way as to render them much less capable of success. If this is the case, we may be justified in assuming that one class of sperm may often, even under normal conditions, be at a disadvantage as compared with the other. It is even more probable that under modified conditions the two morphologically different classes of spermatozoa will not be affected to equal degrees.

In conclusion, then, it seems highly probable that the peculiar sex-ratios shown by the several groups of treated animals recorded in table 6 are in part due to differential sex mortalities during early prenatal stages, on account of the close correlation between the sex-ratios and the average litter sizes. This difference in early prenatal mortality between the sexes does not, however, completely satisfy the case. The sex-tendency of the animals considered and the possibility in the case of delicate treatment of affecting the two types of spermatozoa in different ways or degrees are certainly factors to be recognized in the production of the results obtained.

Pearl found that for fowls treated with alcohol the relative proportions of the sexes produced were not significantly different from normal control series. Our results for the sex-ratio of the total alcoholic series agree with Pearl's findings. The sex-ratio of the 594 alcoholic animals considered in the present paper is 105.6, which, in view of the numbers involved, is not significantly different from the control series. Yet studying separately the several groups shown in table 6, we find strikingly wide differences in the sex-ratios and the arrangement of these differences is decidedly consistent. From the standpoint of the above discussion it seems to us legitimate to consider the six groups individually, or at least as three classes, since there is a probability that different processes or conditions are affecting the results in the different cases. Several recent experiments on the modification of the sex-ratio would tend to strengthen such a probability.

12. THE BIRTH WEIGHTS AND RATE OF GROWTH IN THE NORMAL AND THE ALCOHOLIC SERIES

In the present section the birth weights and ability to grow of the animals born in the normal and the alcoholic series may be compared. Here again comparisons must be made between animals born in litters of the same size. It may be expressed generally, as was done above for the mortality rate, that the birth weight of an animal, either normal or alcoholic, varies inversely with the size of the litter in which it is born. The average daily increase in weight during the first month varies in the same way. So that when one month old the weight of a guinea-pig also as a rule varies inversely with the size of the litter in which it was born. This condition holds up to three months, at which time the guinea-pig is mature. But the daily gain in weight during the second and third months after birth ceases to be greatest for the members of small litters. Yet the advantage in growth rate comes to the members of the large litters at so late a time that they are unable to make up their disadvantage sufficiently to equal in size the members of small litters within three months. All of these statements apply equally to both the alcoholic and normal series, and thus the influence of the litter size in general is the same in both cases.

The question then arises whether there is an actual difference in birth weights and growth rates between the two series. Table 8 contains the birth weights of 225 normal control and 531 animals of the alcoholic series. This alcoholic group, as the foregoing tables show, not only includes F_1 animals, or offspring from directly treated parents, but also their descendants for several generations, F_2, F_3, and F_4. The animals of both series are arranged in table 8 according to the size litters in which they occur.

A review of the table shows that the normal series is superior in the average birth weight of the individual and the average birth weight of the entire litter, as well as the average birth weight of the individual born in each of the five different-size litters.

TABLE VIII
BIRTH WEIGHTS AND RATES OF GROWTH OF
NORMAL AND ALCOHOLIC YOUNG

	Normal					Alcoholic				
	1	2	3	4	5	1	2	3	4	5
Weight at birth	1085 / 10 / (108.5)	2119 / 46 / (82.15)	6367 / 87 / (70.88)	4125 / 67 / (61.56)	941 / 15 / (62.73)	4028 / 41 / (98.42)	13867 / 168 / (82.54)	14741 / 225 / (65.51)	5200 / 92 / (56.52)	244 / 5 / (48.80)
	(Average 77.16) Average productivity 197.12				225	(Average 70.35) – 9.23% (of the mean) Average productivity 170.0				531
Weight at the end of the first month	2228 / 7 / (318.28)	8902 / 31 / (240.59)	14288 / 68 / (210.11)	6750 / 37 / (182.43)	2302 / 12 / (191.83)	8633 / 29 / (297.62)	30461 / 131 / (232.52)	26398 / 133 / (198.48)	6881 / 40 / (172.02)	338 / 2 / (169.0)
	(Average 228.64)					(Average 213.94) – 6.63%				
Average daily increase in weight during the first month	6.99	5.38	4.64	4.02	4.30	6.64	4.99	4.43	3.85	4.00
	(Average 5.04)					(Average 4.78)				
Weight at the end of the third month	4013 / 8 / (501.62)	16454 / 38 / (433.0)	25643 / 62 / (413.54)	12552 / 33 / (380.36)	4764 / 12 / (397.0)	14264 / 31 / (460.12)	44635 / 105 / (425.09)	41751 / 102 / (409.32)	11749 / 32 / (367.15)	718 / 2 / (359.0)
	(Average 425.11)					(Average 404.13) – 5.06%				
Average daily increase in weight during the 2nd and 3rd months	3.05	3.20	3.39	3.29	3.41	2.70	3.20	3.51	3.25	3.16
	(Average 3.26)					(Average 3.16)				

The average birth weight of the individual in the normal series is 77.16 grams against 70.35 grams for the alcoholic, and the average litter weight is 27.12 grams heavier among the normal animals. The average weight of the individual in a given size litter is shown in parentheses below the litter number; this is obtained by dividing the total weight in grams of all such litters by the total number of animals composing them. For example, in the alcoholic series there are 168 animals born in litters of two and their total birth weight was 13,867 grams, which gives an average weight of 82.54 grams per individual. The average weight of the individual is lower in the large litters than in the small ones in both series.

The second line of the table shows in a similar way the total weight at the end of the first month of all individuals in the several-size litters and below this the number of individuals concerned in each case. The quotient obtained by dividing the total weight by the number of animals is given in parentheses as the average weight of the individual in each litter at one month old. At this age the average weight of normal animals

in litters of one was 318.28 grams against 297.68 grams for the alcoholic litters of one. The general average weight at one month for the normal series was 228.64 grams against a general average of 213.94 grams for the alcoholics.

The average daily increase in weight during the first month is given in the third line of the table. It shows a mean daily increase for normal animals of 5.04 grams and for alcoholic animals only 4.78 grams. Members of small litters in both groups gained more rapidly than members of large litters.

The weights at the end of the third month, when the animals are about mature, are given in the fourth line of the table. Normal animals born one in a litter average over 500 grams, while comparable alcoholic animals weigh only 460.12 grams. The average normal animal at three months old weighs 425.11 grams against an average of 404.15 for the alcoholic animal.

The last line shows that the average daily gain in weight during the second and third months was about as great for the alcoholic animals as for the normals. A much greater selection or elimination has taken place previous to this time among the alcoholic series than among the normal, as a reference to any of the mortality tables will show.

All in all, table 8 would seem to indicate that in every case the normal offspring weigh more and grow more rapidly shortly after birth than do the young alcoholic specimens.

The several points considered above and their general meaning may be much more clearly expressed in the diagram, figure 9. On the left side of the diagram are shown the records for the alcoholic series and the normal records are on the right. The shaded right-angle triangles represent the difference in average weight between the individuals in litters of one, two, three, four, and five at birth, at one month old, and at three months old from the two series. The altitudes of the right triangles measure the magnitude of the differences.

Animals born one in a litter in the alcoholic and the normal series, as the bottom short triangle indicates, show a greater difference in weight than those from any other size litter except that consisting of five individuals as the low long triangle repre-
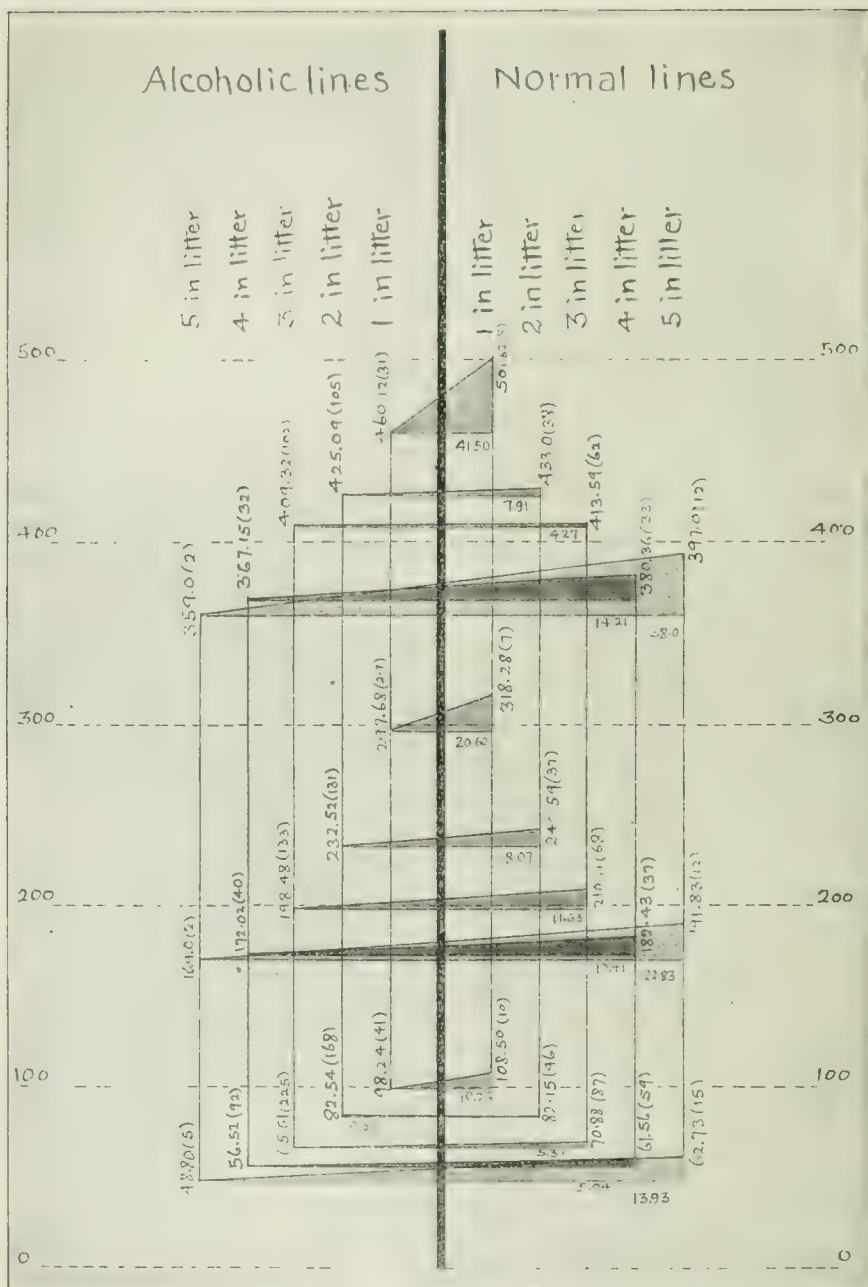
Fig. 9 Diagram illustrating the differences in weight between normal and alcoholic line animals born in litters of the same size. The weights are given at birth, at one month, and at three months old. Further explanations are to be found in the text.

sents. There is little difference between the birth weights of normal and alcoholic animals born in litters of two, three or four.

When one month old the middle group of triangles representing by their position the weights in grams again show the largest differences between alcoholic and normal animals in litters of one, the short triangle, and litters of five, the long triangle. The normal animals in litters of one have passed the 300-gram line in weight, while the average alcoholic member of a litter of five weighs only 169 grams. Members of the two series in litters of two, three, or four do not show very great weight differences.

The top triangle shows a very large difference in weight at three months between normal and alcoholic animals born one in a litter. The triangles for two and three in a litter animals are almost flat at three months, indicating very little difference between such normal and alcoholic animals. Alcoholic members of litters of four are somewhat smaller in average than normal, while alcoholic from litters of five are far below the normal in weight as the long triangle shows at three months.

We have here an example of the influence of the alcohol effect combined with the action of a normal condition, the condition being the size of the litter in which the animal is born. From a consideration of the diagram we may, therefore, conclude, first, that normal-stock animals born one in a litter are so strong as to run far ahead of the one in a litter alcoholic animals, although the latter at birth, at one month, and at three months are much heavier than all normal animals born in larger litters at similar periods. Consequently, the advantage of developing alone in the uterus is sufficient, so far as birth weight and rate of growth are concerned, to overcome the disadvantages resulting from alcoholic ancestry to such a degree that these individuals are better than control animals developing in larger litters. Yet in birth weight and growth rate these singly born alcoholic animals are further behind the singly born control than are the alcoholics from any other size litters behind the control from the same size litters. Thus, although being born alone tends to overshadow the alcohol effect, nevertheless the effect is still shown by comparison with control specimens born alone.

If we now recall the fact that alcoholic animals produce more small-size litters than do the control, and recognize that members of small litters in all cases weigh more, grow faster, and are more apt to survive than members of larger litters, it becomes evident that the production of a high percentage of small litters is a fortunate provision tending to preserve the alcoholic stock by counterbalancing to some degree the magnitude of the effects induced by the alcoholism.

Second, animals born in litters of two or three have a tendency to weigh the same at birth and to grow at a similar rate during the first three months, whether they are from the normal or alcoholic stock. In other words, being born in litters of this size gives no great advantage to the normal animals over the alcoholics, as does being born in litters of only one. Or stated reversely, members of litters of two or three are not placed at a great disadvantage so far as birth weight and growth rate are concerned on account of their alcoholic ancestry, as is found below to be the case for the members of larger litters.

In the third place, when animals are born in litters of four the alcoholic stock are at a disadvantage in birth weight when compared with the normal. The rate of growth of the alcoholic animals from litters of four is also slower than that of the comparable control animals.

Lastly, in the fourth place, alcoholic animals born five in a litter are very small and weak and only a few survive, yet these selected few fall far behind the normal animals from litters of five in their rate of growth. Thus at three months there is a greater difference in average weight between the alcoholic and control members of litters of five than between the members of any other size litters in the two series, except the animals born singly. The alcoholic animals as a group are at a disadvantage in birth weight and rate of growth, but when born in large litters of four or particularly five, this disadvantage is greatly exaggerated by the handicap which befalls the members of all large litters, the control as well as the alcoholic.

13. THE RECORDS OF NORMAL MALES AND FEMALES PAIRED SUCCESSIVELY WITH NORMAL AND ALCOHOLIC MATES: THE CRUCIAL DEMONSTRATION OF THE EFFECTS OF ALCOHOLISM ON THE OFFSPRING

When the records of any group of experimental animals are compared with the records of a normal group, the possibility presents itself that some selection either conscious or unconscious may have played a part in forming the groups. Such a source of error is no doubt practically eliminated by many well-known methods of choosing control and experimental animals from a given population. We believe such a defect is entirely insignificant in the foregoing records which have involved many animals through several generations from the same stocks in the case of both the experimented and the control. It is, nevertheless, satisfactory to consider the records of the same normal animals paired successively with control animals and with animals of the alcoholic lines. Table 9 presents all of the mating records of fourteen normal males and fifteen normal females that have been paired in this way. This table gives a most perfect control and shows most clearly the alcohol effects.

TABLE IX

NORMAL MALES AND FEMALES PAIRED SUCCESSIVELY WITH NORMAL AND ALCOHOLIC MATES

	Individual matings of 14 normal males, each one mated successively with		Individual matings of 15 normal females, each one mated successively with	
	Normal females	Alcoholic females	Normal males	Alcoholic males
Number of matings	36	44	26	23
Total number of young	86	100	59	50
Negative result	2 (5.55%)	4 (9.09%)	1 (3.84%)	5 (21.73%)
Lived over 3 months	65	58	51	30
Total dead or died within 3 months	21 (24.41%)	42 (42.0%)	8 (13.55%)	20 (40.0%)
Defective	0	6 (6.0%)	0	5 (10.0%)

The fourteen male animals are in no sense selected; they are all of the normal males in our series of animals between the numbers 613 and 1909 which have been mated with both normal and alcoholic females. The record numbers of these males are 665, 666, 667, 669, 670, 676, 677, 679, 681, 682, 683, 854, 914, and 1052. The fourteen males, as the table shows, have been mated in all eighty times. The fifteen females recorded include also every normal female among the animals considered in this paper that has been paired with both normal and alcoholic males. The record numbers of the females are 645, 646, 650, 652, 657, 661, 662, 671, 674, 675, 703, 722, 760, 890, and 1043. These have been mated in all forty-nine times.

There has been no selection or choice in mating these animals or in estimating the results, since it was only decided to arrange such a table after beginning the present study of the data.

The first column of table 9 shows the results of thirty-six matings of the normal males with normal females. Two of the thirty-six matings failed to produce results, or 5.55 per cent, and the remaining thirty-four matings gave rise to eighty-six young. Sixty-five, or 75.59 per cent, of these lived to reach maturity, while 24.41 per cent died within three months. None of the eighty-six offspring showed any gross structural defects.

When these same normal males were mated forty-four times with alcoholic females, the second column shows that four matings failed, or 9.09 per cent, almost twice as many as the failures with normal females. The forty successful matings produced one hundred offspring, only fifty-eight of which were capable of survival to maturity. Thus 42 per cent of the young animals died within three months against only 24.41 per cent of those from the normal mothers and same fathers. Six per cent of the young from the alcoholic mothers possessed noticeable structural defects.

In every respect the matings of the fourteen normal males produced greatly superior results when paired with normal females, as compared with their records by alcoholic females. The numbers are comparatively small, but the differences are large and the inferior records are consistently in the same column.

The third and fourth columns contain similar records from the matings of the fifteen normal females with normal males and with alcoholic males. The twenty-six normal matings gave only one failure, while the twenty-three matings with alcoholic males failed to give results in five cases, or in 21.73 per cent of the trials. The alcoholic males always give a high percentage of mating failures even with normal females and, as this case shows, with females giving only a low per cent of failure by normal males.

The normal matings produced fifty-nine young, fifty-one of which survived while only eight, or 13.55 per cent, died within three months. This is an unusually low mortality record and proves the ability of these females to produce strong viable young. None of the offspring from the normal matings were defective.

The same females produced by alcoholic males fifty young, only thirty of which lived to maturity. Therefore, 40 per cent of them were non-viable, which is three times more than was the case with offspring from these females by normal fathers. Ten per cent of the fifty offspring were defective. The contrast between the two groups of results from the same females is so great that the possibility of the difference being due to the smallness of the numbers involved would seem to be completely eliminated. The records in the entire table are perfectly consistent and very clear cut.

It would seem only proper to interpret such results, along with the mass of evidence in the foregoing pages, as showing that alcoholic guinea-pigs, whether directly treated or descended from treated individuals, have had their ability to produce strong, viable offspring definitely and decidedly lowered. And it may be added in this connection that evidence from purely male treated lines as well as that given by later generations from the female treated and mixed lines, points directly to the fact that the germ cells have been affected. The effects of this modification are transmitted through several generations, only to be lessened by the elimination through death and sterility of the weakest individuals from the mating records and the constant introduction of more and more normal germ plasm into the line by matings with the normal stock.

14. THE CONTRASTED QUALITIES IN THE CONTROL AND THE ALCOHOLIC SERIES

The earlier reports on these experiments have given in the general text the various differences between the alcoholic and control lines; the case is made much clearer, however, if all the contrasted qualities be arranged together in summary fashion. In Pearl's recent report on the influence of alcohol inhalation on the progeny of the domestic fowl, he has given a concise arrangement of the differences between the records of the experimented and control lines. The several qualities he has compared such as mortality records, fertility, abnormalities, etc., are the same as those considered in our previous papers. We have here constructed a similar table to the one used by Pearl to show the qualities contrasted in the former sections of this paper. Definite numerical values have been presented for fourteen different qualities studied in the two groups of animals. Several of these qualities are closely related, such as weights after different periods of growth and the mortalities calculated at different periods, yet these are stated separately since they were measured in this manner and help somewhat to give a clearer analysis of the entire problem.

Table 10 shows the qualities measured. The first column of figures are the records from the control, the second column are the alcoholic records. In the last column a − sign indicates that the alcoholics are inferior to the control for the given quality; a zero, that the two groups are similar in the given respect, and a + sign would show that the alcoholics are superior to the control. It is seen at once that the alcoholic series suffers by comparison in every case except one, and in this case the two series are equal on account of an earlier unusually large difference.

The alcoholic guinea-pigs are less productive, giving litters of smaller size than the normal, their matings more often result in failure to conceive; associated with these two facts there is a higher early prenatal mortality which is the only quality included in the table that cannot be numerically expressed for reasons brought out in previous pages.

TABLE X.
QUALITIES CONTRASTED BETWEEN THE NORMAL AND ALCOHOLIC PROGENIES

Qualities measured	Normal	Alcoholic	Alc. Sup.+ Alc. Inj.—
1. Size of litter	2.77	2.47	—
2. Failure to conceive	4.45%	13.04%	—
3. Early prenatal death (size of litter, failure, etc.)	low	high	--
4. Proportion late prenatal death	51.92%	70.14%	—
5. Post-natal mortality	10.70%	10.60%	O
6. Total mortality	22.31% (100)	35.52% (189)	—
7. Abnormalities	O	2.52%	—
8. Oversize (+500 grs. at 3 mos.)	5.57%	2.86%	—
9. Undersize (- 300 grs. at 3 mos.)	0.42%	1.34%	—
10. Late generations alcoholic improved, mortality index	22.31%	F_1 42.40% to F_4 17.14%	—
11. Altered sex-ratios	109.60	9 ancestors 86.50	—
12. Av. birth wt of litter	197.12	170.00	—
13. Av. individual birth wt	77.16	70.35	—
14. Av. wt. 1 month old	228.64	213.94	—
15. Av. wt. 3 months old	425.11	404.13	—

The alcoholics have a higher proportion of their total mortality occurring very early, so that there is a great elimination of weak embryos and fetuses; this lowers their later or postnatal mortality to about the normal record. In this case we have an elimination or selection of individuals or zygotes rather than a germinal selection. The total mortality record for the experimented group is far higher than for the control and a greater percentage of abnormal young are produced. The percentage of abnormalities is lower than in our former records, as is also the total mortality rate. The improved mortality rate is partly due to better methods of breeding and caring for the animals. Yet the mortality record of the alcoholic group is very high, and when corrected for the normal rate on the basis of the size litters concerned it becomes 189 against the control as 100. Among the

normal animals of the same general stock as the alcoholics, not one grossly deformed individual has been born in over 400 cases, and, as stated above, this is a remarkable record which argues strongly for the perfection of the stock. In considering the defective young, one must also keep in mind the fact that these are not worse, but, on the contrary, are better organized than individuals which die during early stages of development.

At three months old, as No. 8 in the table indicates, fewer alcoholic than control animals were larger than usual or over size, though some were, while the next line shows that more alcoholic animals were small or under size, weighing less than 300 grams.

The later generations of the alcoholic stock are improved by the continued elimination of weak and defective individuals which die or are unable to breed, and also by the introduction of more and more normal germ plasm from generation to generation until a mortality rate of 42.4 per cent for the F_1 generation becomes only 17.14 per cent for the F_4 generation. This is a clear demonstration of the alcohol effect and may also serve to show the action of increased germ dosage. The earlier generations being nearer the directly treated animals receive higher doses than do the later generations where in most cases the dose has been considerably diluted by a mixture of normal germ plasm.

The sex-ratio in the alcoholic group seem to have been modified in ways which we have attempted to explain.

The average weight of the alcoholic litter is less than the normal and the average individual birth weight of an alcoholic specimen is also less than for the normal. The average weight of the alcoholic individuals at one month old is below the normal and the average weight at the age of three months, when guinea-pigs are about mature, is still below the weight of the control animals.

Therefore, in the fourteen measured points considered, the offspring of the alcoholic series are below the normal control in thirteen cases and apparently equal to the control in only one.

The qualities are largely the same as those we have considered

in former papers though analyzed in further detail. They are also very similar to those recorded by Pearl ('17) in his table 14. From a physiological standpoint it seems to us that these qualities are all closely associated and finally come down to the three related qualities: ability to develop normally, grow rapidly, and live to maturity. An animal possessing such qualities is usually termed a vigorous individual. At present it can only be stated that these properties are due to the vigor of the germ cells from which the individual arose. The qualities discussed might all involve a limited range of physiological factors so far as present knowledge permits a separation of such factors and they only show on the part of the alcoholics a reduced capacity of development and growth. The same underlying cause may actually account for the abnormal sex-ratios, as has been pointed out in an earlier section.

Leaving the environment out of account, the normal development, growth and length of life of a zygote varies with the perfection or vigor of the germ cells from which it originated. An experimental treatment may act upon the germ cells of an animal so as to modify them in some general way which lowers their ability to react normally in combination with germ cells from another individual. Thus zygotes are produced which tend to develop abnormally, grow slowly, or die during early stages of their existence, depending upon the degree of modification the treated germ cells have suffered. We are fully embarrassed by the unsatisfactory nature of such statements, but have been unable to gather scientific facts that would permit any more definite estimate of the situation.

All of our experiments on the modification of the germ cells have given results which express themselves in some such general fashion. Yet the germ plasm has been definitely modified and the subnormal condition is transmitted through a number of generations beyond the animals directly treated. This result is original on the complex material used, and is of primary importance, although it may be disappointing in that it has not shown a modification in the mode of behavior of some particular character known for its Mendelian inheritance.

The experimental modification of the inheritance of definite characters by a treatment of the germ cells is a future possibility. It must be recognized, however, that one is able to produce grotesque monsters by a treatment of eggs or spermatozoa, and yet all of the known characters which Mendelize in such an individual may be expressed in a perfectly normal fashion. This may be due to the fact that comparatively few such characters are known. Aside from the future definite modifications of inheritance, it would seem from the present study that the 'general qualities,' for lack of a more suitable term, of an organism may be affected, on account of an experimental modification of the germ plasm from which it arose. The modification may have taken place several ancestral generations ago. This is really the inheritance of pathological conditions which were induced upon and transmitted by the ancestral germ plasm. Such a type of inheritance is no doubt important in its relation to the normal processes of development and inheritance.

15. GENERAL CONSIDERATIONS

A discussion of the literature bearing on the influence of various chemical substances on the egg and spermatozoön has been given in former papers of this series, particularly Stockard ('12 and '13). In all cases only the effects of the treatments on the zygotes immediately resulting from the modified spermatozoa or eggs have been studied. There has been no experimental investigation of later generations arising from the affected specimens. And indeed, in almost all cases the developing individuals were lost during early embryonic stages as in the X-ray experiments of Bardeen and the radium studied of Oskar Hertwig which are the most satisfactory investigations on the direct injury of the sperm. These experiments really supplied no available material for an investigation of the inheritance or transmission of the induced defective conditions.

Since the beginning of the present experiments other studies have been recorded which bear more directly on the results considered in the foregoing pages. Of particular interest in connection with our supposed differential effects of the alcohol treatment on

the behavior of the X and Y groups of spermatozoa is the ingenious double-mating experiment of Cole and Davis (14) with rabbits. They found that when two male rabbits were mated with a single female, superfetation occurred in most cases, so that part of the resulting litter of young were sired by one male and part by the other. The males differed in their fertilizing abilities, so that one more often sired the majority of young of a given litter, and in the total number of competition matings he sired the greater number of young. This male with the fertilizing advantage was then treated for a month or more with the fumes of alcohol by the inhalation method. As a result of this treatment his spermatozoa became affected in such a way that mated in competition with the same male he normally had beaten he now failed to sire any young. Yet when mated singly or alone with a female he still possessed the power to beget offspring. This is a striking illustration of the debilitating effect of a short alcohol treatment on the physiological behavior of these spermatozoa, thus lowering their fertilizing ability below that of other spermatozoa which were formerly less potent than they.

When it is seen how definitely and readily alcohol treatments affect the behavior of the spermatozoa, we are led to speculate as to whether the treatment might not affect the X and Y groups of sperm differently, and thus be partially responsible for a distortion of the sex-ratios, should such occur. This responsibility may be due in the first place to a lowered fertilizing power on the part of one group of spermatozoa, thus giving rise to fewer individuals of one sex than of the other. Or, in the second place, even though both groups of spermatozoa should be equally capable of fertilizing the eggs, one group might be more affected as to its ability to produce viable zygotes in combination with normal ova, and thus an early differential sex mortality would occur causing a modification of the proportion of one sex to the other among the young born. We have elaborated somewhat on these possibilities in the section devoted to the sex-ratios of the alcoholic guinea-pigs.

Cole and Davis originally devised their experiment as a cru-

cial control for the influence of alcohol treatment on the male germ cells. In mating two males to a single female any defective condition that might arise among the offspring from one of the males, as compared with those from the other, could not be attributed to differences in developmental environment or in the qualities of the ova, as might possibly be the case where different females are used.

Cole and Bachhuber ('14) have employed the same method in a study of the effects of lead on the germ cells of the male rabbit and fowl. Their conclusion in regard to the rabbit is "that the offspring produced by male rabbits which have been poisoned by the ingestion of lead acetate into the alimentary tract have a lower vitality and are distinctly smaller in average size than normal offspring of unpoisoned males." This conclusion is in exact accord with the conditions shown by our F_1 generation of guinea-pigs sired by alcoholized fathers. Cole and Bachhuber have not reported on the transmission of the effects to later generations.

Their results with fowls "are interpreted as indicating that in fowls also poisoning of the male parent with lead results in offspring of a distinctly lower average vitality." This again accords with the results on the offspring when male guinea-pigs are treated with alcohol.

A later more extensive report concerning the influence of lead as a substance producing blastophthoric effects is given by Weller ('15). This investigator has treated both male and female guinea-pigs with commercial white lead. The lead is administered by mouth in gelatin capsules, the same method as was employed by Cole and Bachhuber ('14). The effects from the lead poisoning on the guinea-pigs are very similar to those obtained by treating the rabbits and fowls. Weller has been careful not to overdose the animals and his precautions would make it seem probable that any effect from the treatment which might be shown by the offspring was actually due to the lead poisoning and not to impaired nutrition or other indirect causes.

His conclusions are based on a total of ninety-three matings yielding 170 offspring. There were thirty-two control matings

which produced only fifty-eight offspring.   Whether or not every mating gave offspring is not definitely stated, but if so the average-size litter was unusually small, being only 1.81.   This would indicate either a stock of very low productivity or a high proportion of absorbed embryos and partial abortions, as a final result of which the litters would be small.   In the foregoing tables where the numbers of matings and young are very much greater, not one group shows so small an average litter.   From the thirty-four matings of lead-poisoned males with normal females, sixty-five offspring resulted, an average litter of 1.91, and from twenty-seven matings of normal males with lead females forty-seven young were born, an average litter of only 1.74.

The fact that among the few individual litters recorded there were three cases of litters of four, and five cases of litters of three, makes it seem as though there may have been a high proportion of mating failures, giving rise to the small average litters obtained when the total number of young is divided by the total number of matings.   The distribution and cause of these mating failures, as is pointed out in the text above, may be of considerable importance.

Weller has analyzed his results in some detail.   He takes into account the influence of litter size on the birth weight and gives several individual mating records which illustrate the effects of a treated sire on the birth weight of the young from a normal dam.

Weller has also taken into account the relationship between lead dosage and birth weight of the offspring without finding very consistent correlations.   The relationship between germ dosage and the condition of the offspring in our records may be calculated for every individual born in the alcohol experiments, yet the result is uninstructive so far as at present studied. There are a great number of confusing factors involved in this seemingly simple proposition.

Weller's final conclusions from the study of lead poisoning closely accord with our previous statements regarding the influence of alcohol on the same animals.   He finds that chronic lead poisoning in guinea-pigs produces a definite blastophthoric effect.

This can best be demonstrated upon the male germ plasm, in which case the blastophthoria manifests itself in some instances by sterility without loss of sexual activity, by a reduction of approximately 20 per cent in the average birth weight, by an increased number of deaths in the first week of life, and by a general retardation in development such that the offspring of a lead-poisoned male remains permanently under weight.

These experiments with alcohol and lead on rabbits, fowls and guinea-pigs seem to their authors to modify the male germ cells in a definite manner. The offspring sired by treated fathers are inferior to those from control males. The transmission of the defects to subsequent generations has not been reported.

In addition to the experiments on the direct treatment of the spermatozoa of lower forms, a few attempts have been made to treat the spermatozoa of higher animals directly with certain chemicals. Ivanov ('13) has given a short note on the effects of immersing the spermatozoa of several mammals in solutions of alcohol. He finds that when fertilization is obtained after such treatments a normal development follows and normal offspring are produced. To anyone who has studied the action of alcohol on the free swimming spermatozoa of lower vertebrates such results are not surprising. The most probable explanation is that the spermatozoön has been entirely protected from the action of the alcohol of the strengths used. When any action is obtained the usual effect on the spermatozoön is to render it immobile. To obtain a fertilization the motionless sperm must be activated by the use of some alkaline substance, such as NaOH. Following this activation the spermatozoa may often give normal offspring after union with normal ova, thus indicating that their chemical nature has not been disturbed. It is most difficult to treat the spermatozoön even of the very hardy fish, Fundulus heteroclitus, in such a manner as to injure it and afterwards obtain a fertilization. Dr. Wilson Gee ('16) experimented on the spermatozoa of fishes at Woods Hole for two seasons and found that the difference between an effective alcohol dose and a fatal dose was so slight that it required the most delicate adjustment of solutions in order to injure the spermatozoa to such a degree that the development of eggs subsequently

fertilized was rendered abnormal. Ivanov's report is certainly not sufficiently detailed to satisfy one that his results have any bearing on the problem of the modification of the germ cells by chemical treatment.

There can be no doubt that if a spermatozoön is actually affected by a direct chemical treatment, the egg which it fertilizes will develop more or less abnormally. The radium and X-ray experiments of Bardeen and Hertwig, as well as fertilization by foreign spermatozoa give conclusive evidence on this point.

The statistical research by Elderton and Pearson ('10) has frequently been quoted as if it shows that parental alcoholism was really to some degree beneficial to the human offspring. Their mathematical calculations were based on two series of statistics, the "Edinburgh Charity Organization Society Report and a manuscript account of the children in the special schools of Manchester provided us by Miss Mary Dendy." "Suspected drinkers were included with drinkers," "the parents could be divided into two classes only, those who are temperate and those who are intemperate," and many other such statements make this biological data somewhat unsatisfactory to those interested in an experimental modification of the germ plasm. These authors, however, do not claim to find any effect, either good or bad, of alcoholism on the offspring, and finally state that

On the whole the balance turns as often in favor of the alcoholic as of the non-alcoholic parentage. *It is needless to say that we do not attribute this to the alcohol, but to certain physical and possibly mental characters which appear to be associated with the tendency to alcohol.*[1]

Such a conclusion on the part of the authors themselves would scarcely warrant anyone else in claiming that an effect of alcoholism on the parent had given evidence of its existence in the quality of the children produced. A number of English physicians interested in alcoholism largely from a social and sentimental standpoint opened a bitter attack on the memoir by Elderton and Pearson, not because it claimed a beneficial effect,

[1] Italics are ours.

but merely because no harmful effect was shown. Such criticism is of little interest, yet one very serious point was cited against the data on which this study was based, and Pearson and Elderton ('10) in their reply failed to satisfy the objection. The children considered were in the neighborhood of nine years old at the time the statistics were collected and the fact that some parents were drinking at this time might not necessarily prove that they were drinking nine or ten years ago when the children were conceived. It is very evident that from our standpoint accurate data relating to this particular fact is most essential.

This study really has no bearing in the literature on the chemical modification of germ cells or the developing embryo, as Elderton and Pearson themselves state in the italicized portion of the quotation cited above. No one can confidently affirm that in their data alcoholics are being compared with normals or really whether any alcoholics or normals as such are actually being considered beyond the chance probability that some individuals of both classes creep into the statistics to be included in the two groups arranged.

Very recently Pearl ('17) has published a most thorough analysis of the influences of parental alcoholism on the progeny of the domestic fowl. He states (p. 285):

that a careful study of the present results makes it impossible to assert that the treatment of the parents has had no effect upon the progeny. . . . . The offspring of the alcoholists, as a class, are indubitably differentiated from the offspring of the non-alcoholists.

Such a statement agrees entirely with our results from the alcoholic guinea-pigs. In detail, however, Pearl finds that after treating fowls with alcohol the progeny produced are in some respects superior to the control. This, he believes, is brought about by an elimination of all weaker germ cells through the action of alcohol which thus serves as a selective agent to improve the race. At first sight this would seem to be entirely contradictory to our results, since the guinea-pig progeny is decidedly the worse for the experimental treatment. Yet the treatment in both cases has affected the progeny through its

action on the germ cells.   This is the point of actual importance
and the one of chief interest from the standpoint of these ex-
periments.   We are not here studying the alcohol problem from
a social standpoint and it is immaterial whether the progeny be
benefited or injured by the treatment of parental generations.
Our interest lies in whether or not the germ cells are modified
by the chemical treatment and whether the modification is of
such a nature as to alter the qualities of the individuals which
may compose the subsequent generations.

Pearl, of course, fully agrees with such a position, and states
('16 a, p. 258):

Our results seem to me to be supplementary to those of Stockard,
and to throw an interesting light on the need for caution in reac ing
a correct interpretation of all experiments in which a mildly deleterious
agent acts upon the organism.

He also believes that his results are in no way contradictory to
ours, but recognizes the fact that, although the same chemical
substance may act upon the germ plasm of two different classes
of animals, the visible response on the part of the animals need
not necessarily be the same.   In other words, one is not always
within the realm of legitimate scientific speculation who assumes
that since a given substance acts to induce a certain response
on the part of one animal species that the same substance will
call forth a like response on every other species.   "What is one
man's food is another man's poison."   With this we fully agree;
it is dangerous to draw universal deductions from experiments
on any one or two classes of animals.

Another possibility also recognized by Pearl presents itself in
considering the opposite effects of the alcohol treatment on the
progeny of guinea-pigs and fowls.   Small doses of many sub-
stances, one of which is alcohol, may form a physiological stand-
point produce a stimulating effect, while larger doses produce
decided depression.   There is a possibility that the same may
be true of the action of such substances on the germ cells.   Pearl
has discarded such an explanation after very fair consideration,
and is possibly right in so doing.   The experiences, however,
with the guinea-pigs makes our opinion decidedly prejudiced in

favor of the possibility, that although a sufficiently large dose may have been used, yet it did not act solely to eliminate germ cells as such, but also caused the production of many zygotes which died during early developmental stages.

The amount of dosage is very important. Treating female guinea-pigs with considerable doses of alcohol fumes only shortly before and during their pregnancies certainly does not injure the offspring to any noticeable degree. While the same dose of treatment, if administered for a number of months or years, will render these mothers almost incapable of producing vigorous young, even when mated with normal males.

Pearl ('17, p. 281) finds regarding his 1915 results which were obtained after the treatments had been running for only a few months that considering the number of animals in the experimental series the individual differences are not in every case sufficiently large to be significant in comparison with their probable errors. The control in this case was also not what Pearl had wished. He had originally chosen a carefully pedigreed control, taking as the one control male a half-brother of the three experimental males and using control females that were sisters of the treated hens as recorded in table 5, p. 158 ('17). The only control male, No. 666, proved to be practically sterile and useless. This necessitated the use in paper No. III of an ordinary random sample control instead of the refined control originally planned in Part I of the series of papers, and nullified the statement in the summary of Part I, p. 162, that "Full brothers and sisters of treated are used as control."

For certain qualities, such as the fertility and hatching records of the eggs, the control was not in all cases the same cross as the experiment, which was invariably between Barred Plymouth Rock hens and Black Hamburg cocks. The hatching weight and rate of growth of the experimental chicks on account of want of control data from the 1915 season were compared with chicks from a similar cross hatched and reared in 1913. Different keepers were in charge of rearing the chicks during the two different seasons. These unfortunate conditions, all of which are pointed out with conscientious fullness by Pearl, make it rather difficult

to fully estimate the actual significance of the differences between the experimental offspring and the control groups used.

Fortunately, however, the data from the 1916 season is available (Pearl, '16 b) for comparison with the 1915 results. The alcohol treatments were continued throughout the time so that the 1916 chicks are derived from more highly alcoholized parents. Should the alcohol continue to improve the race by "completely putting out of commission all of the weaker germ cells," the 1916 results should in all respects show a further improvement in the qualities that had been previously benefited.

The percentage of infertile eggs given in the 1915 table may be reversed to per cent of zygotes formed and compared with this column in the 1916 table. The percentage of zygotes formed in the several combinations of alcoholic mating should be less than in 1915, and they are. When both parents were alcoholic in 1915, 40.8 per cent of the eggs formed zygotes, while in 1916 only 21.95 per cent produced zygotes; sire only alcoholic, 74.8 per cent zygotes in 1915 and only 53.52 per cent in 1916. This is in line with the lowered fertility and increased number of mating failures from the alcoholic guinea-pig records. The more decidedly alcoholic the guinea-pigs become, the smaller the litter size from double alcoholic and sire only alcoholic matings, and the greater the number of failures to conceive.

With the guinea-pigs, however, this is not alone due to a destruction of weak germ cells by the treatment, but is certainly in part due to an increased very early prenatal mortality for which much evidence is given in the body of the present paper. The smaller number of zygotes formed by the treated fowls is probably also due in some cases to death in very early stages, as blastulae or gastrulae, before the egg is laid; or in the hen's eggs these weakened zygotes may not be able to withstand the developmental interruption following the laying of the egg. Embryos dying during such stages could not be identified except by a most minute study.

It seems to us in keeping with what is known of biological reactions in general and the guinea-pig histories in particular to take the following position. The alcohol treatment acts on the

germ cell populations of both fowls and guinea-pigs in such a manner that the weakest or least resistant ova and spermatozoa die from the effects of the treatment as germ cells without taking part in zygote formation. The somewhat more resistant ova and spermatozoa are greatly injured though still capable of forming zygotes. The zygotes, however, are so defective as to be capable of only a short period of development and die during stages too early to be definitely detected by gross examinations of either the fowl's egg or the mammalian mother. Still other embryos are capable of development to later stages and are actually found dead, not only as the youngest embryos to be identified, but from these early stages there occurs a continuous series of prenatal deaths up to the full-term still-births. Immediately after birth the postnatal mortality is greatest and gradually decreases until these specimens capable of reaching maturity may often enjoy a comparatively long life.

At the present stage of the two experiments it would seem as though this elimination of defective germ cells and very early embryos was much more intense in the fowls than in the guinea-pigs as a group; so that the late prenatal and postnatal mortality among the fowl progeny was low and those specimens that hatched were the hardy survivors from this early vigorous process of germ cell and individual selection. The records from the double alcoholic and male treated lines among the guinea-pigs forms a second step. The size of the litters and failures to conceive in these lines indicates a rather high degree of infertility or germ cell debility as well as early prenatal deaths, though this is not so extreme as among the fowls, and the late prenatal and postnatal mortality is higher.

Finally the female treated guinea-pig lines produce large litters and have few infertile matings, indicating a low germ cell and early prenatal mortality, and here the late prenatal and postnatal mortality is highest, not entirely on account of the action of the treatment on the developing individual in utero, since the same condition is found among other female generations than the one directly treated.

This presentation of the situation is somewhat similar to that

which Pearl ('17) has illustrated in his diagrams, figures 5 to 7, pages 290 and 291. The chief difference being that we would decrease the proportion of eliminated germ cells and increase the proportion of defective and non-viable zygotes, and thus emphasize the selection of individuals rather than of germ cells.

A further consideration of Pearl's 1916 results as shown in table 1, p. 676 ('16 b), may be used to argue in favor of our position. The 'prenatal mortality' column of this table when compared with the 'dried in shell' column from 1915 records (table 1, p. 244, '17) should show lower percentages according to our interpretation of Pearl's expectation for an improved stock from the alcoholic lines. Instead of this, in only one combination is the prenatal mortality lower. In both parents alcoholic it has been lowered from 26.9 per cent to 11.11 per cent, and here the postnatal mortality as we would expect is increased. In the other cases dam only alcoholic, none of which were reported for 1915 on account of the useless control male, gives 80 per cent prenatal mortality sire only alcoholic increased to 47.08 per cent from 36.6 per cent; sire and one grandparent, 46.84 per cent; one or more grandparents, 46.02 per cent; all alcoholic ancestry, 45.95 per cent, which is a considerable increase over the 1915 records. The control of 1916 also shows a higher prenatal mortality than that of 1915, though it is not stated whether the same breed crosses are used in the two controls.

The postnatal mortality of the 1916 control is, on the contrary, lower than the postnatal mortality of the twenty-two 'random sample matings' of 1915.

While the total mortality for all the alcoholic groups is about the same, 17.6 and 16.5 per cent, for the two seasons, the individual combinations show wide variations. From both parents alcoholic the 1915 postnatal mortality was 10.6 per cent, while for 1916 it rose to 25 per cent, sire only alcoholic fell from 21.1 per cent, 1915 record, to 13.79 per cent, 1916 record. Sire and one grandparent alcoholic gave a postnatal mortality of 28.38 per cent, while the non-alcoholic postnatal mortality was 21.2 per cent.

Considering the numbers involved, the records from the prog-

eny of the 1916 matings after longer alcohol treatment do not seem altogether improved as compared with the 1915 records. A comparison of individual lines in the tables frequently show disadvantages for the 1916 matings. This would seem as though some injured zygotes were present and all of the affected germ cells had not been completely eliminated by the treatment. The percentage of abnormal specimens among the 1916 alcoholics is about the same or slightly more than among the control, while Pearl had counted this point in favor of the alcoholics from his 1915 records.

It would thus seem, as Pearl ('17, 292) himself suggests, that "it might be supposed that with larger administration to the fowls (higher germ dosage) or more years of drinking behind them in the case of Elderton and Pearson's workingmen, the conditions shown in figure 7 would gradually pass over into those shown in figure 5." That is, that not only weak germ cells would be eliminated by the treatment, but that also a considerable proportion of defective individuals would arise to be eliminated during various developmental stages or persist as degenerate specimens. From these conditions we believe that there is a really close agreement between the results on the fowls and the guinea-pigs.

These suggestions are advanced only in a spirit of the most friendly criticism. We have worked long enough in accumulating and considering evidence bearing on the various phases involved in this problem to highly appreciate the masterly manner in which Pearl has considered and analyzed his data; and we are thankful for many suggestions that have come to us through the contribution on parental alcoholism in the fowls. In the end our aims and objects are the same, to affect the germ plasm in so definite a manner as to be able to predict the quality and degree of the modifications subsequently expressed in the generations to follow.

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THE DEVELOPMENT OF THE IDIOSOME IN THE GERM-CELLS OF THE MALE GUINEA-PIG

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TWELVE FIGURES

1. INTRODUCTION

The idiosome was first described by la Valette St. George more than fifty years ago, and since that time a number of investigators have studied the behavior of this structure during the spermatogenesis of many different animals. The various descriptions are by no means consistent, and in the mammals the idiosome has been reported both as a very simple and as a very complex body. This peculiar structure within the cytoplasm of the cells during the stages of spermatogenesis is extremely sensitive in its response to the many different ways of fixation and staining. The observations considered in the present paper have been made possible through the application of a new staining method, which seems to possess particular advantages for the study of the finer structure of the idiosome. These

37

observations, we believe, add important details to our knowledge of the idiosome structure and the genesis of certain parts of the spermatozoön.

The following pages describe the method in full and attempt to give a concise description of the idiosome and its developmental changes during the spermatogenesis of the guinea-pig. A brief consideration of the literature is also presented in order to place the new observations in their proper relation with previous ideas concerning this structure.

2. GENERAL METHOD AND MATERIAL

The general method consists of a combination stain with methylen blue and acid fuchsin following a fixation in Zenker's fluid. The fixation with Zenker's fluid is necessary in order to obtain a good differential stain for the idiosome. Other fixing fluids, corrosive acetic, Flemming's fluid, Hermann's fluid, and Bouin's fluid, used for the same study have not been satisfactory, since the tissues fixed in them fail to stain in a clear differential fashion.

The studies have been made on the testes of guinea-pigs, from both normal and alcoholic stock. The age of the animals ranged from six months to four years. The living animals were castrated and the testes from both sides were cut in thin, longitudinal pieces and fixed in Zenker's solution for eighteen to twenty-four hours. The pieces were imbedded in paraffin and cut in sections 3 to 5 micra thick. The details of the staining will be described after considering the parts of the cells which are to be best demonstrated with different modifications of the method.

3. DESCRIPTION OF THE DEVELOPMENT OF THE IDIOSOME

A. *The idiosome in the spermatogonia*

The term idiozom, as proposed by Meves ('99), from ἴδιος (own) and ζῶμα (belt), should be better changed to idiosome, from ἴδιος (own) and σῶμα (body), as suggested by Regaud ('10), as will be evident from the descriptions of this structure which follow.

The idiosome in the spermatogonia shows many variations in form. Its shape may be spherical, or oval and flattened and cap-shaped. Its boundary is usually clear and distinct, but sometimes seems to pass insensibly into the surrounding cytoplasm. In some cells the idiosome presents a vacuolar structure, while in others there is a distinct differentiation into a peripheral and a central part. It is possible that the idiosome in the spermatogonia may be divided into two zones, as is the case with the idiosome of the first spermatocytes described in the next section. This point may be decided by future studies. The present paper only attempts to give a description of the idiosome, beginning with the primary spermatocyte stage and passing through its subsequent phases.

B. *The idiosome in the primary spermatocytes*

The idiosome of the primary spermatocyte is differentiated into two distinct parts, a peripheral, which may be termed the idioectosome, and a central, the idioendosome.[1] The relationship of the two parts is that of one slightly oval body being enclosed within another. The endosome lies within a central cavity of the ectosome and is connected with the ectosome by a number of processes or prolongations from the periphery of the endosomatic mass. As these processes pass into the substance of the ectosome, a vacuolar aspect is produced, as shown in figure 1. The position of the endosome within the ectosome is generally a little eccentric, being nearer the side towards the spermatocyte nucleus. The idioendosome is the more essential part of the idiosome persisting through all stages of development, while the idioectosome is later to be eliminated and dissolves or disappears in the cytoplasm.

During the preparation for division of the primary spermatocytes, the idioendosome exhibits a peculiar transformation. Its periphery breaks up into a number of at first large and then

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[1] These terms and others which follow are proposed by us not merely to burden the present confused terminology, but on account of an actual lack of technical words to sufficiently or exactly designate the structural details presented by the idiosome during its several developmental phases.

smaller granules, as shown in figure 2. These we name the idio-granulomes. As they form the granulomes lose connection with one another while still lying within the idioectosome. Such an arrangement is to be seen during the prophase of the primary spermatocyte division. As the process of division progresses, the idioectosome loses its regular spherical or oval form to become irregular in shape and begins to break up. The pieces scatter in the protoplasm where they lose their identity. The idiogranulomes, after the breaking or disintegration of the idioectosome, are now set free in the protoplasm and become slowly dispersed throughout its substance. In this way the old idiosome is destroyed and its constituent elements, viz., small pieces of ectosome substance and idiogranulomes derived from the transformed idioendosome, are scattered throughout the cytoplasm.

The staining reaction of the ectosome remains the same during all these phases, whereas the endosome changes its color reaction with its structural transformation. The idioendosome, as such, shows a violet color after the combined fuchsin-methylen blue stain, while its derivatives, the idiogranulomes, have a greater affinity for the red acid fuchsin stain, thus presenting a dark red color. In the beginning the idioendosome, therefore, resembles the idioectosome in its color reaction.

After the end of the primary spermatocyte division, a new idiosome is reconstructed in each secondary spermatocyte. The idiogranulomes, which were dispersed in the protoplasm, migrate toward a place near the nucleus and close to the side of the old spindle remnant.

Around these idiogranulomes a new idioectosomatic substance is slowly collected. This substance shows the same staining reactions as the old ectosome, and is possibly the same substance being reformed or reconstructed. It seems improbable, yet another possibility is, that the new idioectosomatic substance is formed by the idiogranulomes.

The concentration of new idiogranulomes and new idioecto-somatic substance progresses until a new idiosome is formed, having very probably been built from about half of the material

of the old one in the primary spermatocyte.   All of these stages
are the same as those illustrated in figures 4 and 5, which repre-
sent the corresponding phases in the division of the secondary
spermatocytes.

C. *The karyogranulomes*

From the earliest stage of the spermatogonia down to the
latest stage of the spermatids, the nuclei of the germ cells con-
tain a number of granules which may be designated karyogranu-
lomes.   These granules are distinguished very clearly from all
other constituents of the nucleus and they show the same stain-
ing reaction and the same general structure as the idiogranulomes
(figs. 1 to 11).   The karyogranulomes seem to be independent
of the chromatic substance in the nucleus and are the only ele-
ments of the nucleus which show the red acid fuchsin reaction
just as the idiogranulomes are the only elements outside of the
nucleus which exhibit the same staining reaction.   All other
parts show a bluish or violet reaction.

Are these karyogranulomes of the same origin as the idio-
granulomes?   Or, is there any relation between these two kinds
of granulomes?   These questions cannot be answered in a defi-
nite way.   That karyogranulomes come out through the nuclear
membrane and go over to the idiosome or vice versa is very im-
probable.   It is possible, however, that, during the process of
division when the limits of the nucleus are broken down, some
of the idiogranulomes may pass into the nucleus and some karyo-
granulomes may escape into the cytoplasm and later be incor-
porated by the idiosome.   The karyogranulomes during the
division process may be seen among the spindle fibrils or
on the chromosomes, while the idiogranulomes are dispersed
throughout the protoplasm, see figure 4.   Since no obstruction
exists to prevent the mixture of the two kinds of granulomes
during such a stage, it is very possible that some of the idio-
granulomes may pass into the spindle and be later brought
into the nucleus, or the opposite may occur and karyogranu-
lomes may be detached from the spindle and left behind in the
protoplasm when the division is over.   Such a migration is al-

most impossible to prove positively, as both kinds of granulomes show the same staining reaction and have the same structural appearance.

The number and size of the karyogranulomes seem to differ in the different stages of the developing germ cells. They are greater in number and smaller in size in the stages represented by figures 3 to 6, during which stages the idiogranulomes are also small in size and very numerous. In the later stages, figures 8, 9, and 10, the karyogranulomes are generally less numerous and of larger size. This increase in size and decrease in number is probably the result of a fusion similar to that shown by the idiogranulomes in figures 7 and 8, where all of them have somehow run together to form a large spherical body.

It is thus seen that karyo- and idiogranulomes show many analogies during the different stages of their development. The finest granulation prevails in both karyo- and idiogranulomes during the stages illustrated by figures 3, 4, 5, and 6. This is what would be expected if one should suppose that the fine granulation represents a process to secure a distribution of the granulome material in the protoplasm and the nucleus during every division, as will be discussed beyond.

The similar ways in which both kinds of granulomes react during the same stages strongly suggests some genetic relationship between them. Indeed it is probable that both sets of granules are the same things only located in different places.

The karyogranulomes persist through all stages of the development of the germ cells as can be seen in the figures. In the ripe spermatozoa, however, they seem to be dissolved, as is the chromatic substance to disappear in the head of the spermatozoön.

It is also of importance to note that karyogranulomes may be occasionally seen in the nuclei of the Sertoli cells.

D. The idiosome of the secondary spermatocytes

The idiosome of the secondary spermatocytes, illustrated in figure 3, is a perfectly reconstructed, large, spherical, or slightly oval body, consisting of an idioectosomatic substance having the

same color reaction as the idioectosome of the primary spermato-
cytes and being filled with a great number of idiogranulomes. It
seems that all idiosomes of the secondary spermatocytes have this
granular appearance. The idiogranulomes have a tendency to
be concentrated into one group of more or less circular outline,
as if they were preparing to form a central sphere similar to the
idioendosome of the primary spermatocytes. The idiosome in
the secondary spermatocytes probably shows this constant
granular type since the next division follows so quickly, little
time being allowed for the idiogranulomes of this stage to fuse
together as they do in all other more permanent stages.

During the division of the secondary spermatocytes, the idio-
some undergoes the same changes as the idiosome of the primary
spermatocytes. These changes are illustrated in figures 4 and
5. The idioectosome becomes irregular and begins to break
into small pieces, while the idiogranulomes are dispersed in the
cytoplasm. After the division a new idiosome is formed in the
same way as was described during the corresponding stage fol-
lowing the first spermatocyte division. The idiogranulomes
flow together and a new idioectosome is slowly formed around
them. During the reconstruction of the nucleus in the sperma-
tids the number of the idiogranulomes increases and the idio-
ectosome gradually becomes larger, assuming a regular spherical
shape. In this manner the new spermatid idiosome is formed.

In the nuclei of the secondary spermatocytes the number of
karyogranulomes is large, corresponding to the great number of
idiogranulomes. During the division of the secondary sperma-
tocytes, these karyogranulomes are to be seen on the spindle
fibrils or on the chromosomes, as illustrated in figure 4.

E. The idiosome of the spermatids

The idiosome of the spermatids presents, during its early
formation, a type similar to that of the idiosome in the secondary
spermatocytes, there being a great number of small granules
enclosed in a large idioectosome (fig. 6). At the same time the
nucleus contains a comparatively large number of karyogranu-
lomes.

This granular stage is of short duration in the spermatids, since the process of granular fusion begins very quickly after the reconstruction of the idiosome is completed. The small idiogranulomes fuse with one another to form first a smaller number of larger granulomes as shown in figure 7. This fusion process continues until ultimately a single large spherical body is produced, which we have termed the idiosphaerosome. And it, like the granulomes from which it arose, exhibits a very intensive acid fuchsin reaction, as illustrated in figure 8.

The idiogranulomes of the spermatids differ from those of the secondary spermatocytes in that each is contained within a distinct small vacuole, the idiogranulotheca, the origin of which is very difficult to decide. It is possible that these vacuoles are formed by the idioectosome, but it is more probable that they are produced by the idiogranulomes themselves. These idiogranulothecae flow together when the idiogranulomes fuse, forming larger vacuoles around the larger granules, until finally a single large vacuole that may be designated the idiosphaerotheca surrounds the final idiosphaerosome; the steps in this process are seen in figures 6, 7, and 8.

At times the idiosphaerosome or some of the larger idiogranulomes are connected with the wall or surface membrane of the idiosphaerotheca or of the idiogranulothecae, as the case may be, by one or more processes or prolongations. These prolongations extend in different directions, sometimes towards the nucleus and sometimes away from it (figs. 7 and 8). The idioectosome in this stage is concentrated more and more on the upper periphery of the idiosphaerotheca, as shown in figure 8.

The idiosphaerosome is a very changeable body. As soon as it arises it gives off a substance from its periphery mainly on the superior surface, which seems to have a different structure and different chemical qualities. This substance is distinctly vacuolar and its color reaction with the combined acid-fuchsin and methylen-blue stain is blue, thus presenting a striking contrast to the red color of the idiosphaerosome and the violet of the idioectosome.

In this way the idiosphaerosome becomes differentiated into two distinct parts; one, an idiocryptosome, being more or less spherical in form, lies very close to the cell nucleus, while the other, the idiocalyptosome of our terminology, rests in the form of a cap over the idiocryptosome on the side away from the nucleus, as shown by figure 9. Both of these bodies, derivatives of the idiosphaerosome, are surrounded by the idiosphaerotheca.

The idioectosome which, during the idiosphaerosome stage over-capped the idiosphaerotheca, begins now to assume a more concen-trated cap-like form and at the same time moves along the wall of the idiosphaerotheca, which it finally leaves to migrate along the surface of the nucleus to its posterior pole. This body is to be finally eliminated, and it perishes with the remains of the pro-toplasm during the metamorphosis of the spermatid as a sepa-rate spherical body, the idiophthartosome, shown in figure 10, *id.phth.*

During all these changes the karyogranulomes are to be seen in the nucleus, but apparently in smaller number and of a somewhat larger size than in earlier stages, as shown by figures 8 and 9. These karyogranulomes are most frequently found in close proximity to the nucleolus, but may also be seen in other places.

In a later phase the idiocryptosome fits down closely upon the cell nucleus, and in so doing loses its spherical form to be-come somewhat discoidal or cap-shaped. The idiocalyptosome continues to increase in size, probably through some kind of con-stant reaction, and finally becomes a large body completely covering the cryptosome and a greater part of the nucleus, as is shown in figure 10. Its structure remains vacuolar. Some-times small pieces become detached from the cryptosome, crypto-granulomes, and are to be seen in the substance of the calyp-tosome, figure 10. The idiophthartosome continues to move towards the posterior end of the nucleus, as figure 10 also shows.

At this stage the karyogranulomes are generally very large and few in number. Exceptionally, however, they are small in size and more numerous. Some of them are to be seen in that portion of the nucleus immediately beneath the cryptosome,

while others are nearer the posterior pole, as shown in figure 10. In some cases where great numbers of cryptogranulomes are present, as will be described later, the karyogranulomes which lie in that portion of the nucleus covered by the calyptosome may easily be confused with the cryptogranulomes contained within the substance of the calyptosome itself, since they are superimposed. At other times the karyogranulomes come in such close apparent relation to the cryptosome that they seem to fuse with this body. It is highly improbable, however, that any fusion of the karyogranulomes with the cryptosome or any migration of these granulomes into the calyptosome through the wall of the nucleus ever takes place. The karyogranulomes later seem to dissolve in a fashion similar to the dissolution of the chromatic substance and are probably contained within the head of the spermatozoön in this dissolved condition.

In its later development the calyptosome gradually attains an elongate shape until it forms a long cone which comes into contact with the prolongation from a Sertoli cell. At the same time it becomes more and more homogeneous, losing its original vacuolar condition.

The cryptosome follows this change in shape of the calyptosome and forms a smaller cone enclosed within the conical calyptosome, while its wide base rests upon the nuclear membrane as illustrated in figure 11. At this stage the body of the cryptosome presents an irregular, granular structure (fig. 11). The idiophthartosome is now separated from the wall of the nucleus and passes into the cytoplasm with which it later disappears.

As mentioned above, the calyptosome often contains within its mass small cryptogranulomes. This is probably due to a tendency on the part of the cryptosome to again break up into smaller granules. Such a tendency is not very strongly expressed in some animals, as, for example, the one which is taken as a type for the main description. Yet in other animals this tendency may be so strong that the cryptosome is broken up into a great number of cryptogranulomes, as shown in figures 11a to 11c and 12a to 12d. All of these figures represent different degrees of cryptogranulosis observed in one and the same animal.

This breaking up into granules begins very early, even during the formation of the calyptosome. When such is the case the calyptosome is, throughout its development, being filled with small cryptogranulomes, while a relatively small central cryptosome is left behind. In rarer cases this granulation begins even earlier at the stage when the idiosphaerosome is still present. The idiosphaerosome then consists of a number of granulomes which lie in a substance of semifluid appearance and are enclosed within the idiosphaerotheca.

As mentioned above, this breaking-up process is only slightly expressed in some animals, while in others it is very prominent. Thus we may distinguish two different types of development, a massive, as in figures 11 and 12, and a granular type, as in figures 11a to 11c and 12a to 12d. Of the ten animals examined in this study, seven show the massive type and only three the granular. Of these three, two were treated with alcohol, one for four years and the other for three years, while the third was a normal but inbred animal (Stockard and Papanicolaou, '16). This merely suggests a possibility, and from present data it is only a possibility, that the granular type may represent a disturbance of the normal type caused by the influence of some injurious factors, such as the alcohol treatment or inbreeding are found to be. It may be, however, that this deviation from the usual type is a normal variation due to some as yet unknown cause, and we have recently found a normal animal showing the granular type.

During still later stages of development, the calyptosome loses its elongate shape and becomes more flattened, forming a cap over the upper part of the nucleus, which now appears almost homogeneous and is soon to form the head of the spermatozoön (fig. 12). The cryptosome, which is enclosed beneath the calyptosome, shows a tendency to form a unique homogeneous body.

In the massive type the cryptosome changes its shape to form a smaller cap lying beneath the calyptosome cap, as seen in figure 12. Small granules on its surface soon disappear, and during the development of the spermatozoön all granular struc-

ture is lost and the cryptosome again presents an homogeneous appearance, as figure 13 will show.

In the granular type of cryptosome the granules finally come to lie in a group at the base of the cap-like calyptosome and here fuse together, forming a body of the same conformation as in the massive type (figs. 12a to 12d).

The heads of the ripe spermatozoa are thus covered by two caps, an inner, the cryptosome cap, and an outer or superior, the calyptosome cap. Without a special stain these two caps give the appearance of a single body, the spermiocalyptra. However, with the staining methods to be explained in a following section, it is possible to differentiate the two parts of the calyptra; one as an intense red cryptosome cap and the other as a decidedly blue calyptosome cap, as the figures illustrate.

The spermiocalyptra is covered by a theca or membrane, the spermiocalyptrotheca, which is directly formed by the development of the idiosphaerotheca. This theca continues to exist through all stages of the transformation of the spermatids and becomes very large in size, covering the entire calyptra and a great part of the head of the spermatozoön (fig. 13).

F. The relation of the centrosomes to the idiosome

Since the special methods used in this study of the idiosome do not stain the centrosomes, we have tried to study their evolution and especially their connection, if any, with the idiosome, by staining a number of the specimens with iron haematoxylin. The only stage during which the centrosomes are connected with the idiosome is that of the primary spermatocytes. In all primary spermatocytes, at the stage illustrated by figure 1, the center of the idiosome is occupied by two dumb-bell-shaped centrosomes, as described by Meves ('99). We have never observed more than two centrosomes in one idiosome. As the centrosome stain does not furnish a clear differentiation between the ectosome and endosome, it is difficult to decide whether or not the two centrosomes are confined within the endosome sphere. In most of the cases, however, the two centrosomes appeared

to be enclosed within the endosome cavity, being usually in contact with its wall.

When the endosome breaks up to form the idiogranulomes, the stage shown by figure 2, the centrosomes begin to migrate toward the cell nucleus, passing through the ectosomatic area and leaving the idiosome to perform their active rôle during the division process of the primary spermatocytes. This behavior of the centrosomes and their later changes are described in detail by Meves ('99), and our own observations agree very closely with his descriptions.

The facts of particular interest in the present consideration are, first, that the centrosomes, on account of their specific staining reactions and their peculiar elongate slightly dumb-bell shape, should not under any circumstances be confused with the idiogranulomes; second, in no stage later than the primary spermatocytes do the centrosomes show any connection with the idiosome. This temporary connection or association between the idiosome and the centrosomes and their later completely independent and different activities throughout the process of spermatogenesis, along with their different staining reactions, suggest that the idiosome and the centrosomes, as well as their derivatives, are bodies of different natures with only early temporary topographical connections. Niessing ('96) has undoubtedly confused the idiogranulomes with the centrosome, and this is probably the reason he sometimes finds more than two centrosomes. It also seems evident from his figures that what he has designated as a 'Verklumpungsfigur der Centralkörpergruppe' has nothing to do with the centrosomes, but is the endosome in process of transformation or granulation to form the idiogranulomes. Meves ('99) has also criticized this point in Niessing's work.

4. THE SPECIAL STAINING METHODS

The manner of application of the fuchsin-methylen blue staining method differs for the examination of the different parts of the idiosome in the various stages of its development.

For the study of the idiogranulomes and of the karyogranu-
lomes a satisfactory method is a single stain with acid fuchsin as
follows: *Method 'A.'* Bring the sections through xylol and alco-
hol into water, cover for a few seconds with Lugol's solution, and
then wash in water until the yellow color begins to fade out;
then place in a saturated aqueous solution of acid-fuchsin for
one-half to one minute, after which bring through the alcohols
to carbo-xylol and mount in Canada balsam.

With this method most of the cell structures stain a very light
rosy tint, while the idio- and karyogranulomes have a decidedly
dark red color. The idiosphaerosome and the cryptosome are
also dark red, while the idioectosome and the calyptosome have
the much lighter rose tone. The chromatin stains very lightly.
When the chromatin does take on a dark color it indicates
that the fixation is not very good. A good fixation with Zenker's
fluid allows only the idioplasmatic substances to stain deeply
with the above method.

Method 'B.' After the staining with acid-fuchsin, as in method
'A,' bring the sections into 80 per cent alcohol and then for a
few seconds, ten to twenty, place in a saturated solution of
methylen blue in 80 per cent alcohol. With this treatment the
calyptosome takes on a blue color, while the cryptosome main-
tains its dark red acid-fuchsin reaction. The karyogranulomes
are not so prominent after this stain.

Method 'C.' Bring the slide through xylol, absolute, 95 per
cent and 80 per cent alcohol into a saturated solution of methylen
blue in 80 per cent alcohol for several minutes. Then pass
through the alcohols to water and leave for a few minutes in a
2 per cent solution of iron-alum until a pronounced bluish tone
is obtained. Wash thoroughly in running water until the blue
tone begins to disappear and cover for a few seconds with Lugol's
solution. After this, place again in running water and leave for
a few minutes, until the yellow color begins to fade; then stain in
acid-fuchsin for from one to two minutes, and from this pass
through the alcohols to carbo-xylol and mount in Canada
balsam.

With this method the granulomes, the idiosphaerosome, and the cryptosome are stained intensely dark red, especially the karyogranulomes and the cryptosome, which·take a very dark color, almost black. The calyptosome is light red and transparent, showing distinctly within it the granulomes and the cryptosome. The endosome and the ectosome of the primary spermatocytes are well differentiated and have a pronounced violet color. The ectosome of the spermatids and the phthartosome are also violet. The chromatin has a light bluish or violet reaction, contrasting strongly with the dark red karyogranulomes.

If a heavier chromatin stain be desired, the slides are left for a longer time in the methylen blue and less washed out in running water. In this way a blue chromatin stain may be obtained. The chromatoid Nebenkörper is also stained blue. The granulomes, however, are no longer so prominent and all other parts take a dark, heavier color.

Method 'D.' This method is the same as 'C' with the difference that after the fuchsin stain the slides are brought into 80 per cent alcohol and then to the methylen blue for a very short time, only a few seconds, and from this to carbo-xylol and mounted in Canada balsam. This method, like method 'B,' gives a good contrast between the cryptosome, which becomes dark red, and the calyptosome, which becomes blue. The granulosomes show the same color as the cryptosome, but sometimes are not so prominent.

The two parts of the calyptra are usually clearly differentiated by this method, as also by method 'B.' In the stages of the conical prolongation of the calyptosome, however, when the cryptosome and the cryptogranulomes are enclosed within the calyptosome, it is sometime difficult to see both parts clearly, since the calyptosome becomes very dark. For a better differentiation in this stage it is advisable to stain previously a shorter time in methylen blue, only a few seconds, or to use pure xylol instead of carbo-xylol for clearing the sections. All of these methods require a good fixation with Zenker's fluid. If the fixation is not good, the different parts are not so nicely differentiated and one obtains a darker, rather diffuse stain.

The history of the idiosome begins with the studies of la Valette St. George ('65 '67), to whom is attributed the first description of this body. La Valette St. George gave a simple description of the idiosome (Nebenkern) without going into the details of its transformations during the process of spermatogenesis.

Merkel ('74) gave a description of the idiosphaerosome, Spitzenkörper, which he described as being formed through a partial condensation of the idiosome. The spermiocalyptrotheca, Kopfkappe, derived as described in the foregoing text from the idiosphaerotheca, was described by Merkel as being formed through a transformation of the nuclear membrane.

V. Brunn ('76) regarded the idiosphaerosome, Spitzenkörper, as formed within the nucleus, probably from the nucleolus. The Kopfkappe was regarded by him as formed by the cytoplasm, and he believed it to be a temporary or perishable structure.

Renson ('82) also described the idiosphaerosome, bouton terminal, in rats and rabbits as a product of the nucleus. He described the formation and the disappearance of the idiophthartosome, corpuscule accessoire. The spermiocalyptrotheca was described by him also as formed from the separated nuclear membrane and was regarded as a transient structure.

H. H. Brown ('85) gave a description of the idiosome in rats after the spermatogonial stage. He records also the changes of the idiosome, accessory corpuscle, during the division of the spermatocytes. Brown observed that the idiosome during the division of the cells broke into pieces and disappeared, and that a new idiosome was formed in the spermatids, probably from the remains of the old sphere. He also described the formation and the fate of the phthartosome, lunula stage, and the formation of the spermiocalyptra or cap from the idiosome.

Hermann ('89) gave a clearer and more detailed description of the idiosome, Nebenkern, in the spermatocytes. He described it as being an uncolored oval body with another smaller body colored with gentian violet closely attached to one of its poles. It

is very probable that this smaller body is nothing else than the
chromatoid Nebenkörper. This body, which gives the same
staining reaction as the nucleolus, is often in very close connection
with the idiosome. Hermann believed that the idiosphaerosome
was derived from the nucleus.

Benda ('91 '92) gave a detailed description of the develop-
ment of the idiosome, Archoplasm, starting from the spermato-
cyte stage. He described the dissolution of the idiosome during
the last division of the spermatocytes and its reformation in
the spermatids. He also described the formation of the idio-
sphaerotheca, vacuole, and the appearance of the idiosphaero-
some, kornartiger, stark färbbarer Körper. He further recorded
the formation and disappearance of the idiophthartosome,
Archoplasma-rest. The spermiocalyptrotheca was correctly de-
scribed by Benda as formed from the idiosphaerotheca, Vacuole.

Moore ('94) recorded in rats that the idiosphaerotheca, archo-
plasmic vesicle, was formed by the fusion of many smaller
vesicles. In each of these vesicles is formed a small central
granule, archosome. All these archoplasmic vesicles and archo-
somes flow together and finally form a single archoplasmic vesicle,
our idiosphaerotheca, and a unique archosome, our idiosphaero-
some. The idiosphaerotheca was regarded as a temporary for-
mation.

In 1896 Niessing gave a rather detailed description of the de-
velopment of the idiosome in guinea-pigs. He described the
idiosome, Sphäre, of the primary spermatocytes, Mutterzellen,
as consisting of a darker peripheral layer, Rinderschicht, and a
central substance, Markschicht, in the midst of which lie the
centrosomes, central Körper, two to three or sometimes more.
According to Niessing, the idiosome is traversed by a number of
fibrils passing radially from the center to the periphery. In the
peripheral layer Niessing observed a sharp and darker stained
layer of granules, Körnerstratum. At times these granules were
very numerous and formed in some cases as many as eight cir-
cular layers within the peripheral portion. Sometimes the gran-
ules extended into the cytoplasm beyond the limits of the idio-
some. Niessing held that these granules were connected with

the center by means of the radial fibrils, their positions being probably regulated through the contractions of the fibrils.

Judging from this description by Niessing, the peripheral layer of the idiosome corresponds probably to our idioectosome and the central portion to our idioendosome. The granules observed in the peripheral part are the idiogranulomes and the radial fibrils are the prolongations from the endosome, as described above, page 39. All observers, who studied the idiosome before or after Niessing, failed to find any trace of these fibrils. We were never able to see such fibrils in the idiosome and think it probable that Niessing observed nothing more than the processes mentioned in our description as passing from the endosome through the ectosome.

Niessing was not able to obtain a distinct stain for the idioendosome, and for this reason he described the central portion of the idiosome as lighter than the peripheral, and thus failed to note the existence of the central body with clearly differentiated limits, the idioendosome. The description of Niessing leaves unsolved the origin and significance of the granules. He did not follow the distribution of the granules in the protoplasm during the divisions of the primary and secondary spermatocytes, nor their reunion in the formation of the new idiosome in the secondary spermatocytes and in the spermatids.

The present description makes it clear, we believe, that the breaking-up process is probably a preparatory stage for the division of the idiosome and a means to carry out this division. The fine granulation secures a distribution of the idioplasmatic substance in a way comparable to that by which the chromosomes serve to secure an exact distribution of the chromatic substance in every cell division. Fine granulomes accomplish a distribution, but evidence is entirely wanting to show that the distribution of the granulomes is equal in the daughter idiosomes as the chromosomal distribution is in the daughter nuclei.

Niessing goes further with the description of the idiosome, Sphäre, in the spermatids, Tochterzellen. He states that the idiosomes immediately after the formation of the spermatids show all the elements described in the idiosome of the sper-

matocytes, viz., centrosomes, radial fibrils, and idiogranulomes.
He describes the idiogranulomes, Microsomenstratum, as being
largely concentrated near the periphery.   In our preparations the
existence of radial fibrils is not demonstrated and the idiogranu-
lomes not only occur near the periphery but also in the central
part of the idiosome and very often close to the surface of the
nucleus.   We never have observed the centrosomes connected
with the idiosome at this stage or at any other stage during the
development of the spermatids.

During the metamorphosis of the spermatids the idiosome
undergoes the following transformations according to the de-
scription of Niessing.   The radial arrangement of the idio-
granulomes, Microsomenstrata, disappears.   The small idio-
granulomes run together and thereby form fewer granulomes and
finally a single large idiosphaerosome, Mitosom.   At the same
time the idiosphaerotheca, dünne glashelle Membran, is formed
probably from the idiosome substance.   The idiosphaerosome is
still connected with the wall of the sphaerotheca by a fiber which
extends towards the nucleus.   Through the contraction of this
fiber the idiosphaerosome comes into contact with the nucleus.
As shown in the descriptive part of this paper, such prolongations
of the idiosphaerosome may extend in all directions, sometimes
directly away from the nucleus.

The idiosphaerosome, Niessing believed, differentiated later
into two parts, a central part which takes a black color with iron
haematoxylin, probably our cryptosome, and an external part
which takes a gray color, probably our calyptosome.

We say probably, because we are not altogether certain that
the peripheral lighter zone of the idiosphaerosome, as illustrated
by Niessing and others, corresponds completely with the
calyptosome.   In our preparations the calyptosome is formed, as
described above, in the shape of a cap resting on the superior
pole of the idiosphaerosome (fig. 9), and is not of the nature of a
theca or zone.   It seems more probable that the outer lighter
zone of Niessing is a part of the idiosphaerosome and not the
first indication of the idiocalyptosome.   Duesberg ('11) gives a
figure showing the same differentiation into two zones in a large

idiogranulome before the idiosphaerosome is formed through the complete fusion of all idiogranulomes, his figure 57. In our preparations, stained with the fuchsin-methylen blue method, the idiosphaerosome and the large idiogranulomes also show a differentiation in some cases into two zones; the outer one being generally somewhat darker in color, but always red. The blue calyptosome, with its distinctly vacuolar structure, is formed gradually from the anterior pole of the idiosphaerosome in the shape of a cap. In those of our preparations stained with iron haematoxylin, the differentiation into two zones was more apparent, with a central zone black and a peripheral zone gray, exactly as described by Niessing who used the same staining method. In some slides, stained with iron haematoxylin and differentiated with iron alum, we have observed certain places on a slide, where the differentiation with iron alum was slight, which shows the entire idiosphaerosome black; in other places, where the stain was further differentiated, the idiosphaerosome is separated into two zones, as already described, a central black and a peripheral gray; while in still other places even more extracted the whole idiosphaerosome is gray. The same phenomenon may be observed in the staining and extracting of the idiogranulomes, some of them being black and others gray, on account of a less or more complete differentiation. This is probably one of the reasons that Niessing, as stated above, has confused the centrosomes with the idiogranulomes. The peripheral gray zone of the idiosphaerosome in our iron haematoxylin preparations does not represent the first formation of the calyptosome. We cannot make this statement for the preparations of other investigators, but must point out the possibility that the peripheral zone of Niessing may not be the early beginning of the calyptosome, but a differentiated part of the idiosphaerosome.

This external part is described by Niessing as homogeneous and not as having a vacuolar structure. The cryptosome, dunkler Teil des Mitosoms, grows until it lies as a disk on the nucleus. The calyptosome, äusserer teil, also grows until it is separated from the cryptosome. The development of the idiosphaerotheca, helle Membran, into the spermiocalyptrotheca,

Kopfkappe, is also described by Niessing.   He further described
the formation of the idiophthartosome, Sphärenrest, which mi-
grates to the posterior pole of the nucleus and disappears with
the cytoplasmic remains.

Niessing described very well the conical transformation of
the calyptosome and he illustrated the existence of the crypto-
some in this stage.   Also in the transformed spermatozoön he
showed the persistence of the same body enclosed in the calypto-
some.   He did not give sufficient importance to the separation
of these two bodies, and for this reason he failed to designate
them by different names.   From his description one derives the
impression that he did not regard the cryptosome and the
calyptosome as two different things, just as he did not fully
recognize the central and the peripheral parts in the idiosome
of the spermatocytes as two separate bodies differing in structure
and developing independently of one another.

Niessing recorded the idiosome of the spermatocytes as a
unique body showing a differentiation into two zones, an ex-
ternal and an internal, in the same way as he described the
spermiocalyptra, Spitzenknopf, as a unique body showing a
differentiation into two zones, an external lighter gray in color and
a central of a darker color.   He did not observe that the calypto-
some always presents a distinctly vacuolar structure in contrast
with the homogeneous or granular structure of the cryptosome.
He failed to notice the existence of the endosome as a separate
body, its dissolution to form the idiogranulomes, the persist-
ence and the rôle of these granulomes during the divisions, and the
continuity of their development from the idioendosome to the
spermiocryptosome.   In a general way in spite of this Niessing
gave a most detailed description of the idiosome, and his ob-
servations mark an important step in its study.

v. Lenhossék ('98) studied the development of the idiosome in
rats and guinea-pigs.   He also observed in the idiosome of the
spermatocytes a differentiation into two zones, an external dark
and an internal pale zone.   This differentiation was not always
distinct and sometimes the internal zone was eccentric in posi-
tion.   Although using the same method as Niessing, he was

unable to observe the radial protoplasmic fibrils or the concentric strata of granules described by Neissing.   Lenhossék noted frequent granulations in the idiosome without giving their definite distribution.   He did not believe the idiosome to be of a fibrillar or granular structure, but to be a homogeneous concentrated mass of protoplasm, and held that the granulations often observed in it are not regular or typical.   He also observed the centrosomes in rats, invariably two in number, as is the case in guinea-pigs.

Lenhossék found the idiosome appearing for the first time in the spermatogonia, in some of which it may still be absent, while in others only its early beginning can be seen.   Only in the spermatocytes does it show a high development and a differentiation into a peripheral and a central zone.   During the division of the spermatocytes the centrosomes come out of the idiosome, while the latter assumes irregular shapes and later breaks up to be dispersed in the cytoplasm.   In the secondary spermatocytes and in the spermatids a new idiosome arises from the remains of the old one.   The newly formed idiosome of the spermatids is described by Lenhossék as homogeneous.   At the border only he sometimes observed granules of different sizes staining black with iron haematoxylin and eventually uniting to form a continuous outline.   He emphatically denies the existence of any granules or fibrils, such as described by Niessing.

Later on a vacuole is formed within the body of the idiosome, our idiosphaerotheca, and in this vacuole appears a central granule, the acrosome, our idiosphaerosome.   The central granule appears suddenly, 'wie durch einen Schöpfungsakt,' through a spontaneous concentration and differentiation of its substance. This acrosome later shows a differentiation into a peripheral zone and a central zone.

Lenhossék further describes the formation and the fate of the idiophthartosome, the form changes of the calyptosome and the formation of the spermiocalyptrotheca, Kopfkappe, from the idiosphaerotheca, his vacuole.   No further mention, however, is made of the cryptosome.   The calyptosome through its entire development he described as homogeneous with no trace of

differentiation. The spermiocalyptra he also described as an homogeneous mass resulting from the growth and transformation of the idiosphaerosome, Acrosoma, during whose growth the idiosphaerosome loses its affinity for the iron haematoxylin stain. He noticed also the existence of a sickle-shaped area between the spermiocalyptra and the head of the spermatozoön, this area being filled by a pale substance, Kittsubstanz. It is possible that this area corresponds to our spermiocryptosome.

From his description it is evident that Lenhossék, although using the same methods as Niessing, did not observe so many details as the latter. The idiogranulomes seen by Niessing in the spermatocytes and the newly formed spermatids were not observed by Lenhossék. The formation of the idiosphaerosome and the idiosphaerotheca were incorrectly described as appearing suddenly, 'wie durch einen Schöpfungsakt.' The differentiation of the calyptosome and the cryptosome he observed only during its first stage, while Niessing followed this differentiation to the ripe spermatozoön. In a general way the observations of Lenhossék concerning the idiosome of the guinea-pigs confused, rather than advanced the subject.

One year later, Meves ('99) also gave a description of the development of the idiosome in the guinea-pigs. Meves observed the granular structure within the idiosome of the primary and secondary spermatocytes and in the spermatids. He found no granules in the spermatogonia nor in the cells of the growth period. In addition to the granulations he also observed in the idiosome of the spermatocytes the two elongate, slightly dumb-bell-shaped centrosomes. Meves described correctly the formation of the idiosphaerosome, acrosoma of Lenhossék, through the fusion of the granules existing in the idiosome of the spermatids, as well as the formation of the idiosphaerotheca by a fusion of the vacuoles, Bläschen, surrounding these granules. He also described correctly the formation and disposition of the idiophthartosome and the transformation of the idiosphaerotheca into the spermiocalyptrotheca, Kopfkappe. He observed the separation of the idiosphaerosome into two zones, an external, Aussenzone, and an internal, Innenkorn.

Meves observed these two zones with different staining methods and expressed the opinion that they must represent actually different structural products. His staining methods, however, did not permit him to trace the persistence of the cryptosome, Innenkorn, through all the later stages to the ripe spermatozoön. He, therefore, believed that the entire cryptosome, Innenkorn, was transformed into the calyptosome, Aussenzone. The calyptra was described by Meves as being homogeneous, and he recorded the cryptosome as later disappearing completely.

The descriptions of Meves are very accurate, but do not bring out facts other than those observed by previous investigators, especially Niessing. The granules in the spermatocytes and the spermatids, the formation of the idiosphaerosome and the idiosphaerotheca, the formation and disappearance of the idiophthartosome, the separation of the calyptosome and the cryptosome, and the formation of the Kopfkappe had all been described before.

Niessing is the only observer who followed the separate existence of the calyptosome and the cryptosome up to the spermatozoön.

The probable reason for so little essential progress after Niessing ('96) in the investigation of the idiosome is the fact that the major attention of investigators, studying the spermatogenesis in different animals, has been concentrated on changes in the nucleus and especially in the chromatic substance. The idiosome has been more or less considered in many of these studies, but it has not been made the central point of attack. It would be out of place to discuss here all the papers in which the development of the idiosome has been partly described in different animals. It is not our purpose to give a comparative review of the idiosome in the different animal classes, but only to consider the progress made in the study of this body in certain animals and especially in mammals, in order to convey some idea of the proper position of the present results.

In the light of the above review, we may now state the case of the idiosome in the spermatogenesis of the guinea-pig in the following summary manner.

6. CONCLUSIONS AND NEW POINTS

1. The idiosome in the spermatogonia is not a fixed structure, but shows great variability in form and appearance. In some cases it is distinctly vacuolar and in others there is a beginning of a differentiation into two zones, a central and a peripheral.

2. The idiosome in the primary spermatocytes has a definite structural division into two parts; an internal or central body, the idioendosome and a surrounding larger sphere, the idioectosome. The endosome sends processes or prolongations of its substance into the surrounding idioectosome, giving the latter a vacuolar appearance.

3. When the primary spermatocytes prepare to divide, the idioendosome breaks up into a number of granules, the idiogranulomes. As the division of the spermatocytes proceeds the idioectosome likewise breaks up into smaller pieces which are gradually dispersed in the cytoplasm. This liberates the idiogranulomes which now also become scattered in the cytoplasm of the spermatocytes.

4. After the division of the primary spermatocytes a new idiosome is formed in the secondary spermatocytes through a reunion of the idiogranulomes and possibly of the original dispersed pieces of the idioectosome.

5. The idiosome in the secondary spermatocytes maintains at all times a granular structure, consisting of a number of idiogranulomes enclosed within the ectosome sphere.

6. During the division of the secondary spermatocytes the idiosome again breaks up in the same manner as during the division of the primary spermatocytes.

7. A reconstruction of the idiosome again takes place in the spermatids immediately after the division. The idiosome exhibits a number of idiogranulomes enclosed within the ectosome, but each idiogranulome seems now to be surrounded by a small vacuole, the idiogranulotheca. It is not certain whether these vacuoles are formed by the ectosome or by the idiogranulomes.

8. The idiogranulomes and the idiogranulothecae run together and fuse into larger and larger granulomes enclosed in larger and

larger vacuoles until finally one large granulome, the idiosphaero-
some, is surrounded by one large vacuolar wall, the idiosphaero-
theca.

9. The idiosphaerosome produces from its upper surface a
new body in the form of a cap, having a vacuolar structure and
different staining reactions. This is the calyptosome. The re-
maining part of the idiosphaerosome is the idiocryptosome.
These two bodies now develop separately and persist up to the
formation of the spermatozoön.

10. The idioectosome detaches itself from the idiosphaerotheca
and migrates along the surface of the cell nucleus to its posterior
pole and there passes into that part of the cytoplasm which is
lost during the metamorphosis into the spermatozoön.

11. The idiosphaerotheca persists through all later stages and
develops into a membranous cover for the cap and head of the
spermatozoön.

12. There are two types of development shown by the crypto-
some, one massive or concentrated and the other granular.

13. During all stages of spermatogensis a number of granules,
karyogranulomes, having the same appearance and staining re-
actions as the idiogranulomes, are to be seen in the nucleus.
During the last metamorphosis of the spermatids, when the
nucleus is transformed into the head of the spermatozoön, the
karyogranulomes seem to be dissolved within the sperm-head in
the same way as is the chromatin.

14. During the divisions of the germ cells the karyogranulomes
are to be seen on the spindle fibrils or on the chromosomes, while
the idiogranulomes are scattered throughout the cytoplasm.
No membranes or structures separate the two classes of granules
during division, and it is possible that some idiogranulomes may
migrate into the spindle area and thus be carried to a daughter
nucleus, or that karyogranulomes may be detached from the
spindle and become free in the cytoplasm to form later part of
the idiosome. A direct observation of such an exchange is al-
most impossible, since the size variations, the appearance and
the color reactions of the idio- and karyogranulomes are too
closely the same.

15. The breaking up into small granules may be considered as a process to secure a distribution of the idioplasmatic substance during cell divisions.

The new points brought out by the present study are the following:

1. A description for the first time of the idioendosome.

2. A description of the formation of the idiogranulomes through the breaking up of the idioendosome.

3. The persistence of the idiogranulomes during the divisions of the primary and secondary spermatocytes, their distribution in the cytoplasm, and their subsequent reunion during the reconstruction of the daughter idiosomes. Idiogranulomes have not been previously observed during the division of the spermatocytes.

4. The existence of the karyogranulomes which had always been overlooked and their persistence through all stages of spermatogenesis.

5. The exact manner of formation of the calyptosome and its vacuolar structure. This body had previously been described as homogeneous.

6. The great variability in the development of the cryptosome, the formation of the cryptogranulomes and the granular consistency of the cryptosome during the stages shown by figures 11 and 12.

7. The double nature of the idiosome consisting of an ectosomatic and an endosomatic substance, the development of the two substances being independent of one another.

8. The rôle of granulation in serving to distribute the idioplasmatic substance during cell division. No theory until now has been advanced concerning the significance of the idiosome granules.

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PLATES

All figures are camera-lucida drawings of the objects magnified by paired oculars No. 10 and oil-immersion objective 1/9 mm. of Bausch & Lomb.

Abbreviations used:

cal., calyptosome	*id.gr.*, idiogranulomes
car.gr., karyogranulomes	*id.gr.th.*, idiogranulotheca
crypt., **cryptosome**	*id.phth.*, idiophthartosome
crypt.gr., cryptogranulomes	*id.sph.*, idiosphaerosome
hd., head of spermatozoön	*id.sph.th.*, idiosphaerotheca
id.ect., idioectosome	*n.id.*, new idiosome
id.end., **idioendosome**	*sp.th.*, spermiocalyptrotheca

Figures 1 to 13 are from one animal and figures 11a to 11c and 12a to 12d are from another.

PLATE 1

Fig. 1   A primary spermatocyte showing the idioendosome and the idioectosome and with karyogranulomes in the nucleus.

Fig. 2   A primary spermatocyte showing the formation of the idiogranulomes from the idioendosome.   Two granulomes are to be seen in the protoplasm and four others in the nucleus.

Fig. 3   A secondary spermatocyte showing the idioectosome, the idiogranulomes and a number of karyogranulomes.

Fig. 4   A secondary spermatocyte in division showing a piece of the ectosome filled with idiogranulomes, while other idiogranulomes are scattered in the protoplasm; karyogranulomes are seen on the spindle fibrils and on the chromosomes.

Fig. 5   A spermatid immediately after the secondary spermatocyte division, showing the reconstruction of the new idiosome, *n.id.*

Fig. 6   A spermatid showing the reconstructed idiosome with the idiogranulomes enclosed in vacuoles, the idiogranulothecae.

Fig. 7   A spermatid showing two large idiogranulomes formed by the fusion of smaller granulomes and another small idiogranulome.

Fig. 8   A spermatid showing the large idiosphaerosome which resulted from the fusion of all the idiogranulomes; the idiosphaerosome is enclosed within the idiosphaerotheca, surrounding which is the idioectosome.
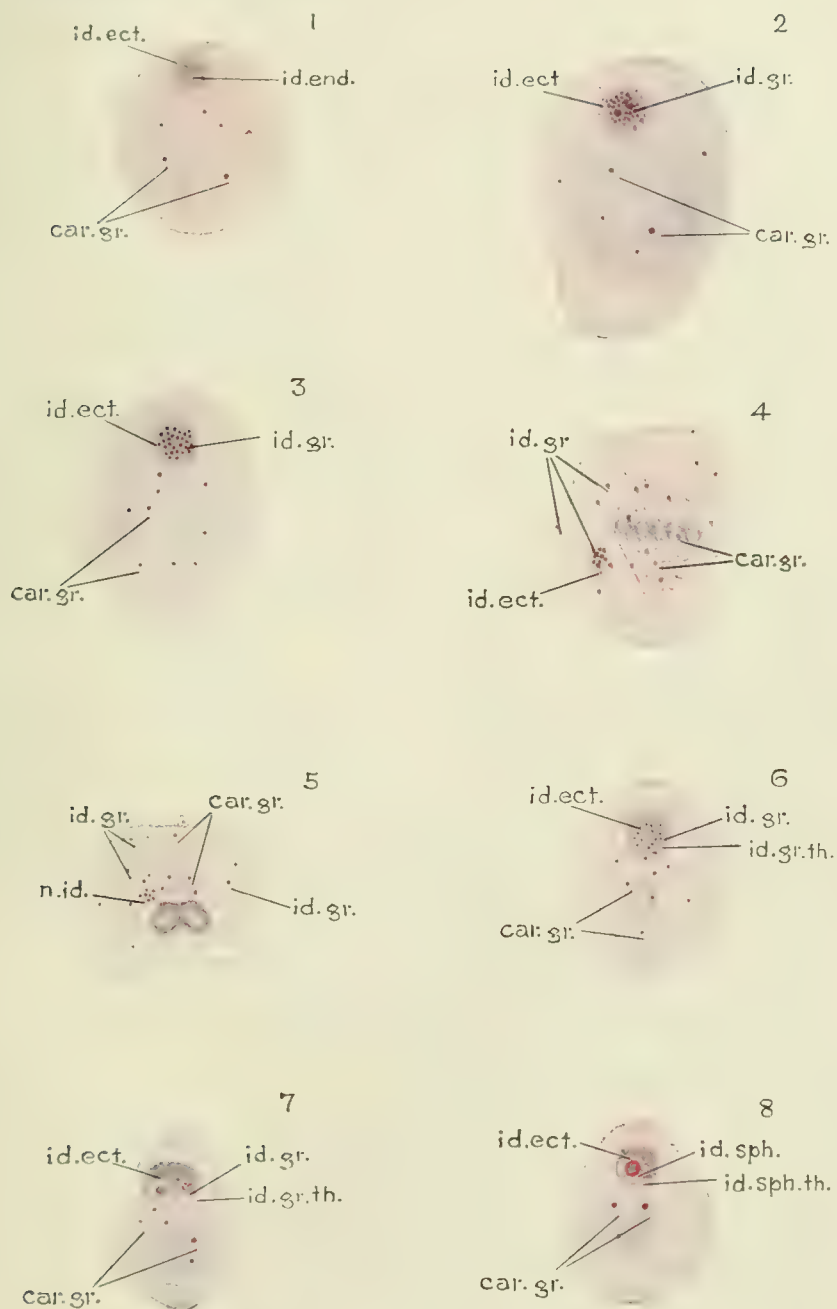
67

PLATE 2

Fig. 9 A spermatid showing the separation of the calyptosome, *cal.*, and the cryptoson e. *crypt.*, both are enclosed within the large idiosphaerotheca. The idiophthartosome derived from the idioectosome begins to migrate towards the lower pole.

Fig. 10 A later stage in the development of the calyptosome and the cryptosome. In the calyptosome are two small cryptogranulomes. The idiophthartosome, *id.phth.*, is near the lower pole. The chromatic substance begins to be dissolved, concentrating about the lower pole. Two large karyogranulomes are to be seen, one beneath the cryptosome and another near the lower pole.

Fig. 11 A later stage showing the conical elongation of the calyptosome and the cryptosome. The cryptosome has a granular appearance. One cryptogranulome is near the apex of the cone. The karyogranulomes and the chromatic substance are dissolving in the nucleus.

Figs. 11a to 11c Similar stages showing different degrees of cryptogranulosis. the granular type.

Fig. 12 The head and calyptra of a spermatozoön in process of development as seen in a seminiferous tubule. The cryptosome still has a granular structure.

Figs. 12a to 12d Similar stages showing different degrees of cryptogranulosis. the granular type. in one and the same animal.

Fig. 13 A mature spermatozoön head from the epididymis. The calyptra composed of the calyptosome and the cryptosome. as well as a large part of the head are covered by the spermiocalyptrotheca
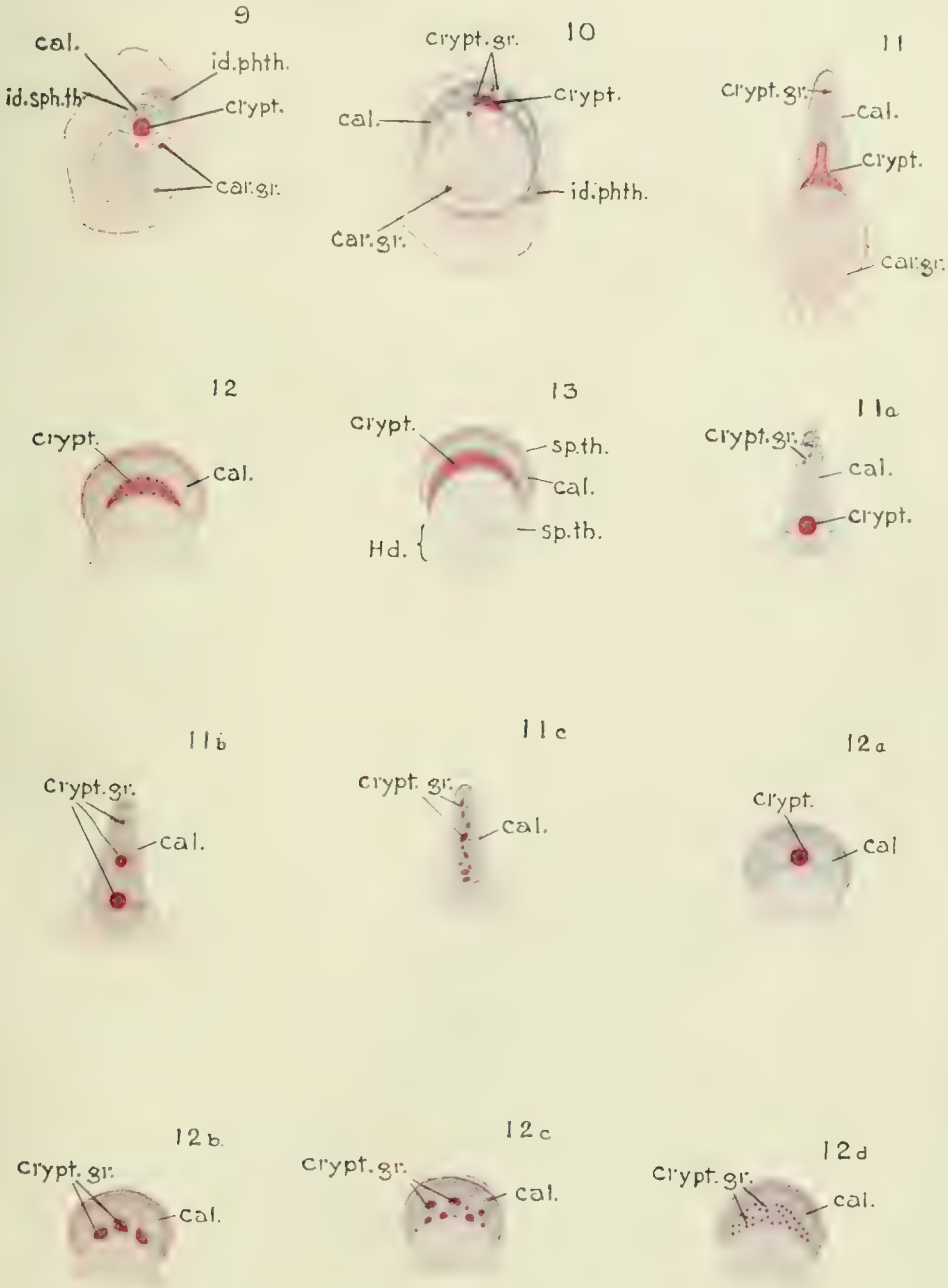
69

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THE VAGINAL CLOSURE MEMBRANE, COPULATION,
AND THE VAGINAL PLUG IN THE GUINEA-PIG,
WITH FURTHER CONSIDERATIONS OF THE
ŒSTROUS RHYTHM.

THE VAGINAL CLOSURE MEMBRANE, COPULATION, AND THE VAGINAL PLUG IN THE GUINEA–PIG, WITH FURTHER CONSIDERATIONS OF THE ŒSTROUS RHYTHM.

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Two years ago we recorded the results of a detailed study of the œstrous cycle in the guinea-pig. A rather full description of the histological and physiological changes which take place in the ovary, uterus and vagina during the "heat period" was presented. We emphasized particularly the importance of changes occurring in the microscopic composition of the vaginal fluid as indicative of the exact conditions in the uterine wall and ovarian follicles at corresponding moments.

Since that time we have somewhat extended the analysis of these phenomena. It has been found that a membrane covering the orifice of the vagina furnishes a most valuable and simple means of diagnosing certain periods in the œstrous cycle. This we have termed the "vaginal closure membrane." The exact moment of copulation and the conditions in the walls of the vagina and uterus at this time have been carefully followed, along with a consideration of the formation and significance of the vaginal plug. In the present paper a discussion of these several topics will be undertaken.

Certain points in the literature will also be discussed, a more complete review having been given in the previous article.

I. THE VAGINAL CLOSURE MEMBRANE.

In the former communication attention was called to the fact that "the external vaginal orifice, which during the period of œstrous activity is more or less open, actually showing in many cases a little fluid or some blood, closes and becomes less accessible after the period." During ovulation the vagina is open, but the fact of its being open is not unmistakable proof of the time of

ovulation unless the open vagina also contains what was described as second or early third stage œstrous fluid.

At that time the method of closure of the vagina following the œstrus was not explained nor was its actual significance fully appreciated. The vagina is now found to be closed by a remarkable cellular membrane and in a very definite way.

The external orifice of the vagina is crescentic in shape and the urethral opening lies in front of it in the mid line. The anterior and posterior lips of the crescent-shaped opening come together, and a delicate epithelial membrane grows over the opening and unites the lips. This occurs shortly after the heat period in females that have not copulated and in those that have copulated the closure follows the expulsion of the vaginal plug, a process to be considered beyond. The closure begins at the tips of the crescent-shaped opening and progresses toward the midpoint. The lips do not approximate so intimately at the midpoint and the membrane here seems to be under more tension than at other parts, even after the entire orifice has closed. The opening of the orifice by a tearing of the epithelial membrane begins at the strained middle part and extends from there laterally until finally the vaginal lips are freely separated. The midpoint is, therefore, the last to close, and the first to open as a general rule, although at times the opening may begin at either side of the midline.

The epithelium completely unites the lips of the vagina so that nothing can escape from or enter into the vaginal lumen without tearing this closure membrane. Such a membranous closure of the vaginal orifice is unknown to us in any other mammal. In many species the sides of the vaginal opening may be approximated or cemented together by some hardened fluid or secretion so that the lips are not readily pressed apart, but a membranous growth closing the orifice after each heat period is apparently unique.

This membrane also completely closes the vaginal opening throughout pregnancy and only becomes ruptured when the vulva swells shortly before parturition.

Such an obstruction or closure of the vaginal lumen at once suggests the hymen of the human vagina. But this, of course,

is quite a different structure in its origin as well as in its later history. The closure membrane in the guinea-pig not only exists in the young immature animal but is regularly destroyed before and reformed after every heat period that takes place during the life of the female. The formation or growth of this membrane might also be compared in some respects to the membranous growths tending to extend across and close the pharynx and other canals under pathological conditions.

The membrane is thin and delicate in structure and when stretched by slightly pressing apart the lips of the vagina with the fingers it is seen to be almost transparent, the outline of the vaginal lumen showing through. The closure membrane is of the same glossy appearance as is the surface epithelium covering the region of the vaginal lips with which it is continuous. It is composed simply of stratified squamous epithelium which has grown from the borders of the lips over the orifice and contains no vessels or blood.

When the membrane is torn or broken by accident during the dioestrum, or period of sexual rest, it reforms sometimes within a day, or within a few days, and remains until the beginning of the new period of heat or œstrus. A recognition of this membrane is then a great convenience in determining the onset of the œstrus in a group of female guinea-pigs. Daily smears of the vaginal fluid are not now necessary to find when the œstrus is about to begin in animals examined for the first time and whose rhythm is therefore unknown.

Although the presence of the closure membrane is a definite aid in recognizing the condition of the œstrous cycle, it must be remembered that this membrane often persists up to the first stage of œstrus, at which time the lumen of the vagina is filled with a mucous fluid and first stage cells. This is actually the "heat" time and the normal moment for copulation as we shall explain below. When the closure membrane still persists until the vaginal lumen is so filled it may be distended and rounded out resembling a blister membrane on the point of bursting. Puncturing this the vaginal fluid oozes out through the break. As a general rule, however, the vulva becomes inflamed and very slightly swollen immediately before œstrus and the stretched

membrane breaks. Thus the membrane has reached the break-ing point or has actually broken at just about the time the female is in heat and ready to copulate.

While the presence of this membrane is a reliable index of the œstrous condition, the open vagina, or its absence, is by no means indicative of the œstrous state. Although the vagina is always open during what we have termed the second and third stages of œstrous, and, therefore, at the time of ovulation whether copula-tion has taken place during the first stage or not, it is nevertheless frequently open at other times. It is not permissible to assume that the open vagina indicates a state of heat or the time of ovulation in a guinea-pig. Only when the open vagina contains fluid showing on examination the cells described as second or early third stage is the ovary almost exactly in the condition of ovulation. It may be stated parenthetically that after long experience one is able as a rule to diagnose the stages of the vaginal fluid by slight difference in color and consistency without microscopic examination.

Finally, then, when the vagina is open one may only be certain of the uterine and ovarian conditions by examining the contents of its lumen, but, on the other hand, if it be closed by this mem-brane one may be certain that the time of the new ovulation has not yet arrived.

2. The Time and Manner of Copulation and the Conditions in the Reproductive Organs of the Female at this Moment.

It is well known that female guinea-pigs in common with other animals of their class, and in fact most mammals, have a definite limited time during which they accept the male, the so-called "period of heat." This period, very slightly revealed by external signs at the mouth of the vagina, but chiefly by the act of copulation has been the starting point in all previous studies on the reproductive activities of the guinea-pig. In order to prevent the modifying conditions of pregnancy following copulation, various operations have been resorted to, as in the case of some of Loeb's experiments. Such operations might com-plicate or even vitiate the results which follow.

In the present account we wish to describe the exact moment at which copulation takes place during the sexual cycle and to show definitely the conditions of the vagina and uterus at this moment. From the condition of the vagina or uterus the ovarian condition is readily estimated, as we have shown in the former paper. After determining the exact œstrous condition of a female at the moment she is ready for copulation we may then recognize a corresponding moment in any female by an examination of the vagina without the necessity of introducing the male or permitting copulation to occur.

In order to designate the copulation time exactly, we must review briefly the characteristics of the four very clearly defined stages of the œstrus or "heat period" proper. During stage one the uterine epithelium swells, the cells becoming distended with an abundant mucous secretion which very soon pours into the lumen and reaches the vagina. At this time a desquamation of the epithelial cells from the lower part of the vagina also begins. The second stage shows a great accumulation of leucocytes below the uterine and vaginal epithelium with a slowly progressing desquamation of epithelial cells. The third is the stage of exodus of the leucocytes, myriads of them coming through the epithelial lining of the walls of the uterus and vagina, with an accompanying extensive destruction of the epithelium. During the fourth stage the broken down epithelium falls away in masses and at the same time a regeneration of epithelium takes place beginning from the mucosa of the uterine glands.

The Graafian follicles of the ovary rupture and ovulation occurs at the end of the second stage or the beginning of the third stage, while during the fourth stage the recently ruptured Graafian follicles are already well under way in their development into new corpora lutea.

A recent abstract by Long ('19) seems to indicate that four similar stages may be recognized during the œstrus in the rat, and that these stages agree almost exactly in significance with the comparable ones in the guinea-pig: Ovulation also occurring in the rat at the end of the second or beginning of the third stage.

During our initial investigation we made no attempt to locate the exact moment of copulation and, of course, did not describe

the corresponding vaginal or uterine stage. The description by Loeb ('14), however, of the great leucocyte migration in the wall of the uterus twelve to twenty-four hours after copulation would indicate that the true "heat" or copulation occurs before the beginning of the destructive changes in the uterus, and this we now find to be true. Here and in the following section we wish to point out just how the uterine changes seem to be associated with the act of copulation, the retention of the sperm in order to insure the fertilization of the eggs, and after this the means of ridding the vagina of the excessive seminal accumulation.

A number of females have been placed with males while in one or another of the above mentioned four stages, as well as during different times of the dicestrum, or interval of sexual rest. The results show that a copulation is never accomplished except during the first stage of œstrus about twelve hours before the second stage begins. Long ('19), also finds copulation to take place in the rat during the first stage. At this stage in the guinea-pig the vagina contains a clear, foamy, saliva-like fluid in which desquamated epithelial cells of the first type are present. Differing from all other stages and times there are now no leucocytes to be found in the vaginal fluid,—compare our former Figs. 1 and 2 with Figs. 5 and 6. Even during the resting period the vagina contains some mucus but this is very scant and filled with many leucocytes, being pus-like in appearance and consistency.

To locate even more accurately the normal time of copulation the first stage may be subdivided into two shorter periods: a preparatory interval, the early beginning of the first stage, when the vagina is almost dry and contains only a scant amount of loose cells of the first type; and, second, what may be designated the true first stage, a more advanced period when the frothy mucous secretion has already begun to accumulate. Copulation takes place during this second phase of stage one and never during the first.

During stage one the vagina is characterized by two important conditions, both of which contribute to the success of copulation. In the first place the existence of a mucous secretion evidently

facilitates the act of copulation. This abundant frothy mucous accumulation is limited to the first stage, particularly to the time when copulation takes place, and it never occurs in the vagina at other times. The second contributory condition is the complete absence of leucocytes in the mucous fluid. The leucocytes begin their migration through the epithelium of the uterus and vagina at the end of the second stage. They are extremely abundant in the lumen during the third stage, while from this time on they are found in the uterus and vagina in smaller or larger quantities up to the approach of the next first stage. Two days before the first stage begins leucocytes are still plentiful, but from this time first stage epithelial cells gradually become more abundant and the leucocytes decrease in number until finally when the first stage has actually arrived no leucocyte exists in the vaginal fluid. The mucous content of the vagina during the first stage hence lacks the pus-like appearance of the vaginal fluid of the "intermenstrual" time and is clear and foamy.

The absence of leucocytes from the vaginal lumen at the time of copulation is important, since, if present, they might by their dissolving powers or phagocytic action exert an injurious effect on the spermatozoa and thus interfere with their normal function. Later a special purpose of the leucocytes seems to be to destroy the excess of spermatozoa remaining in the uterus. This frequently occurs by an interesting process of phagocytosis. A leucocyte comes in contact with a spermatozoön which with its tail is longer than the leucocyte. The leucocyte by stretching and contracting finally takes into itself the entire spermatozoön, the tail being wound in circular fashion within the cell body. The leucocytes, however, apparently accomplish most destruction by their dissolving or disintegrating action.

It seems that the migration of the leucocytes through the walls of the uterus and vagina, though not increased in extent, is accelerated by the act of copulation and the entire œstrous process is shorter than in non-copulated females. About six hours after copulation the third stage is in full development, while under virgin conditions a comparable stage is reached only after at least twelve hours from the time when copulation might have occurred. It may be said that copulation tends to

hasten ovulation, or that the act itself may facilitate the bursting of the Graafian follicles, which is a very old conception.

The act of copulation is short, lasting a few seconds only, while the preceding time of sexual excitement leading up to it is rather long. The male becomes excited by the presence of the female some time before she reaches the proper condition for copulation. A male after long isolation from females becomes sexually excited by the presence of any female irrespective or her sexual condition, and he invariably attempts to copulate. Nevertheless, the excitement of the male is not so strong nor prolonged when in the company of a female during sexual inactivity as with one during her sexual season. When the female is nearing œstrus the male is extremely excited and tries again and again to copulate, while at other times he soon tires and loses interest and ceases his aggressive behavior.

The male and female never fight during the long period of aggressiveness on the part of the male, which often lasts for many hours. The male tries to induce the female to copulate by irritation and excitement rather than by forcing her. The female may at times become nervous and attempt to bite the male, but an actual fight such as occasionally occurs between two males never takes place. No mating by force is observed; the consent of the female is necessary for the completion of copulation. Copulation is followed by a state of relaxation similar to that observed among mammals in general, and immediately afterwards both male and female may spend some time in cleaning their external genitalia.

3. The Vaginal Plug, Its Formation, Length of Existence and Manner of Discharge.

The spermatic fluid of the guinea-pig, especially that portion derived from the seminal vesicles, on entering the vagina of the female coagulates to form the *bouchon vaginal*, a rigid plug, filling the lumen of the vagina. This plug prevents the outflow of the sperm after every copulation. Such a vaginal plug has been described in many species of rodents and seems in general to be characteristic of this class of mammals. It was first observed in the guinea-pig by Leuckart in 1847. He correctly

described it as a Pfropf (plug), formed by the coagulation of the secretion from the seminal vesicles and serving to fill the vagina and prevent the flowing out of the sperm after copulation.

Bischoff, in 1852, verified the observations of Leuckart and accepted his conclusions regarding the rôle of the vaginal plug in the copulation process. Reichert, 1861, differed with these two original descriptions in failing to find the formation of a vaginal plug after every copulation, and concluded that its presence was not a general phenomenon. Later, however, Hensen in 1876 brought new evidence confirming the observations of Leuckart and Bischoff.

Landwehr, in 1880, examined the seminal vesicles and found their secretion to contain twenty-seven per cent. of fibrinogen to which its coagulation reaction is due. Coagulation may occur as soon as the secretion of the seminal vesicles comes in contact with a small amount of blood.

Héron-Royer, 1881, observed the vaginal plug in *Pachyuromys duprasi*, but gave no satisfactory explanation of its formation. According to him the vaginal plug was formed in the vagina before copulation and was pulled out or loosened by the hooks on the penis during the act of copulation. These observations were entirely contrary to all earlier records, according to which the plug is formed after copulation and falls out some hours later. Blanchard made histological examinations of the vaginal plugs collected by Héron-Royer and found them to consist of two parts, a central, *partie centrale*, composed chiefly of great numbers of spermatozoa, and a peripheral part, *couche corticale*, formed of hardened mucus.

Lataste, in 1882, after examinations of the vaginal plug in the same species, *Pachyuromys duprasi*, came to quite different conclusions. He states that the vaginal plug, *bouchon vaginal*, as he termed it, is not formed as Héron-Royer claimed, before copulation, but immediately after, and in the same way as was known for other rodents. Regarding its function he accepted the old opinion of Leuckart that it serves to prevent the spermatozoa from flowing out of the vagina after copulation. He also mentions an instance in which a vaginal plug-like formation was found when there had been no previous copulation. From our

observation on the œstrous discharge in the guinea-pig it is probable that this plug-like structure was nothing else than a concentrated accumulation of such a discharge, it having become unusually dense or dried out. In fact, as will be shown beyond, the superficial portion of the vaginal plug is actually the sluffed-off vaginal epithelium surrounding the coagulated seminal fluid. Thus the plug is partly of vaginal origin.

In later papers Lataste makes many contributions to the knowledge of the vaginal plug. In 1883 he described the vaginal plug in other rodents and pointed out that this formation was evidently not limited to a few species but was characteristic of the entire class.

Regarding the function of the vaginal plug, he slightly modifies his former position and concludes that its rôle is not only to prevent the sperm from flowing out of the vagina but rather by a filling up to push the sperm into the uterus. He extended the observation of Blanchard that the vaginal plug consists of two parts, differing in structure, a central core and a superficial envelope. He described the central part as consisting chiefly of the coagulated secretion of the seminal vesicles and also of a quantity of mucus, 1888a, while the superficial portion, *enveloppe vaginale*, was formed of stratified epithelial cells. The *enveloppe vaginale* is produced in the female by a rapid exfoliation of cells from the uterine glands and the vaginal walls on account of the irritating presence of the coagulated core. (His conception of the cause of the exfoliation is entirely incorrect.) The envelopment of the core by loosened epithelium from the vaginal wall serves to make easy the expulsion of the vaginal plug. This epithelial production he thinks is probably of a pathological nature and may be compared to the condition in women known as *vaginite exfoliante*.

These studies of more than thirty years ago by Lataste are in most respects surprisingly correct and it is only the nature of the process by which the outer epithelial envelope is formed with which we would materially differ.

Tafani, in 1888, described the vaginal plug in the mouse and found it to fall out about thirty hours after copulation.

Steinach ('94) found that the removal of the seminal vesicles

from rats did not influence their sexual instincts or ability to copulate, but decidedly impaired the power of the male to fertilize the female.

Sobotta ('95) also has studied the formation of the vaginal plug in the mouse and found it present after every copulation. Histologically it consists of an homogeneous mass which is surrounded by an envelope of vaginal epithelium. Spermatozoa are more abundant in the central mass at its upper end or that portion near the uterus as the plug lies in the vagina. He confirms the observations of Tafani regarding the fate of the vaginal plug, finding that its surface gradually becomes soft and loose and the entire mass falls out of the vagina about twenty to thirty hours after copulation. Sobotta states that the vaginal plug in the guinea-pig falls out much sooner than in the mouse, being eliminated from the vagina within from four to nine and a half hours after copulation. The longer interval is approximately correct. He claims to have at times observed another copulation following the expulsion of the first vaginal plug.

Camus and Gley ('96) studied the coagulation process in the formation of the vaginal plug. They claim coagulation to be due to the influence of a prostatic enzyme, "vesiculase," upon the secretion of the seminal vesicles. The action of the prostatic enzyme is specific towards the seminal vesicle secretion of any rodent. The prostatic enzyme of a rat will coagulate the seminal fluid of a guinea-pig and vice versa.

Rubaschkin ('05) returns to the old opinion of Reichert, 1861, in claiming that the vaginal plug is not a constant formation in the guinea-pig following copulation. His statements are as follows: Bei der Maus (Sobotta), und nach Bischoff und Hensen auch bei Meerschweinchen bildet sich nach dem Coitus ein charakteristischer Vaginal-pfropf, der auf einen vorausgegangenen Coitus hinweist. Ich muss hier die Beobachtungen von Reichert bestätigen, dass beim Meerschweinschen ein solcher Vaginalpfropf sich meistens nich bildet. Von aussen konnte ich einen klaren Pfropf in der Vagina niemals erkennen; in einigen Fällen liessen sich einige Schleimstreifen bemerken, die aber ganz unregelmässig und nicht immer zu Tage traten. In seltenen Fällen wurden nach dem Secieren Vaginalpfröpfe gefunden,

welche zum Teil aus verdichtetem Schleim, zum Teil aus Epithel-
zellen bestanden. Unter diesen Verhältnissen ist die Bedeutung
des Vaginalpfropfs beim Meerschweinchen ganz nichtig, und am
Anfange meiner Arbeit habe ich, durch diese Angabe Bischoff's
irregeführt, einige Tiere verloren, weil sie zu spät getötet wurden.

Königstein ('07) described the vaginal plug in rats and agrees
with the observations of Lataste, Tafani and Sobotta. He finds
also the vaginal plug to consist of two parts, a central and a
superficial. The vaginal plug contains in addition to the secre-
tions of the male genital glands, mucus, detritus, many leucocytes,
squamous epithelial cells in large numbers and a granular eosin-
phil staining secretion.

From this review the knowledge of the formation of the
vaginal plug is found to be rather complete, although disagree-
ments as to facts are expressed by several authors. It seems
well established that the formation of a vaginal plug following
copulation is a general phenomenon among the various species
of rodents. The plug proper consists of a central core formed
mainly by coagulated fluid from the seminal vesicles and this
is surrounded or enclosed by a mass of flat epithelial cells,
apparently derived from the vaginal wall. The coagulation of
the seminal fluid may be due to the action of a prostatic enzyme
although it is claimed that the coagulation occurs without the
presence of such an enzyme. The vaginal plug as a whole
falls out of the vagina a few hours after its formation.

On the other hand it is not clear from the literature just
how or why the peripheral part of the vaginal plug, *enveloppe
vaginale*, of Lataste is formed. And the manner and cause of
the separation of the epithelial lining from the wall of the vagina
are also unknown. These points could not be clearly understood
without a knowledge of the changes occurring in the wall of the
vagina and uterus during the œstrus, at which time copulation
and the formation of the vaginal plug take place.

As we pointed out in our description of the œstrous changes,
there is a stage in the cycle when immense numbers of leucocytes
accumulate immediately below the epithelium lining the uterus
and the vagina. From this position the leucocytes attack the
epithelial cells and at the same time dissolve or destroy the

connection between the mucosa and the subjacent connective tissue over extensive areas. This reaction is taking place a few hours after copulation during the latter part of stage one and throughout stage two of our description. A few hours later, during stage three, the leucocytes have made still further progress in their invasion of the mucosa and the destruction of its connection with underlying tissues. In certain sections of the uterus the entire mucosa filled with immense numbers of leucocytes is completely separated from the uterine wall and lies within the lumen, while in other regions the epithelium is loosely connected but still hanging to the wall. This disconnected and degenerating mucosa loaded with leucocytes breaks into small fragments during the fourth stage and is expelled from the uterus and vagina, while a new mucosa begins to regenerate from the mouths and the regions about the uterine glands and from the deeper layers of the vaginal epithelium. This is the fate of the mucosa when no copulation has taken place.

There is, then, no pathological "vaginite exfoliante" due to an irritation of the vaginal wall by the seminal fluid as Lataste thought. But a simple periodic œstrous breaking down of the uterine wall under leucocyte invasion, entirely independent of whether copulation takes place or not.

When copulation has occurred the loss of the epithelium follows a somewhat different course. Immediately after copulation the coagulated seminal fluid forms a mass within the lumen of the vagina and partly extending into the uterus. Around this mass the mucosa forms a close fitting envelope, thus preventing its early dislocation. The envelope serves to retain the plug in the vagina until the fourth stage of the œstrous cycle at which time the enveloping epithelium becomes completely separated from the vaginal wall by the dissolving effects of the leucocytes. The epithelium is now expelled as one continuous tube forming the cover around the vaginal plug instead of sluffing off in smaller pieces as occurs during the fourth stage when a copulation has not occurred. However, the vaginal epithelium may occasionally be shed en masse without copulation. In one striking case the epithelium was pulled out of the vagina as a conical sheath, enclosing the speculum that had been introduced for examination.

It is clear, therefore, that what was termed by Lataste the "enveloppe vaginale" is the layer of epithelium separated from the underlying connective tissue by the dissolving action of the leucocytes which invade the walls of the uterus and vagina at this time. It is also readily understood how the plug, after its short sojourn in the vagina and cervix of the uterus, is finally separated from its adhesion or tight connection with the wall and expelled as a mass from the vagina.

A possible function or effect of the vaginal plug in addition to those before mentioned has recently been suggested by Long ('19). He states that a stimulation of the cervix of the uterus in rats, by merely inserting a glass rod during stage one of the œstrus, prolongs the next cycle, and suggests that the vaginal plug may also act in this mechanical way. We have not tested the prolongation of the cycle in guinea-pigs following copulation without conception as compared with its length in virgin animals.

4. The Œstrous Rhythm.

In our earlier review of literature it was pointed out that the knowledge of the actual time of ovulation in the guinea-pig was decidedly inexact. Nothing scarcely was known of the periodic recurrence of the œstrus stages in a given female. In short the moment of ovulation in the guinea-pig was not available for accurate experimental purposes and no definite criterion or method had been devised for detecting the œstrous condition. And this was true in spite of a very long list of studies pertaining to the reproductive activities of these animals.

Reichert, as long ago as 1861, had found that the Graafian follicles rupture about nine to ten hours after copulation. This, in general, approaches correctness, but in cases where copulation has not taken place, or failed to be observed, such knowledge is of little consequence. Rubasckhin ('05) had more recently claimed that the vagina was open and the vulva somewhat inflamed ten to twelve days after parturition, but this is certainly too short an interval to indicate an actual return of heat. It must be remembered that the female guinea-pig goes into "heat" and accepts the male almost immediately after the delivery of her litter. This fact makes the length of Rubaschkin's interval still more improbable.

The most valuable and extensive investigations of the repro-
ductive activities of the guinea-pig were those made by Leo Loeb
('11, '14).   But here the data were derived almost entirely from
examinations of the uterus and ovaries after their removal from
the body of the female.   While such studies did give a means of
comparing the conditions found among different individuals at
different times, and made it possible to estimate approximately
the length of the sexual periods, yet this estimate could not be
transferred with certainty to any one living individual.   We
further objected to Loeb's method of study since it failed to
permit an investigation of the recurring œstrous periods in a
number of unoperated females.   The results of such an investiga-
tion would be most important in determining the influence of
any unusual or experimental conditions introduced with intent
to modify the intervals between ovulations or other periods of
the sexual cycle.   These are just such problems as Loeb had
under consideration.

The entire literature showed that any such thing as a regular
œstrous flow was completely undiscovered for the guinea-pig.
It became necessary, however, for our studies to have an accurate
knowledge of ovulation times, and to determine this, extensive
investigations of the sexual cycle in the guinea-pig were under-
taken.   A simple method of examining the vagina of the living
animal proved to be of the greatest value.   Virgin females were
selected and the fluid present in the vaginæ was taken daily by
means of a small nasal speculum and cotton swab.   This fluid
was smeared on slides, stained and studied microscopically.
The method is fully described in the former paper.

  It very soon became evident that the vagina generally con-
tained little or no fluid, but that periodically a great accumulation
of mucus and cells was to be found.   This excessive amount of
mucus and cells is to be recognized as a typical œstrous flow.
The constituent elements of the fluid change in their relative
abundance in a definite manner from the beginning to the
cessation of the flow.   Four clearly marked stages, as mentioned
above, could be separated by microscopic examination of the
fluid smears.

These changes in the composition of the vaginal fluid were

found to be associated with comparable changes in the structure of the epithelial walls of the uterus and vagina. And not only was this the case, but the changes in the vaginal fluid proved to be most reliable indices of definite processes taking place in the ovaries in connection with the rupture of the Graafian follicles and the expulsion of the ova. It is, therefore, evident that by an examination from time to time of this fluid, one may know the exact condition of the ripening follicles in the ovary and very nearly the exact moment of ovulation.

The œstrous cycles in a group of guinea-pigs were followed for a number of months in order to establish the normal periodicity or rhythm. The amount of variation that might exist in the length of the cycles in a given female was studied as well as the variations in cycle lengths among different individuals. An attempt was further made to discover any seasonal variations that might exist.

Only slight time variations were found in the periodic rhythm of a given female. For example, in one animal the record of six consecutive periods shows the œstrous flow to begin on the sixteenth day five times and on the fifteenth day once. In another case of seven consecutive periods the flow began on the sixteenth day six times and on the seventeenth day once. For further cases the reader is referred to the table given in our former paper.

There is only a limited variation in the length of the œstrous cycles among different individuals, ranging between fifteen and seventeen days in younger animals. In exceptional cases the period is slightly lengthened in older multiparæ, sometimes reaching eighteen days. These limits of fifteen and eighteen days for the lengths of the œstrous cycles have never been violated under normal conditions during the several hundred observations which we have now recorded. The method of examining the vagina for the closure membrane above described, and, in the case of its rupture, for the composition of the fluid contained within the lumen, renders these individual variations of no consequence in determining the exact "heat period" and time of ovulation.

Slight, if any, seasonal variations are shown by our animals. This may be due, however, to the uniformly warm temperature maintained in the breeding rooms during the winter months.

For a full description and photographs of the structural changes occurring in the œstrous fluid, the vagina, uterus and ovaries, the reader is referred to the original account.

After the publication of our results it was found that one of the last papers by Leo Loeb ('14), bearing on a related subject, had unfortunately been overlooked. We regret this, since a discussion of his methods and results would have been somewhat clearer in connection with our full consideration of the œstrus given in the previous paper than in the present connection. In earlier papers Loeb ('11) had completely failed to establish a definite length or periodicity for the sexual cycle in the guinea-pig. In the last paper, however, the length of the cycle was more nearly determined and a very thorough description was given of the microscopic changes taking place in the uterine wall during the heat period. Our independent account of the structural changes in the uterine wall fully confirms Loeb's description. But we are unable to agree exactly with the lengths of the sexual periods as estimated from his examinations of the removed uteri. In a still more recent article Loeb ('18) repeats his 1914 estimates and claims the lengths of the sexual cycles to vary between thirteen and a half and nineteen days.

In all cases Loeb's investigations had centered in a study of the sectioned uterus and ovary, thus necessitating their removal by operation or the death of the animal. Either procedure permits only one observation on a given female. No investigation of the uterus and vagina in the living animal had been made and no continuous observations on the consecutive cycles of given individuals were carried out.

As mentioned before, we recorded not only the structural changes of the uterus, but almost equally as marked changes in the wall of the vagina. And what we consider to be of still more importance from an analytical or experimental standpoint as a means of estimating the moment of ovulation, was the complete record of the changes in the microscopic composition of the vaginal fluid during the different stages of the sexual cycle. The removal and examination of this fluid is made without in any degree injuring the uterus or vagina and does not interfere with the further use of the female for ovulation and breeding

records.   This knowledge of the definitely changing structure
of the vaginal fluid made it possible to study the œstrous cycles
in many living females and reduced the time element of ovulation
in the guinea-pig to a certainty.

We considered in a somewhat different manner the connection
between the uterine reaction and the secretion of the corpora
lutea, though essentially we share Loeb's ideas of the functions
of these bodies.   It was concluded that the duties of the corpora
lutea are probably about what Beard ('97) long ago argued in his
monograph on "The Span of Gestation and the Cause of Birth."

The development and the degeneration of the vaginal and
uterine mucosa were found to follow very closely the development
and degeneration of the corpora lutea in the ovaries.   The case
was stated as follows: "The breaking of the Graafian follicles
occurs during the œstrus as a result of congestion which began
in the theca folliculi at about the same time as the congestion
of the stroma of the uterus and vagina.   And finally when the
regenerative growth of the uterine mucosa sets in, the ovaries
then possess new corpora lutea in an active state of differentia-
tion which have been derived from these recently ruptured
follicles."   The presence of the new active corpora lutea sup-
presses the final steps in the development of the almost mature
Graafian follicles in both ovaries, whether the corpora lutea be
located in only one of the ovaries or both.   When the corpora
lutea become less active and their degeneration has proceeded
to a certain extent, another ovulation may then take place.
Therefore, the functions of the corpora lutea are probably, first,
by their presence and activity to inhibit ovulation or to determine
its time, and, secondly, to preserve the structure of the uterine
wall and prevent its degeneration.

Loeb has attacked the problems of corpora lutea function in
the guinea-pig in a more direct experimental way than have other
investigators.   Yet while studying the effects of corpora lutea
removal on the length of the ovulation period, he has been
handicapped by the fact that his animals, after the initial
operation, were later killed for examination and thus were no
longer available for a continuation of the experiment.   Only
one observation was obtained from any particular female.   The

effects on the lengths of the ovulation periods of the removal of corpora lutea or the application of its extracts could be investigated to great advantage on guinea-pigs in which the œstrous cycles are definitely known and followed through a number of consecutive periods. This could readily be done by the method before described. This method is also of value in locating the early stage of developing eggs and in making exact matings for studies on fertilization, etc.

Attention may be called to further slight objections that might be raised in considering Loeb's last paper. He studies the conditions in the structure of the uterine wall removed from females that had copulated shortly before, as well as, uteri from uncopulated females, and states, page three: "The sperm fluid present in the lumen of the uterus exerts a pressure on the surface epithelium and may thus contribute to the harmful influence of the leucocytes." This idea is incorrect since it may be clearly shown that the action of the leucocytes is equally as harmful in the destruction of the uterine epithelium during the œstrous period of virgin females.

In a similar connection Loeb also finds, page 11, that the number of leucocytes in the uterine mucosa is much smaller in animals that have not copulated. Again, page 16, "A few leucocytes can also be seen in the uterus of animals in which copulation had been prevented. . . . In such cases (non-copulated animals) also some degenerative changes occur in the uterine epithelium, but they are less marked than in animals which had copulated." These statements are not entirely in accord with our findings since there is no such marked discrepancy between virgin females and those that have copulated. Such conclusions are probably due to the fact that the uteri examined were not removed from the non-copulated females at the maximum moment of leucocyte migration and degeneration of the uterine wall (our "third stage"). Loeb had no exact means of knowing the comparable stages in copulated and non-copulated females.

The uterine mucosa of our virgin females may show leucocytes to be equally as abundant at comparable stages as the uteri from specimens after copulation. It must be recognized in this con-

nection that each stage in the condition of the uterine wall during the œstrous is of short duration and unless the uterus be removed at a given time, the abundance and position of the leucocytes and the condition of the uterine wall will be changed. Chance was against Loeb's removing the uteri from the non-copulated females at the moment of maximum leucocyte migration, since he had no exact means of knowing when this would occur without having first observed copulation.

Active migration and accumulation of leucocytes may be observed in the entire absence of sperm fluid. The rôle of this fluid and the modification of the shedding or sluffing off of the vaginal and uterine epithelium in its presence was fully brought out in the discussion above of the vaginal plug.

Loeb's estimation of the ovulation times and uterine changes from microscopic examination of fixed specimens does not make it possible to know within a few hours, or even days, of the exact moment of ovulation in a given living individual. He states, however, on page 31, that to determine the effects of the removal of the corpora lutea on the duration of the sexual cycle, it "was necessary to determine the length of the cycle in the normal guinea-pig." Not only is this necessary, particularly in view of the wide variations Loeb finds in the normal sexual cycle among different individuals, but it is better or even necessary, to know the actual length and variations of the sexual cycle in the given specimen experimented upon. As evidence of the correctness of the last statement, we may cite Loeb's method and results in determining the normal cycle lengths. This was done "by observing the time of heat of a guinea-pig and by examining the uterus and ovaries at known intervals" (after removal from different individuals). Such examinations were made on many specimens that had to be either killed or operated upon. The following ovulation intervals were thought to be the normal sexual cycles, page 31, "We found the length of the sexual period to be usually sixteen to eighteen or nineteen days; sometimes the new ovulation may take place as early as fifteen days after copulation. In two exceptional cases we observed the new ovulation as early as thirteen and a half to fourteen and a half days." The sexual cycle, therefore, varies in length from

thirteen and a half to nineteen days, a range of almost six days.

On this basis it is seen to be practically impossible to state within a day or so of when the next ovulation will take place in a female that has just passed the "heat period." And the "heat period" was very indefinitely known unless copulation had been observed. Thus, as a usual practice, in order to prevent pregnancy following a copulation used to prove the existence of heat, the oviducts were previously tied. Such a procedure might easily modify to some degree the sexual cycle and is inconvenient for further study of the animal.

Any significant experimental modification of the ovulation intervals could be readily detected by a simple examination of the microscopic structure of the vaginal fluids collected from a guinea-pig.

Finally, the "signs of heat" recorded from a report by Miss Lathrop, an animal breeder, are, according to our experience in examining and mating guinea-pigs, generally inaccurate and of little value for use in experimental studies.

5. SUMMARY.

1. The œstrous cycle in the guinea-pig is very definitely limited in length. Ovulations follow one another every fifteen to seventeen days in younger individuals, while in old females the period is slightly lengthened, in exceptional cases to eighteen days.

2. These individual variations are readily controlled by the method of vaginal examination described in this and a former article, so that the actual moment of ovulation in any given female may be determined to within almost an hour of the rupture of the Graafian follicles.

3. During the period of sexual inactivity, the dioestrum, as well as during pregnancy, the orifice of the vagina is completely closed by an overgrowth of epithelium which we have termed the "vaginal closure membrane." This membrane ruptures just before or during the first stage of the œstrus in non-pregnant females and before parturition in the pregnant. It always re-forms to close the vagina shortly after the "heat period" has

passed. The presence of this closure membrane is therefore positive evidence that the time of a new ovulation has not been reached. When the membrane is ruptured and the vagina open, the ovarian condition may then be determined by examination of the fluid content of the vagina as described. A knowledge of this closure membrane greatly facilitates the examination of females in locating the beginning of the œstrus.

4. Copulation takes place in the guinea-pig during stage one of the œstrous period and ovulation occurs, as we previously showed, at the end of the second stage or the beginning of the third. Long ('19) finds exactly the same to be true for the rat.

5. At the time copulation occurs, the vagina is filled with a clear foamy mucus, and this is the only time at which no leucocytes are present in the fluid. Both the nature of the vaginal fluid at this time and the absence of leucocytes contribute to the success of copulation and fertilization.

6. A vaginal plug is formed a few minutes after copulation, during the first stage, and remains in the vagina for only a short time, being expelled during the fourth stage of the œstrus.

The core or center of the vaginal plug is composed chiefly of coagulated seminal fluid. This is enclosed within an envelope of epithelial cells, being simply the sluffed-off mucosa from the wall of the uterus and vagina. This epithelial cover which is thrown off at every œstrous period is loosened or dissolved away from the uterine and vaginal wall by an enormous invasion of leucocytes which progresses from the first to the third stage. The breaking away from the vaginal wall of this enveloping epithelium causes the plug to fall out of the vagina during the fourth stage. After the expulsion of the vaginal plug, the mouth of the vagina is closed by the growth of the vaginal closure membrane.

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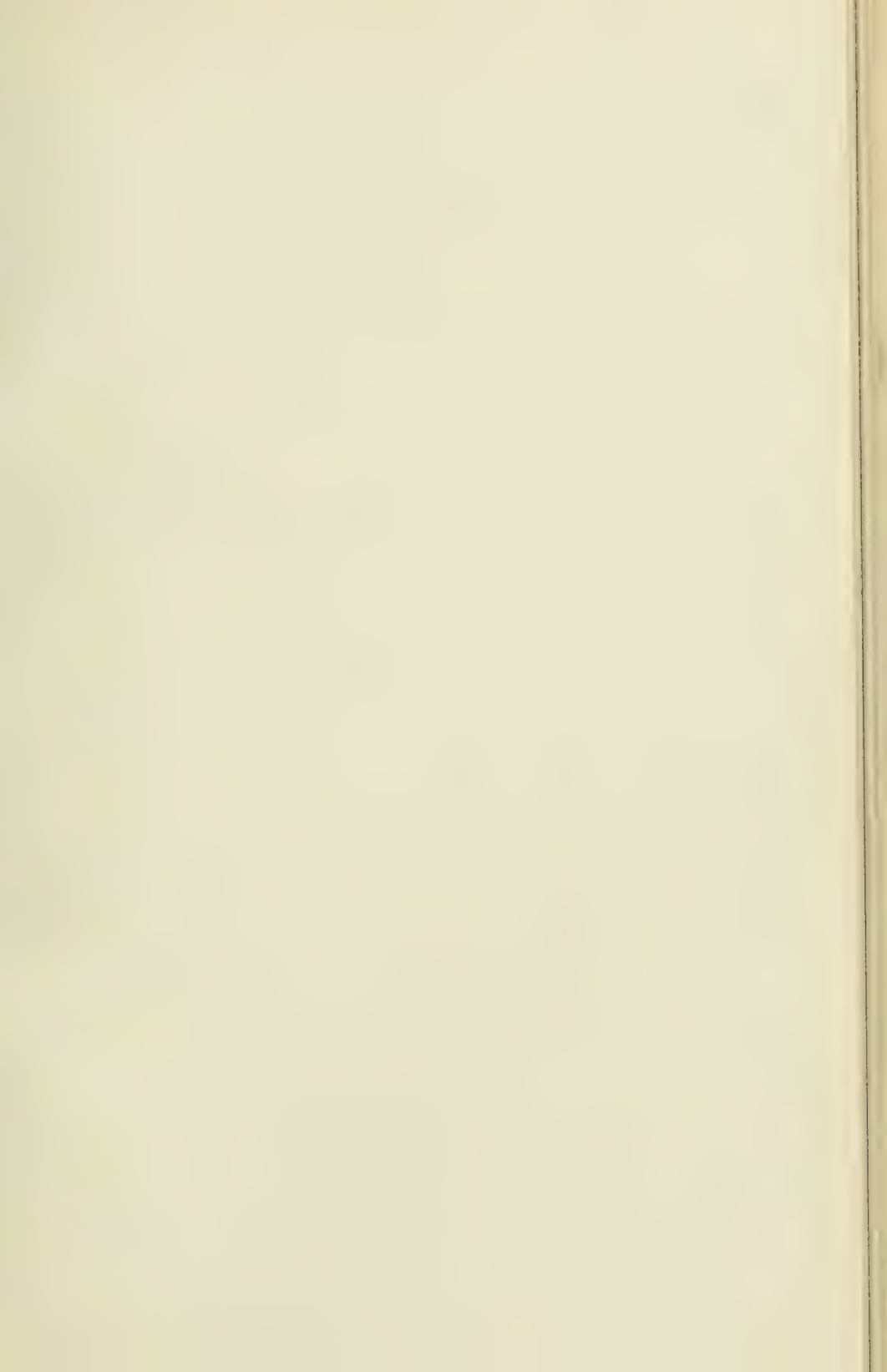
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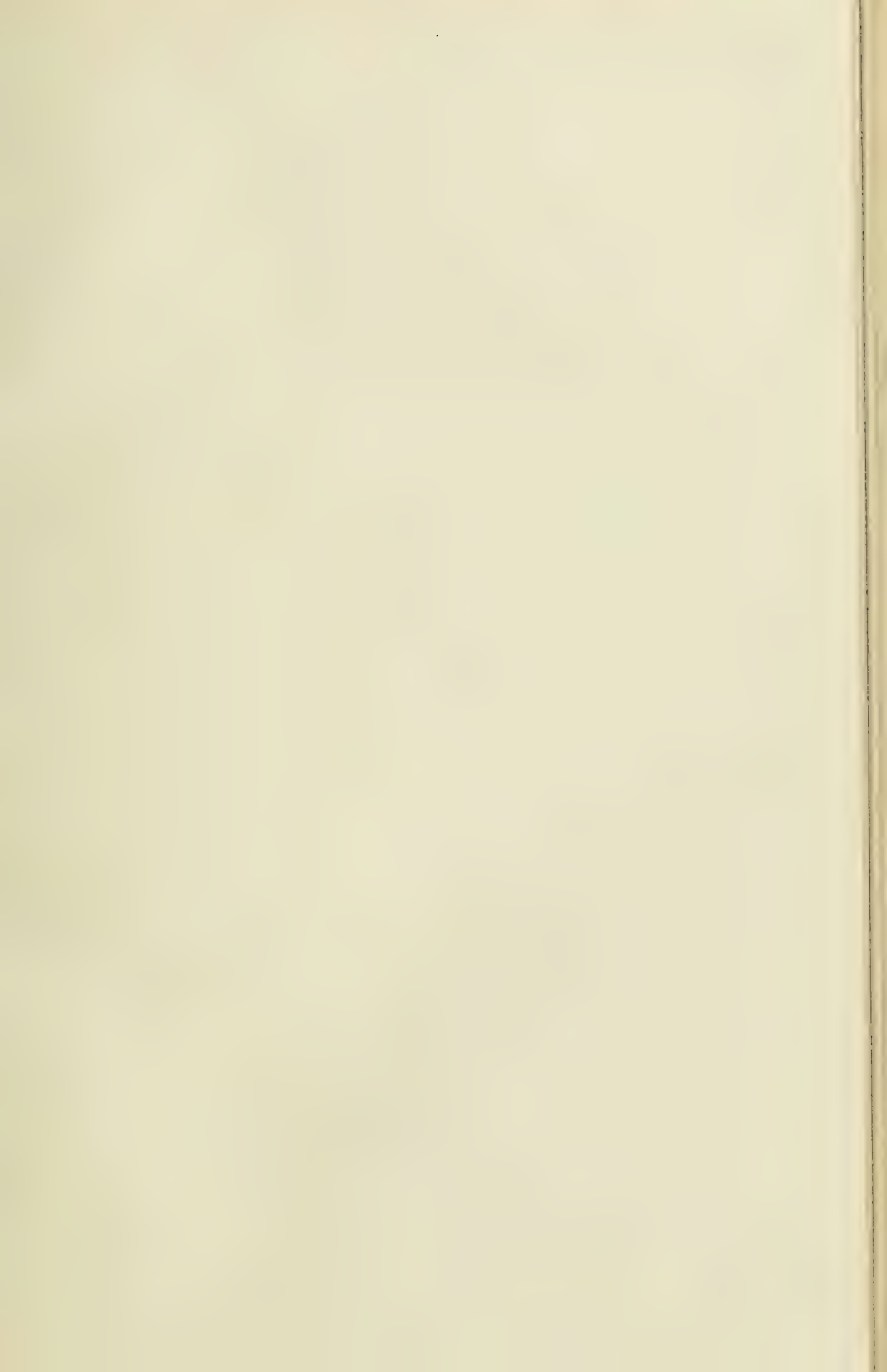
Developmental rate and the formation of embryonic structures.

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The eggs of different species develop at specifically characteristic rates. These rates vary for a given species within certain limits. Variation in the direction of increased rate is far more limited and more difficult to bring about experimentally than is the opposite variation towards a decreased or slower rate.

The slight acceleration of developmental rate that may be induced in early embryos does not seem to cause any marked deviation from the normal course of development, on the contrary such embryos are unusually well developed. It might be said that the ideal rate of development is probably somewhat faster than the so-called normal average usually followed. In other words, the normal conditions of development are not entirely the best possible conditions.

The limits of retardation in developmental rates are extremely wide. The rate may be slowed down to almost zero or development may actually be to all appearances stopped for long or short periods of time in many species without noticeably injuring the embryo. Such an interruption normally occurs during the development of certain eggs as those of birds and some mammals. In eggs having a continuous development, such as those of fish, the rate of development may be slowed to apparent stoppage at many stages and held in such a condition for some time without injury to the embryo which results after the inhibiting influence has been removed. However, when the rate of development is retarded but not entirely stopped at certain critical periods and development is allowed to proceed at this diminished rate for some time, most serious structural anomalies are induced.

Double monsters of varying degrees of doubleness may actually

be produced by slowing the rate at a time when the primary embryonic bud should arise. Normally the initial appearance of the primary embryonic bud probably suppresses the appearance of other buds which potentially exist, but when the primary bud is delayed in its appearance it becomes possible for more than one bud to arise, usually two. The distance apart of these two buds on the blastodisc determines the degree of doubleness of the resulting individual. Buds arising close together give two-headed monsters, while buds arising at opposite points on the periphery of the disk, 180° apart, each give rise to a complete individual, in such a case twins result.

When the two buds arise simultaneously they have equal chances in development and symmetrical double monsters result. If, however, one bud obtains the start over the other bud this start constitutes a supremacy which almost invariably makes it possible for the leading bud to develop into a perfectly normal specimen, and invariably defeats the possibility of normal development on the part of the slower bud.

An investigation of a large series of such double fish embryos lends strong support to the interpretation that the late bud is inhibited in its rate of development on account of the presence of the leading bud, just as the first bud to grow out from a notch on the leaf of *Bryophyllum* inhibits the growth of other buds as Loeb has so strikingly shown. The inhibited rate of development in the lesser component tends to suppress and interfere with the normal origin and development of certain organs, especially the eyes and other head parts. Organs may entirely fail to arise, or develop abnormally after they do arise.

In the series of double fish when both individuals or both heads, as the case may be, are of equal size they are both normal, but whenever one component is larger than the other, the larger one is almost invariably normal and the smaller is *invariably* defective. This is not only true in the present series of specimens but also in all illustrations and descriptions of double monsters which I have been able to collect from the literature.

These embryos furnish material for an analysis of the causes of many common structural defects about which there has been considerable discussion, a consideration of this phase of the subject will be given in the complete review of the experiments.

SYMMETRY REVERSAL AND MIRROR IMAGING IN MONSTROUS TROUT AND A COMPARISON WITH SIMILAR CONDITIONS IN HUMAN DOUBLE MONSTERS

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EIGHT FIGURES (FOUR PLATES)

In the extensive literature on the development of monsters there are frequent references to reversed symmetry and mirror imaging of unpaired organs. This latter condition is most frequently seen in double monsters of the dicephalous type, one component of the monstrosity often exhibiting a partial or complete situs inversus viscerum. As far as I am aware, mirror imaging in the viscera has only been found, or at least described, in human monsters, although there is no morphological reason why monsters in the lower vertebrates should not occasionally exhibit this condition. Recently an opportunity occurred to examine the question. In a collection of newly hatched trout[1] containing many double monsters a number were found in which the abdominal viscera of one component showed reversed symmetry in some degree. The mirror imaging was practically perfect in some cases, while in others it was only slightly indicated or irregularities appeared which made the interpretation difficult. The conditions found in these fish, although not entirely novel, seem sufficiently interesting to merit a brief description. Their theoretical bearing will be considered in conjunction with similar conditions in higher forms.

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[1] I am indebted to Professor Stockard for the use of this material which he has under investigation from a somewhat different standpoint.

SYMMETRY REVERSAL IN MONSTROUS FISH

The collection of monsters under consideration exhibited various degrees of doubling. The less pronounced cases showed externally a double-headed condition with the rest of the body single. At the other extreme were duplicate twins attached face to face on a single yolk. Between were the varying degrees of anterior and posterior duplicity. In the figures (pls. 1 and 2) all the specimens are viewed from the ventral or ventrolateral surface with the yolk dissected away.

The normal asymmetry of the viscera is shown in each of the twin fish of figure 6. The stomach first bends slightly to the left, then sharply to the right, passing to the pyloric end where it turns posteriorly into the intestine. The swim bladder ($S.B.$) lies dorsal to and slightly to the left of the stomach. The liver ($L.$) lies to the right and in the hollow formed by the bending of the stomach. The urinogenital system need not be considered here.

In the monsters the doubling of the viscera corresponds in degree with the external duplicity. In the specimens shown in figures 1, 2, 4, and 5 where the head and a considerable part of the trunk are double, there are two stomachs, two swim bladders, and two livers. The intestines are separate anteriorly, but unite posteriorly to form a common rectum which opens in the usual position through a single vent. Also, though not brought out in the figures, there are two hearts and two complete pairs of pectoral fins. In the specimen shown in figure 3, the duplicity does not extend as far posteriorly as in the foregoing. In this case there are two stomachs and two swim bladders, but the intestines are united immediately beyond the stomachs into a common bulbous enlargement ($C.I.$) from which a single tube leads straight backward to the vent. The liver ($L.$) is a single, irregular mass nearly twice the size of a normal liver and probably formed by the union of two separate liver buds. It lies on the right side of the monster considered as a unit. The swim bladder corresponding to one of the components (lower in the figure) passes backward on the dorsal side of the liver

and is almost concealed from view. The tip of it can be seen at *S.B.* The swim bladder of the other component is in the usual position. The specimens shown in figure 6 are twins which have been dissected away from a single yolk uniting them face to face. Each has the normal complement of organs.

It will be convenient to designate the two components of a monster as A and B. The lower component in each figure is A and the upper is B. If one imagine the figures turned so that the heads are toward the top of the page then A is on the left of the observer as he faces the ventral surface of the specimen and B is on the right. The scheme then conforms to that which Wilder ('04, '08, '16) has adopted for human monsters. If figures 1, 2, and 4 are now examined, it will be seen that in each case the asymmetry of one component is the reverse of the other, as far as the principal abdominal viscera are concerned. Component B (upper or right-hand) has the normal asymmetry—the stomach bends first to the left, the swim bladder (*S.B.*) dorsal and slightly to its left, the liver (*L.*) on the right side.[2] Component A (lower or left-hand), however, has reversed asymmetry—the stomach bends first to the right, the swim bladder (*S.B.*) dorsal and slightly to its right, the liver (*L.*) on the left side.

The transposition of viscera in one component is practically complete in the three specimens shown in figures 1, 2, and 4. In several other specimens (not figured) a partial reversal was indicated. Figure 3 shows a case in which the doubling involves only the head and extreme anterior end of the trunk. There are two hearts, as the figure shows, and four complete pectoral fins. In the abdominal cavity the two stomachs are crowded together and very much contorted, though both seem to bend toward the same side. There is a single large liver (*L.*), probably formed by the union of two liver buds. The swim bladder of component B (upper or left-hand) is the normal position. Its mate of the opposite side, for the most part hidden by the liver,

[2] The rights and lefts are used here with reference to one component, not to the entire monster.

does not exhibit the relations to be expected if mirror imaging
were present, as a comparison with the position of the swim
bladder in the corresponding component of figures 1 and 2 will
show. It is difficult to draw any positive conclusion from this
specimen, owing to the crowded condition in the abdominal
cavity; but from the position of the stomachs and swim bladders,
I am inclined to think that no reversed asymmetry is present in
either set of organs.

It is to be noted that the monsters showing complete reversal
of symmetry on one side are all in the same stage of duplicity
(figs. 1, 2, and 4). No sign of reversal was found in the more
complete stages of doubling. In the twins from the same egg
(fig. 6) each has the same (normal) asymmetry. In the case
described in the preceding paragraph, where the degree of dou-
bling was less marked (fig. 3), all indications were contrary to the
idea of symmetry reversal.

The mirror imaging described above, whether complete or
partial, is not by any means the rule in these monsters. Even
in the particular stage of doubling in which it occurs the major-
ity of the specimens show the normal asymmetry in both sets
of visceral organs. Figure 5 illustrates this latter condition.
Here it is obvious that both stomachs bend first toward the left,
forming a bay, which opens toward the right. The liver (*L.*)
lies in the bay; that is, on the right side of the component to
which it belongs. The swim bladders (*S.B.*) lie dorsal and
slightly to the left of their respective stomachs. Summariz-
ing the facts briefly, the abdominal viscera are mirror images of
each other in some cases (figs. 1, 2, and 4) and not in others (fig.
5), though most of the specimens present the same degree of dou-
bling. Discussion of this point may be conveniently postponed
until the conditions in human monsters have been described.

Despite the very simple condition of the gastro-intestinal
tract in fish, the normal asymmetry is very well marked, and
any change from this strikes the eye immediately upon opening
the abdominal cavity. In view of this, it is curious that neither
Windle ('95) nor Gemmill ('01, '12) observed any reversal of sym-

metry in their work on monstrous fish. Gemmill especially, in his study of the anatomy of double monstrosities in trout ('01), made a careful examination of the internal organs, but apparently saw no changes in symmetry, though some of his specimens exhibited the same degree of duplicity as those described in the present paper. Possibly reversal of symmetry is rare in fish, but the total number of specimens examined is too small to form any definite conclusion on this point.

SYMMETRY REVERSAL IN HUMAN MONSTERS

It is well known that some types of human diplopagi exhibit symmetry reversal and consequent mirror imaging in the unpaired viscera. At the time the present study was begun my attention was drawn to a double-headed human monster which had been kept in a museum jar for a number of years in our laboratory. It belongs to the dicephalous variety, which is the one most likely to show mirror imaging in the viscera, judging by previous reports.[3] A photograph of the monster is shown on plate 4 (fig. 8). Using Fisher's ('66) classification, it would be a dicephalus tribrachius dipus. The name is so descriptive that nothing more need be said of the external configuration. It is similar to the Barkow fetus, no 66 in Fisher's list, except that in the present specimen the hands of the median arm are placed palm to palm and the heads are somewhat nearer together. The sex, as in Barkow's case, is female.

The organs of the upper abdomen, with the exception of the liver, show complete mirror imaging (fig. 7). The stomachs are placed with the pyloric ends pointing toward each other. There are two spleens (*Sp.*), two gall-bladders (*G.B.*), two common bile-ducts, and two pancreases (*Pan.*), each set showing the proper relations to the corresponding stomach. (The hepatic ducts are not shown in the diagram.) The liver forms a large compound mass with many irregular lobules (not figured). The small intestines are separate to within two feet of the caecum, at which point they unite to form a common ileum. The

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[3] Fisher ('66); Eichwald ('70); Hirst and Piersol ('93).

large intestine, including caecum (*Cae.*) and appendix, is single. The caecum lies in the right iliac fossa, from which the colon (*A.C.*) ascends in the usual way to the liver. From here the transverse colon crosses to the hypochondrium of the opposite side, the splenic flexure lying in relation to the spleen of the B-component (right-hand, as one faces the monster). The descending colon has the usual course and relations. There is a single pair of large, lobulated kidneys, each with a normal ureter. The uterus, tubes, ovaries, bladder, and rectum are normal in size and position for a single individual.

The viscera are shown schematically in figure 7. It will be observed that the organs of the right-hand component (B) are more normal in shape and larger than those of the opposite side and that they have the normal situs. There is a disparity in size also in the thoracic organs (to be described below), again in favor of component B. The two heads, however, are practically the same size though one, again the right-hand (component B) is placed a little more in the direct line of the compound body.

The thoracic cavity contains two complete sets of organs. There are two hearts inclosed in a single pericardium. They were pressed close together, both apices directed forward and downward. In figure 7 the apices have been widely separated to show the medial surfaces of both hearts. It is obvious that there is transposition in the left-hand heart (component A). The left atrium in this case receives venous blood from the superior vena cava (*S.V.C.*) and hepatic veins (*Vv.h*) and delivers it to the left ventricle from which the pulmonary artery (*P.A.*) springs. The pulmonary veins here empty into the right atrium and the aorta (*A.*) springs from the right ventricle.[4] The reversal of symmetry is thus complete. The right-hand heart shows the normal symmetry and need not be described. The lungs consist of two pairs corresponding to the two tracheae. Those of the right-hand, or B-component, are normal in lobu-

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[4] A detailed description of the vessels in this monster will appear in a forthcoming paper by Mr. H. B. Sutton, of our laboratory.

lation and nearly so in size, while the other pair are much reduced.[5] There is a fair-sized thymus for each side.

The diaphragm is extremely incomplete. Both stomachs with their adnexa are herniated high into the thorax, especially in component A, the fundus of whose stomach lies almost in the root of the neck.

Summarizing the more important features of the monster: The viscera of the right-hand component (B) are the more normal in size and shape and they have the normal situs. Those of the other component (A) are, generally speaking, reduced in size or irregular in shape and display situs inversus. Externally the head and neck of component B are more nearly in line with the axis of the trunk. Obviously it is always the same component (the left-hand or A-component according to the scheme adopted in the present paper) which exhibits transposition of the viscera whether in man or in fish (compare figs. 1, 2, 4, and 7). This point will be discussed in a later part of the paper.

It has been frequently assumed, as pointed out above, that monsters of certain types, especially the dicephali and ischiopagi, may be expected to exhibit mirror imaging. The number of cases on record where actual examination disclosed this condition are however, very few,[6] and most of them are cited by Fisher ('66) in his very comprehensive paper on diploteratology. From his list I have collected the following cases:

Dicephali

Case 50. Ritta-Christina, a dicephalus tetrabrachius dipus (female) which lived about eight months. At autopsy it was found that the pericardium was single, but enclosed two hearts, which were right and left, touching at their apices. The stomachs, spleens, and pancreases were right and left, and placed so that the pyloric ends of the stomachs faced each other, the adnexa conforming as in the specimens described above. The livers, also right and left, were fused, and there

[5] It was impossible to determine the lobulation of this pair of lungs as they were partly destroyed when the corresponding head was removed from the body during delivery.

[6] Bateson ('94) states that Eichwald (Pet. med. Zeitsch., 1870) found some transposition of viscera in thoracopagi, though to a varying extent. The original paper was not available to the writer.

were two gall-bladders which occupied a median position. Other details need not be given here.

Case 60. Dicephalus tribrachius tripus (male). This specimen had two hearts, one right and one left, in a single pericardium; two aortae, one transposed, i.e., lying on the right of the vertebral column. The liver consisted of three portions, two lateral, each of which corresponded to the right lobe of a normal liver, one of them reversed, and a median lobe corresponding to the left lobes of normal livers fused. Each lateral lobe had a bile duct, gall-bladder, common duct, and portal vein symmetrically placed. There were two stomachs with pyloric ends turned toward each other; the fundus of that belonging to 'A' was in the right hypochondrium and therefore reversed, while that of 'B' had the usual position in the left. A spleen was connected with each.

Case 71. Dicephalus dibrachius dipus (Gruber); sex not stated. There were two food passages; two stomachs with the fundus of each turned outward, and two intestines to within five inches of the lower end of the ileum. There was a large compound liver, two gall-bladders, and two bile-ducts; no pancreas; one spleen on left stomach; two hearts, the right small and imperfect; two sets of lungs and tracheae; urogenital organs single and normal.

Case 74. Dicephalus dibrachius dipus (Horner); sex, male. The thorax contained a compound heart. There were normal right and left lungs and a third compound lung due to the coalescence of adjacent lungs of different foetuses. The liver was single, but compound with increased number of lobes. The gall-bladder was double with a common duct which terminated in two orifices, one for each duodenum. There were two stomachs, one on the right, the other on the left, having their pyloric orifices pointing towards each other. The two small intestines, more or less aherent, finally blended into a single tube. The colon was single. There were two pancreases, but only one spleen, which was attached to the larger left stomach. The kidneys were a single large pair; the bladder and genitals were single.

Case 102. Dicephalus monauchenos (White); sex, female. There were two stomachs, the left in the usual place, the right reversed, its larger extremity towards the right. The two were united at the pylorus and opened into a common duodenum. The liver was single and very large.

One further specimen may properly be placed with the foregoing five, namely, a dicephalus dibrachius dipus (female) described by Fisher (case 76) *which possessed a single globular stomach with right and left fundus resulting from the fusion of two stomachs.* An oesophagus from each mouth entered the compound stomach nearly at the same point. The liver and intestines were single.

The cases cited above together with the one given in the present paper, are the only definitely described cases of mirror

imaging in the dicephali. Unfortunately, the position of the viscera is not stated in the reports of Barkow's fetus (tribrachius dipus) and Ruggles' fetus (dibrachius dipus). Both of these had two stomachs, and it seems almost certain that one was transposed. We have, then, six certain cases of transposition and one indication of this condition in the fetus having a compound stomach with right and left fundus.

Incidentally, one rather striking fact is brought out in looking over the various reports. In human monsters the amount of doubling in the viscera does not necessarily correspond with the amount of external doubling, as was the case in trout. Fisher cites one case (no. 64, from Benedina), a dicephalus tribrachius tripus (male) in which the gall-bladder, stomach, pancreas, spleen, and intestines were all single, although there were two hearts, two urinary bladders, and two pairs of kidneys. Compare this with the two cases of dicephalus dibrachius dipus (nos. 71 and 74) in which the digestive systems were double as far as the lower part of the ileum. It must be admitted that Benedina's case, if correctly reported, is very unusual.

Among other classes of diplopagi in which the two components are more widely separated, it is difficult to find definite information on the position of the viscera. Bateson ('94) quotes Eichwald (l.c.) to the effect that the thoracopagous monsters examined by him showed, in almost every case, some transposition of the viscera of one of the bodies, though to a varying extent. The pygopagous 'Carolina twins,' Millie-Christina (colored), were examined while living, and it was reported that "the apex of Christina's heart is on her left side while that of Millie is distinctly felt in the right side." Gemmill ('02) reports a case of ischiopagus tripus (human) in which modified transposition occurred in the liver. His figure 14 seems to indicate transposition of the thoracic viscera of one component as well, but the author does not comment on it. Windle ('94) gives a report on the 'Orissa sisters,' Radica-Doodica, who were united in the thoracic region (xiphopagus or thoracopagus). Regarding the position of the viscera, he states that authorities differ as to whether one was situs inversus viscerum. In the case of

the famous Siamese twins, one of them is stated to have had a partial reversal of viscera. These few reports, meager as they are, show that some trace of visceral transposition or symmetry reversal may occur in monsters other than dicephali.

In the syncephali, including Janus monsters, transposition of the viscera in one component apparently does not occur, though it seems to me in one case a slight indication was observed. Wilder ('08), in describing a case of this kind (the 'Baldwin synote') makes the following statement:— "The common oesophagus leads into a common stomach, though evidently one formed of two components, since *it presents two cardiac enlargements one on either side of the oesophagus*" (italics mine). "The outline of the stomach is thus heart-shaped, but is not quite symmetrical, since the cardiac lobe of component A is a little larger than that of Component B." With regard to the remaining organs the author states that there is no trace of 'looking-glass symmetry.' The stomach of this synote is thus similar to that of Fisher's dicephalus (case 76) mentioned above.

Among other mammals a number of syncephali have been described: kitten, McIntosh ('68); cat, Reese ('11); pig, Carey ('17), but none apparently showed any trace of mirror imaging. Kaestner ('07) has described in detail several syncephalous chick embryos, with especial reference to the heart region, but they were not far enough advanced in development to show the position of the abdominal viscera. Bishop ('08) gives an account of the heart and anterior arteries in several dicephalous reptiles, but as no pronounced asymmetry of the heart is visible in this class of vertebrates, there is little opportunity to look for mirror imaging. In cases where two hearts were present, both aortic arches developed on each side. It is unfortunate that among the large number of double monsters reported so much attention has been paid to external features and so little to the position of the abdominal viscera.

DISCUSSION

The question of symmetry reversal and mirror imaging has been discussed most recently by Wilder ('04, '16), Bateson ('16), and Newman ('16, '17). It seems to be generally agreed that transposition of the viscera does not occur in human duplicate twins. In armadillo quadruplets Newman finds, after examination of a considerable number of sets, that no symmetry reversal is present in the viscera. The same is true in the duplicate twin trout (fig. 6) described in the present paper. Some mirror imaging, however, does occur in human duplicate twins and armadillo quadruplets, but it is confined to the integumentary structures (friction-skin patterns in the former case, arrangement of the scutes and bands in the latter). The integument of young trout, unfortunately, does not present any regular pattern of asymmetry, at least none could be detected, and thus yields no information on this point. In double monsters, however, it is admitted that a certain amount of symmetry reversal in the viscera is to be expected, although it may not occur in every case. Fisher, in 1866, clearly expressed this opinion, and is quoted by Wilder ('04) to this effect. Wilder, though also quoting Bateson's ('94) opinion, in agreement with Fisher, seems unwilling to admit the importance of this phenomenon and gives little space in his earlier paper (l.c.) to its discussion. In a later paper ('16), however, he discusses a very interesting case of mirror imaging in the friction-skin patterns of a human diplopage.

Newman, ('16, '17) has given perhaps the fullest discussion of symmetry reversal, both in multiple births and in monsters. The relations of symmetry observed in armadillo quadruplets are, he considers, "the results of an intricate interplay of three grades of successively operating symmetry systems, the later tending to obliterate the effect of the earlier, but not always successfully." This conclusion is based on the nature of the polyembryonic development observed in these animals and is explained by Newman as follows: "When the primary outgrowths are formed (i.e., fission in the blastocyst stage), they are the product of the antimeric halves of the first embryo and should

therefore show mirror-image relations. But a partial physio-
logical isolation of the two halves permits a certain reorganiza-
tion, or regulation of new symmetry relations, which tends
more or less completely to destroy the original symmetry, yet
often leaving a trace of the latter. Similarly, when the second-
ary outgrowths arise between the primary ones a certain residuum
of the primary symmetry may be carried over that frequently
manifests itself in mirror imaging between twins derived from
one-half of the original embryo. Finally, when each secondary
outgrowth organizes its own bilateral symmetry, it tends to lose,
partially at least, the earlier symmetry relations and to estab-
lish its own mirror imagings of right and left sides" (third grade
of symmetry). It must of course be remembered that in arma-
dillos, mirror imaging between twins is confined to integumentary
structures. In the case of duplicate twins and double monsters,
there would be according to Newman's conclusion, only two
'grades of symmetry systems.' Any mirror imaging present in a
monster would thus be evidence of the potency of a primary
symmetry which had not been overcome by the secondary sym-
metry acquired later by the separate components. If physio-
logical isolation occurs in a comparatively early stage, there
will be, he thinks, very little mirror imaging, as the secondary
symmetry will have more time to operate. Conversely, if it
appears somewhat later, there will be more mirror imaging. In
consequence, double monsters probably arise somewhat later in
ontogeny than duplicate twins, since the former more often show
evidence of mirror imaging.

Newman's suggestion regarding primary and secondary sym-
metry systems is to some extent supported by the conditions
found in trout monsters. However, by far the greater num-
ber of these monsters, of whatever degree of doubling, show no
influence of a primary system of symmetry, that is a symmetry
of the monster taken as a unit. On the contrary, each com-
ponent develops its own system (secondary, according to New-
man) as if it were entirely disconnected from its mate (fig. 5),
and this symmetry (asymmetry), moreover, is the same as that
of a normal fish. It is interesting to note that in the type of

double monster known as autosite-and-parasite, a number of
which occurred in the present collection, the parasite, whenever
it was of sufficient size to possess a complete set of abdominal
organs, always exhibited its own (secondary) symmetry and
never appeared as a mirror image of the autosite. It is only
in a small proportion of the monsters that the primary symmetry
of the whole is still potent, in which case mirror imaging appears
in the viscera (figs. 1, 2, and 4).

Newman's further suggestion that there is a direct relation
between the occurrence of mirror imaging and the period in ontog-
eny at which doubling takes place, does not accord with what
seems to be the mode of origin of monsters in fish. In this form,
the initial doubling probably always occurs at the same period
of development, regardless of the degree of separation of the
two components. This period corresponds with the first appear-
ance of the embryonic anlage at the circumference of the blas-
toderm, as Kopsch ('99) concluded in his analysis of the causes
of fish monsters.[7] In the case of double monsters, two em-
bryonic anlages are formed at the same time. The degree of
doubling will then depend on how near the two anlages lie to each
other. On this view, mirror imaging and the time at which
doubling first appears cannot be causally related. Nor is there
a very precise relation, it seems to me, between the amount of
separation of the two components and the occurrence of mirror
imaging. It is true that there is a stage of doubling more favor-
able than others for exhibiting symmetry reversal in one com-
ponent, but only a small proportion of the monsters even then
show any evidence of this condition (compare figs. 4 and 5).
Furthermore, specimens showing less separation than in the
stage just mentioned might be expected to exhibit more evi-
dence of primary symmetry (symmetry of the monster as a
whole) and therefore more mirror imaging, while in point of fact
the contrary is true (p. 268).

It was pointed out (p. 271) that in both fish and human mon-
sters it is always the same component (the left-hand or A-com-

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[7] This view apparently originated with Lereboullet. Kopsch has developed
it in considerable detail in the paper referred to above.

ponent) which exhibits transposition of the viscera. In this
the writer agrees with Eichwald, as quoted by Bateson ('94),
except that the latter uses the term 'right twin' for what is here
called left-hand or A-component. Bateson himself is in doubt
on this point and quotes Küchenmeister[8] to the effect that in
xiphopagous twins it may not be possible to say which is the
right and which the left. This objection, however, does not
apply to dicephalous forms, whether fish or human. Here the
undivided portion of the monster obviously has dorsal and
ventral surfaces and these may be traced without interruption
into the corresponding surfaces of the two components which
usually face each other to some extent. The right-hand and
left-hand components are thus easily distinguished. In cases
where mirror imaging occurs, the arrangement of the two sets of
organs is always the same[9]—the stomachs bend first toward the
lateral borders of the monster (taken as a unit), then toward
the median plane (plane of union) so that their pyloric ends
face each other; the livers lie close together or are fused. It is
difficult, however, to find an explanation for this fact, for even
if the reverse arrangement occurred, there would still be mirror
imaging—the fundus of one stomach facing that of the other,
the pyloric ends pointing in opposite directions, the livers lying
on the lateral borders of the monster. It has sometimes been
assumed that in normal development the direction of growth
taken by the liver bud determines the plan of asymmetry of the
remaining viscera. In the case of monsters having either two
livers or a composite liver, it might be further assumed that the
two liver buds, having formed independently, were drawn to-
gether by some sort of mutual attraction. If such a movement
took place, the anterior ends of the two intestines together with

[8] Die. angeb. Verlagerung d. Eingeweide d. Menschen, Leipzig, 1883. The
original was not available to the writer.

[9] An exception to this appears to have been found in the famous Siamese
twins where it was Chang, the left twin (right-hand or B-component of the
present paper), in whose body there were indications of situs inversus (Küchen-
meister, quoted from Bateson). This would give the converse of the usual
arrangement. The writer has not had access to the original description of these
interesting twins.

the pyloric ends of the stomachs would be drawn with the livers
toward the plane of union. This would result in the arrangement
found in practically all monsters in which mirror imaging occurs.
While the above assumptions do, to some extent, account for
the facts, there is some evidence to show, as will be pointed out
below, that the factors controlling asymmetry are located in
the primitive gut and become operative before the liver bud has
developed.

It must be admitted that we are still in the dark regarding
the causal factors underlying the conditions of mirror imaging
found in some types of monsters. The question here arises,
why, in a certain stage of doubling, should mirror imaging
occasionally appear and not always? One might assume that
the rate of development in one component of a monster occa-
sionally becomes a little slower than in its mate so that it tends
to fall behind and is unable to develop or express an independent
system of symmetry like that of a normal embryo. In this case
the lagging component might be thought of as sharing with its
more vigorous mate in a single system of symmetry, that is, the
symmetry of the monster taken as a unit. The result would
then be a mirror-imaged condition of the viscera. A suggestion
of inferiority in one component was noted in the human monster
described above where the transposed set of organs were found
to be slightly smaller and more irregular in shape than those
of the opposite side. In the fish monsters, however, no such in-
equality between the two sets of organs was observed. Further-
more, the assumption that a retardation of development in one
component predisposes to transposition of viscera is rendered
improbable by the conditions found in monsters of the autosite-
and-parasite type. Here it may be fairly assumed that the
parasite tends to be weaker than the autosite, and in fact is often
defective; still whatever asymmetry exists in the parasite is
that of a normal fish and never reversed. In other words, the
parasite, even in its failing struggle for existence, retains the
power to develop its plan of asymmetry as a separate individual.

It is extremely difficult to formulate a theory which will
satisfactorily account for a condition so casual in its appear-

ance as mirror imaging in the viscera of monsters, and further
work on the early developmental stages of these forms is neces-
sary before any definite conclusions can be drawn. The solution
will, of course, involve the more fundamental problem of what
determines the normal asymmetry of unpaired organs and why
single individuals occasionally appear with transposed organs.[10]
Very little progress has been made in this direction. The most
suggestive observations in the field are those of Pressler ('11),
on experimentally produced situs inversus in Bombinator. The
material was obtained from Spemann who performed the fol-
lowing experiment: In the neurula stage, a four-sided piece
of the medullary plate together with a portion of the roof of the
primitive gut lying under it was cut out and replaced in reversed
position, so that the anterior extremity of the piece was directed
posteriorly, the posterior extremity, anteriorly. From these
experimental embryos, tadpoles were reared which showed in
many cases a complete situs inversus viscerum. It has some-
times been assumed, as stated above, that the asymmetrical
growth of the liver bud normally toward the right influences
the position of the remaining organs. The question then arises,
what determines the direction of growth of the liver bud? Spe-
mann and Pressler's work seems to indicate that the factors
controlling asymmetry are located in the primitive gut and prob-
ably arranged in such a fashion as to cause the gut in normal
development to bend first toward the left, thus forcing the liver
bud to grow toward the right. We may suppose that when the
arrangement of these factors is reversed, as in the experiment,

[10] Bateson ('94, p. 560) points out that cases of this kind cannot be explained
on the ground that one member of duplicate twins has died or failed to develop,
since it has been shown that in duplicate twins neither member has transposed
viscera. Conversely, Küchenmeister (l.c.) collected 152 cases of transposition,
of which only one could be shown to have been a twin.

A somewhat similar suggestion has been made to account for cases of situs
inversus in single individuals, namely, that this condition results from com-
plete reduction of one component of a monster (autosite- and-parasite) in which
mirror imaging occurred. The autosite in this instance must necessarily present
the reversed asymmetry. In some cases it is thought that the parasite is taken
into the body of the autosite during development, and gives rise to certain kinds
of tumors. As far as I am aware, there is no evidence recorded that individuals
with complete situs inversus have possessed tumors of this sort.

transposition is produced. Pressler's observations are, it seems to me, very important and indicate the direction along which further experiments should be made to determine the cause of asymmetry. They do not, however, throw any light on the cause of transposition in integumentary structures as found by Newman and Wilder.

A very interesting suggestion as to the cause of asymmetry in the viscera is based upon the fact, first pointed out by Crampton ('94), that in certain gasteropods the position assumed by the adult organs is correlated with the early segmentation pattern. In these snails the more usual type of asymmetry with dextral shell is associated with a right-handed spiral cleavage. Some forms, however, such as Physa (Crampton) and Ancylus rivularius (Holmes), have normally sinistral shells and reversed asymmetry in the viscera; this condition was found to be associated with a reversal of cleavage. These observations very naturally led to the view that in gasteropods there is a causal relation between cleavage pattern and the type of asymmetry found in the adult.[11] It is very questionable, I think, whether this conception of the primary cause of asymmetry can be applied to vertebrates. For in monsters, as has been shown, two sets of organs may develop as mirror images of each other, one with normal, the other with reversed asymmetry, though obviously both have arisen at the same period of development from a single blastoderm. It is difficult to imagine how changes in early cleavage pattern, if such occur in higher forms, could bring about the development of two types of asymmetry in the same embryo, as in the case just cited. From the evidence at hand, it seems probable that the primary cause of visceral asymmetry in vertebrates is to be sought for at the completion of cleavage rather than in the period of cleavage itself.

[11] This view, first expressed tentatively by Crampton ('94), was later more fully developed by Conklin ('97, Jour. Morph., vol. 13) and by Holmes ('99, Amer. Nat.; '00, Jour. Morph., vol. 16) on the basis of additional evidence. Conklin more recently (Heredity and Environment, 2nd ed., 1917, p. 177) has expressed the opinion that the correlation between inversion of cleavage and inversion of symmetry observed in certain snails, will be found "*probably in all animals showing inverse symmetry*" (italics mine). I do not believe this latter generalization is warranted for the reasons given in the discussion (see beyond).

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PLATES

1 and 2 Specimens of monstrous trout showing complete mirror imaging in the abdominal viscera, ventrolateral view. *S.B.*, swim bladder; *L.*, liver; the stomach and intestine are not labeled. The position of the viscera in one component is the reverse of that in the other.

3 Specimen in which doubling is less extensive than in the foregoing (1 and 2). There are two stomachs and two swim bladders (*S.B.*) one of which is almost concealed by the compound liver (*L.*) The intestines unite immediately beyond the stomachs into a common enlargement (*C.I.*). Apparently no mirror imaging is present in this case. The two pear-shaped bodies anterior to the abdominal cavity are the hearts.
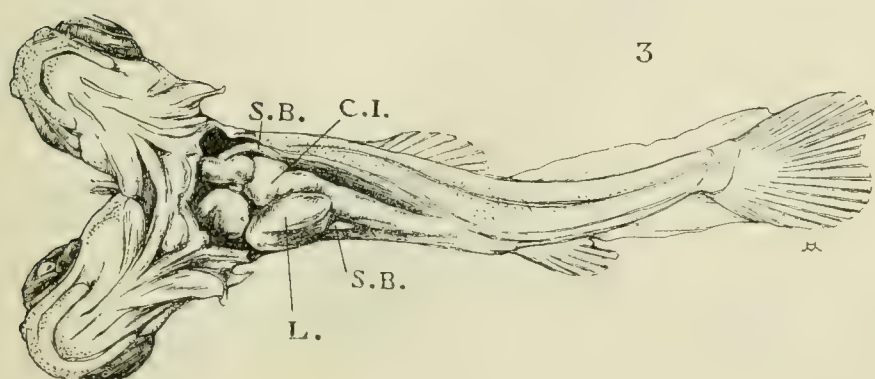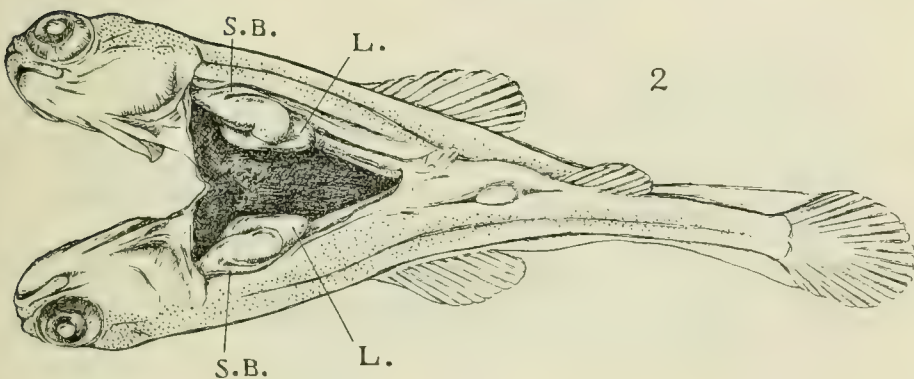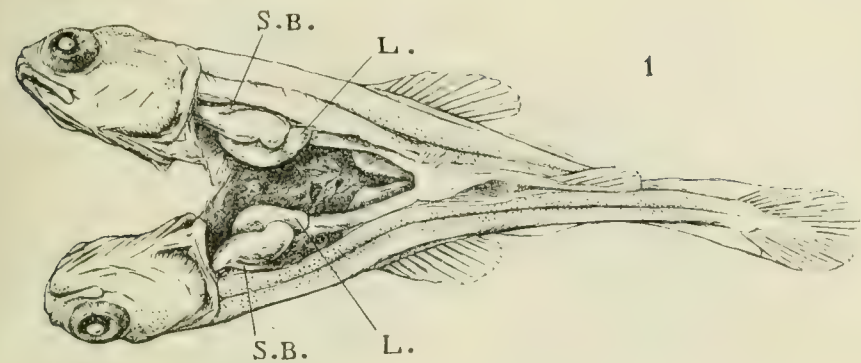
PLATE 1

SYMMETRY-REVERSAL IN MONSTERS
C. V. MORRILL

H. Murayama, del

PLATE 2

4 Specimen showing complete mirror imaging similar to figures 1 and 2 except that the two sets of organs are closer together, the livers (L.) almost in contact; ventrolateral view.

5 Specimen showing the position of the viscera in the majority of monsters in this stage of doubling. The normal situs is present in both components; ventrolateral view. Compare with figure 4.

6 Twins from the same egg. These two specimens lay on opposite sides of a single yolk mass. The position of the viscera is the same in both, the liver on the right, the stomach bulging toward the left as in normal fish (i.e., normal situs in both).
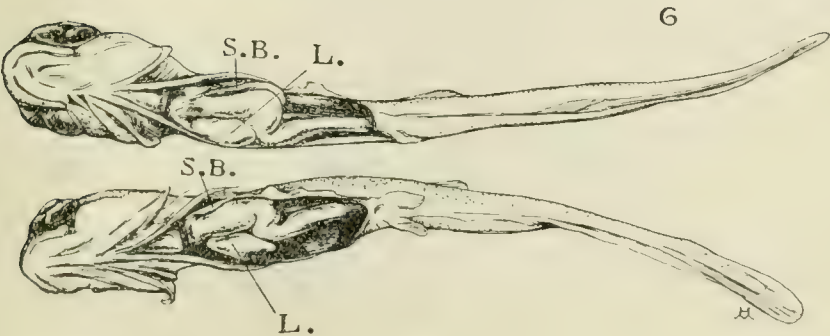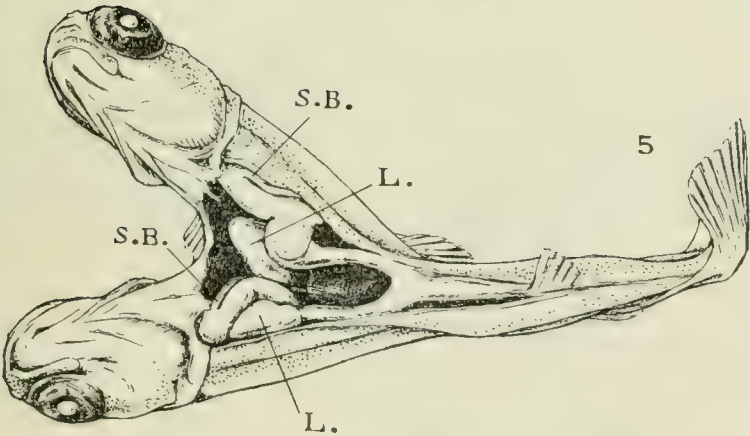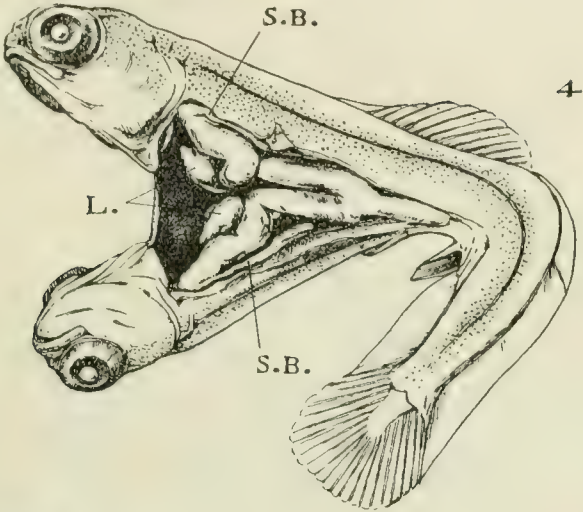
PLATE 3

7 Diagram of the viscera in the human monster shown in figure 8. The compound liver is omitted. The apices of the two hearts are widely separated to show the medial surfaces which were in close contact. *A.*, aorta; *P.A.*, pulmonary artery (origin); *S.V.C.*, superior vena cava; *Vv.h.*, hepatic veins; *Sp.* spleen; *Pan.*, pancreas; *G.B.*, gall-bladder; *A.C.*, ascending colon; *Cae.*, caecum
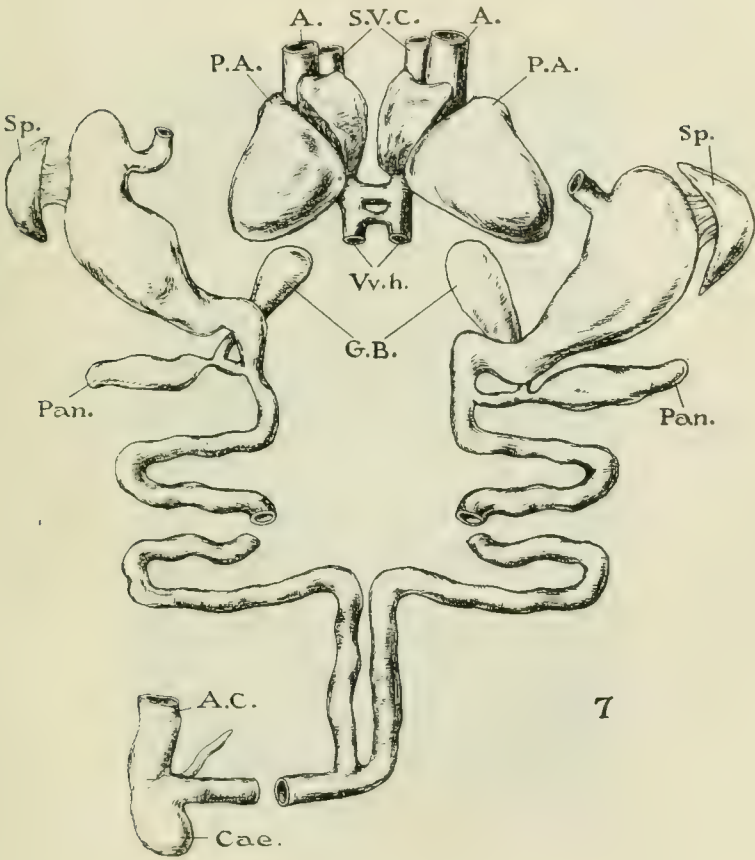
8   Photograph of the human dicephalus tribrachius dipus described in the present paper.

CHANGES IN PROTOPLASMIC CONSISTENCY AND THEIR RELATION TO CELL DIVISION.

By ROBERT CHAMBERS.

(*From Cornell University Medical College, New York City, and the Marine Biological Laboratory, Woods Hole.*)

I. Periodic Changes in Consistency of the Egg Cytoplasm after Fertilization and during Cleavage.

On fertilization an increase in the viscosity of the semifluid cytoplasm of the sea urchin egg was noticed by Albrecht[1] and recently fully demonstrated by Heilbrunn.[2] Heilbrunn based his conclusions on his observation that a greater centrifugal force is necessary to stratify the cell constituents of an egg after fertilization than before. I[3] have presented evidence, from microdissection studies on the sand-dollar egg and the egg of *Cerebratulus*, that the increase in viscosity is associated with the appearance and growth of the aster.

Upon entrance of the spermatozoon into the egg a diminutive aster makes its appearance as a ball of a jelly-like consistency in the immediate vicinity of the sperm head. This aster, with the sperm nucleus, moves inward as it steadily increases in size until, when its center comes to lie in or near the center of the egg, its radiations extend throughout the whole egg. During this migration the sperm nucleus comes into contact with the egg nucleus. The aster then develops completely around the two nuclei, which fuse to constitute the cleavage nucleus.

The development of the sperm aster in the sea urchin egg is at its height within 10 to 15 minutes after fertilization. This is the

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[1] Albrecht, E., Untersuchungen zur Struktur des Seeigeleies, *Sitz-ber. Ges. Morph. u. Physiol.*, 1898, xiv, 133.

[2] Heilbrunn, L. V., Studies in artificial parthenogenesis. II. Physical changes in the egg of *Arbacia, Biol. Bull.*, 1915, xxix, 149.

[3] Chambers, R., Jr., Microdissection studies II. The cell aster: A reversible gelation phenomenon. *J. Exp. Zool.*, 1917, xxiii, 483.

time when Heilbrunn informs me he found the egg substance to be
of maximum viscosity.

The increase in viscosity of the egg cytoplasm is produced by an
influence spreading out in all directions from the center of the aster.
While this occurs the central hyaline area of the aster (the hyalo-
plasmsphere of Wilson) increases in size, and there is strong evidence[3]
that this is due to the accumulation of a hyaline liquid which sepa-
rates out of the semisolidifying cytoplasm and flows in very fine
converging streams to the center of the aster. It is possible that
this and kindred phenomena give to the aster the appearance of
radiations from a common center. The consistency of the cytoplasm
incorporated in the aster diminishes in firmness on passing from the
interior of the aster to its exterior, being greatest in the region border-
ing on the centrosphere and least at the periphery.

The disappearance of the sperm aster, in the opinion of the writer,
occurs through a process of liquefaction. During the liquefaction
the substance of the centrosphere collects into two areas at opposite
poles of the cleavage nucleus. The experiments to be described in
this paper indicate that shortly before cleavage each of these areas
becomes a center around which the cytoplasm commences again to
pass into a semisolid state. The radial configuration about these
areas constitutes the amphiaster. The comparatively firm consist-
ency that the egg now attains for the second time since fertilization
is due to two masses, the two asters, instead of to a single aster as
was the case shortly after the entrance of the sperm. The importance
of this phenomenon in its bearing on cell division is discussed in the
last part of this paper.

Experiment 1.—The consistency exhibited by the protoplasm of the
sea urchin egg at various periods from the moment of fertilization
until the completion of the first cleavage, was ascertained by careful
probing with the microdissection needle.

Immediately after fertilization the cytoplasmic granules readily
flow by the moving needle. After the sperm has entered the egg,
the sperm aster constitutes a comparatively firm mass which gradually
increases in size as it moves to a central position in the egg. When
the sperm aster is at its full development the highly viscous state
of the cytoplasm is detected by the needle. Illustrations of this

are given in a former paper.[3] The cytoplasmic granules, instead of being readily dislocated by the moving needle, are held as in a jelly, and movements of the needle produce torsions of the entire egg substance. This condition is at its height 10 to 15 minutes after fertilization.

15 to 20 minutes after fertilization, the radiations of the aster begin to fade from view, with a reversal in the cytoplasm of the semi-solid to a more fluid state. The cytoplasmic granules are now easily dislocated by the moving needle. The more prominent radiations disappear first, while the finer ones persist for some time, owing prob-ably to the viscid nature which the cytoplasm always maintains. The liquid substance of the central hyaline area now flows over the nucleus to its two poles, beyond which it often extends. This causes the appearance characteristic of this stage, of a hyaline streak plainly visible in the otherwise granular cytoplasm of the egg. Toward the end of this stage, which lasts for about 20 to 30 minutes, the hyaline substance finally collects into two semispherical masses lying at the two poles of the nucleus.

Shortly before cleavage, about 40 to 50 minutes after fertilization, an increase in firmness sets in, spreading radially from each of the two centers situated at the poles of the nuclear spindle. This con-stitutes the amphiaster. The egg elongates, the long axis passing through the two centers of the amphiaster. The cleavage furrow now appears and the egg rapidly divides. The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during this amphiaster stage could be more easily demonstrated by the needle in the eggs of *Echinarachnius* and *Cerebratulus* than in those of *Arbacia*.

After completion of the cleavage process, there are indications that the firmness of the cytoplasm persists in the two blastomeres while they are still more or less spherical. Within 10 to 15 minutes after cleavage the two blastomeres crowd up against one another, each assuming a more nearly hemispherical shape. At this stage their cytoplasm is again quite fluid.

These observations demonstrate a pronounced periodicity in the physical state of the egg subsequent to fertilization and during the first cleavage process. In the immature egg the viscosity is high,

after maturation it drops.   Upon fertilization it begins to rise again, to reach its maximum with the full development of the sperm aster. The viscosity drops again and continues low until the approach of cleavage.   It thereupon rises again to drop only after completion of the first cleavage.   Subsequent to the first cleavage the rhythmic appearance and disappearance of the asters within the blastomeres most probably indicate periodic successions of a process analogous to a jellying and liquefying of the cytoplasm.

The segmentation process may thus be explained as consisting essentially in a growth within the egg of two bodies of material through a gradual transformation of the cytoplasm.   This transformation is associated with a change in the physical state of the protoplasm, two semisolid masses growing at the expense of the more fluid portions of the cytoplasm.

II. Cutting Experiments on the Segmenting Egg.

If it is true that the segmenting egg consists of two rather firm masses which are most fluid at their periphery, and if the physical state of the protoplasm is not affected in the process, one should be able to cut a segmenting egg into pieces without disturbing the cleavage plane.   Cleavage should, therefore, proceed in such a manner as to complete the separation of what remains of the two bodies within each piece.   This is what actually happens.   Some experiments of Yatsu,[4] the results of which he made no attempt to explain, are in full accordance with mine and bear directly on this problem. Yatsu cut the eggs of *Cerebratulus* which were just beginning to segment (anaphase stage) into nucleated and non-nucleated fragments.   He found that the cleavage furrow proceeded in its original plane irrespective of whether the fragments were nucleated or not. In Fig. 1, I have diagrammatically presented some of his results. Fig. 1 *a* represents a segmenting *Cerebratulus* egg being cut in a plane parallel to its long axis and to one side of the daughter nuclei.   The original furrow persisted in the non-nucleated fragment (*b*) and

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[4] Yatsu, N., Some experiments on cell-division in the egg of *Cerebratulus lacteus*, *Annot. zool. japon.*, 1908, vi, 267.

quickly completed its course in the nucleated fragment (*c*). Somewhat later the cleavage of the non-nucleated fragment (*d*) was also completed. Fig. 1 *e* represents a segmenting egg in which the cut
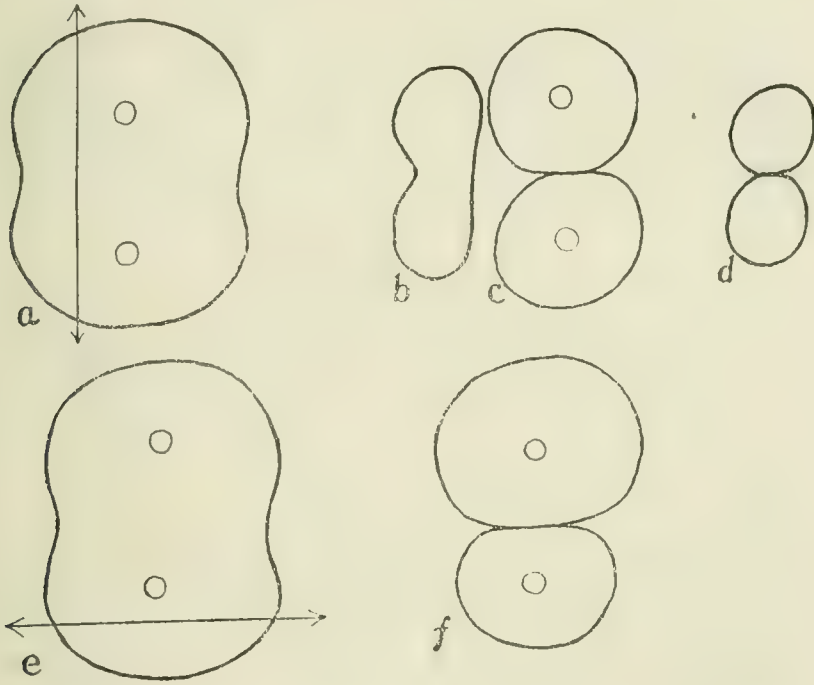
FIG. 1. A diagrammatic representation of Yatsu's results[4] on cutting the segmenting eggs of *Cerebratulus*. The direction of the cut is shown in *a*. The original cleavage furrow completed its course in the nucleated fragment *c* at the same time that it persisted in the non-nucleated fragment *b*. The furrow finally cut through the non-nucleated fragment in *d*. In *e* a cut was made across one end of the segmenting egg. The original furrow completed its course in *f* resulting in two unequal blastomeres.

was made at one end of the egg at right angles to its long axis. The original furrow persisted so as to divide the mutilated egg into two unequal blastomeres (*f*).

My cutting experiments were carried out mostly on the starfish egg, as sea urchins were very scarce during the summer of 1918.

The mature starfish egg averages 0.16 mm. (*i.e.* 160 μ) in diameter.
The needles used for dissection averaged 10 μ in thickness at about
1 mm. from the tip and tapered gradually from there to a point far
below 1 μ. With such a needle one can make a puncture or a clean
cut through the egg in any desired spot or plane without causing
apparent disturbance in the protoplasm of the egg. For cutting
purposes glass needles as shown in Fig. 2 were used.[5] As the egg
lies suspended in a hanging drop the end limb of the needle (Fig. 2 *a*) is
set in such a way as to push the egg against the cover-slip. Con-
striction of the egg is produced by a continued upward pressure of
the needle until the egg is cut in two. The operation does not neces-
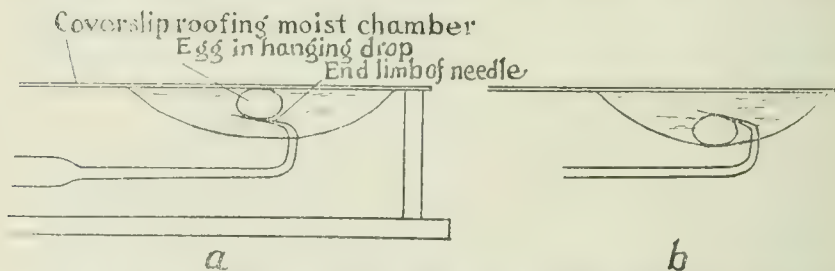
FIG. 2. Methods used for cutting an egg in two. *a*, side view of moist chamber
magnified to show needle in position with its end limb so placed as to compress
an egg between it and the cover-slip. Continued pressure of the needle cuts the
egg in two. *b*, a second method of cutting an egg by bringing the end limb of
the needle down on the egg so as to press the egg against the lower surface of the
hanging drop.

sarily destroy the fertilization membrane which envelops the egg.
The egg may also be cut in two on bringing it (Fig. 2 *b*) between the
end limb of the needle and the lower surface of the hanging drop.
Lowering the needle out of the drop in such a way as to give to the
egg a rolling motion cuts the egg cleanly in two. This second method
is not as satisfactory as the first for cases where one wishes to preserve
the spatial relations of the egg contents, as the rolling motion pro-
duces churning movements within the cell.

Experiment 2.—(Figs. 3 to 7.) An *Asterias* ovum just beginning
to segment and with the amphiaster in full development was cut

[5] Chambers, R., The microvivisection method, *Biol. Bull.*, 1918, xxxiv, 121.

in two in a plane diagonal to the cleavage furrow. The fresh surfaces caused by the cutting form films which prevent reunion of the pieces. The egg was in this way cut into two pieces each consisting of egg substance lying on both sides of the cleavage furrow.
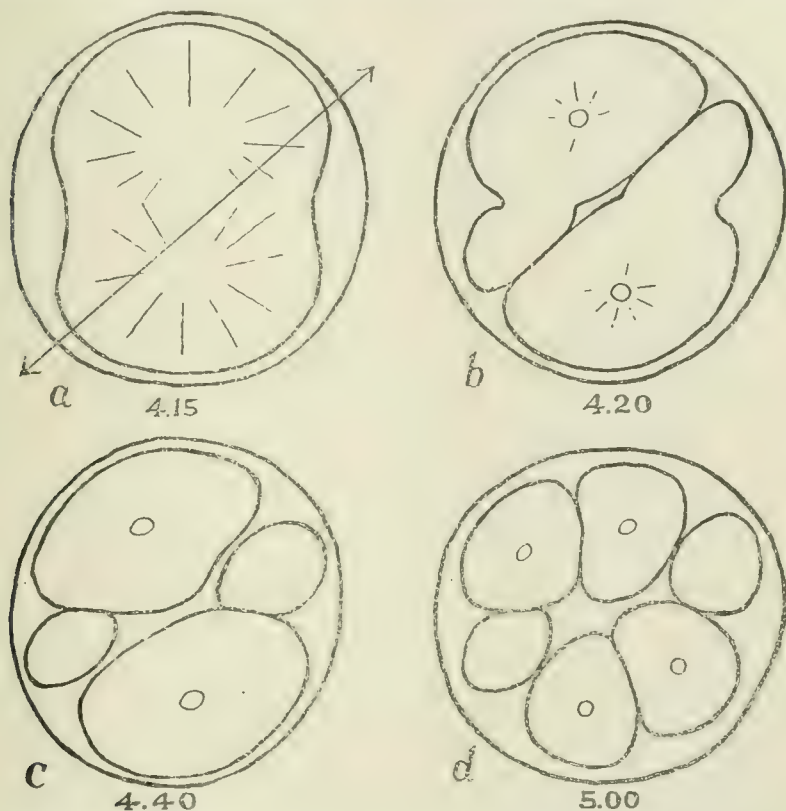
FIG. 3. Effect of a diagonal cut through an *Asterias* ovum beginning to segment in which the cut did not disturb the physical state of the ovum. *a*, operation performed at 4.15 p.m. *b*, 4.20 p.m., persistence of cleavage furrow in the original plane. *c*, 4.40 p.m., non-nucleated fragments pinched off. *d*, 5.00 p.m., nucleated fragments have segmented.

On one occasion the operation was performed on twelve eggs. In nine cases the original cleavage plane was maintained so that each piece pinched off a non-nucleated fragment normally belonging to the other blastomere. Two of them are illustrated in Figs. 3 *a* to *d* and 4 *a* to *d*.

In one case the cut was made at 4.15 p.m. (Fig. 3 *a*). 5 minutes later the cleavage furrow had progressed in the original plane (Fig. 3 *b*). At 4.40 it had completed its course so that each piece was divided into a small non-nucleated and a large nucleated fragment
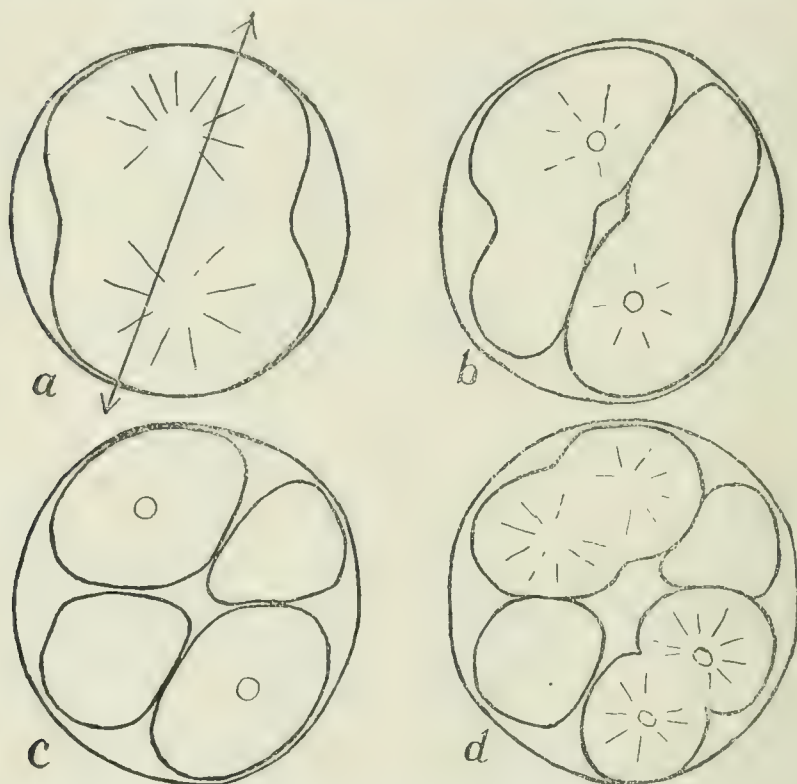
Fig. 4. Similar operation to that shown in Fig. 3 except that the diagonal cut is more nearly perpendicular to the cleavage plane with the result that larger non-nucleated fragments are pinched off by the cleavage furrow.

(Fig. 3 *c*). At 5 p.m. each of the two nucleated fragments or blasto-mere remnants had divided once (Fig. 3 *d*). 1 hour later they had divided once again. By the next morning the egg developed into a double blastula with the two non-nucleated fragments lying as inert masses within the fertilization membrane.

Fig. 4 a to d illustrates a similar case in which the non-nucleated masses are considerably larger than those depicted in Fig. 3. The similar behavior of one of the first two blastomeres in an egg is shown in Fig. 5 a and b.

In the remaining three cases the astral radiations faded out during the operation (Fig. 6 a). The original segmentation furrow gradually filled up and disappeared (Fig. 6 b) and each piece assumed the appearance of a normal blastomere. The nucleus then shifted so as to occupy a more central position in what one may term the reconstructed blastomere and further segmentation proceeded as if the ovum had not been operated upon (Fig. 6 c and d). This procedure always
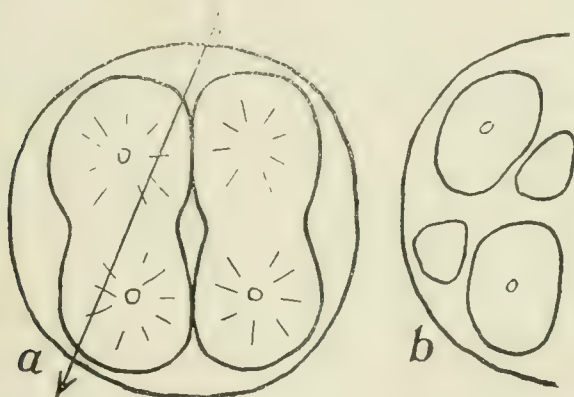
FIG. 5. Effect of a diagonal cut through one of the first two blastomeres of an *Asterias* ovum. a, egg showing direction of cut. b, cut blastomere a few minutes later.

occurred when the ovum was consciously rolled during the operation so as to produce a disturbance evidenced by a churning movement of the egg constituents.

A similar instance in the case of an *Arbacia* egg is shown in Fig. 7 a to c. A piece was cut from one pole of the amphiaster egg. In the process the piece was cytolyzed. The amphiaster in the remainder of the egg disappeared to reappear again in a new position with the result that two equal sized blastomeres were formed.

That mechanical disturbances may cause a reversal of a solid to a fluid state has already been shown.[3] This would make all the

protoplasm on each side of the cut merge into a single fluid mass. The nucleus then comes to occupy a central position. Normal mitosis takes place with the formation of an amphiaster and cleavage
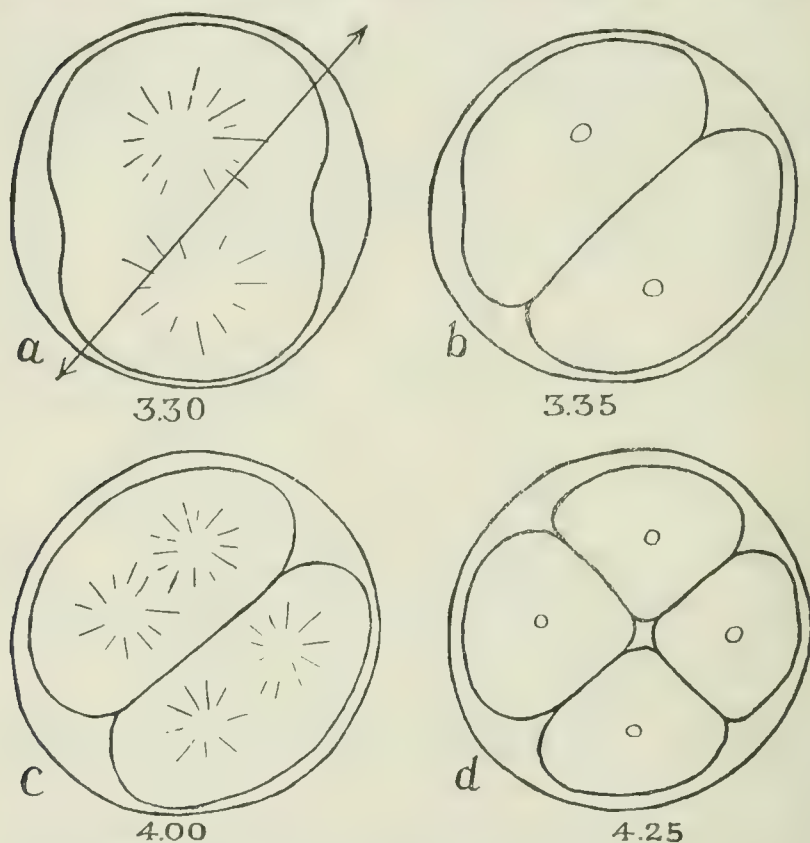
FIG. 6. Effect of a diagonal cut through an *Asterias* ovum in which the cut brought about a change in the physical state of the egg. *a*, operation performed at 3.30 p.m. *b*, 3.35 p.m., original cleavage furrow beginning to be obliterated. *c*, 4.00 p.m., an amphiaster formed in each of the two pieces produced by the cut. *d*, 4.25 p.m., four celled stage in which one cleavage plane was produced by the needle and the other by normal fission.

proceeds along the equator where the boundaries of the two asters are contiguous.

In the nine cases, in which the original cleavage plane persisted after the cutting process, the semisolid state about the two astral

centers was not disturbed. Each of the two pieces resulting from the cut, therefore, consisted of two unequal semisolid masses separated by a fluid area corresponding to the equator of the original egg. As this fluid area is incorporated into the two masses a furrow appears
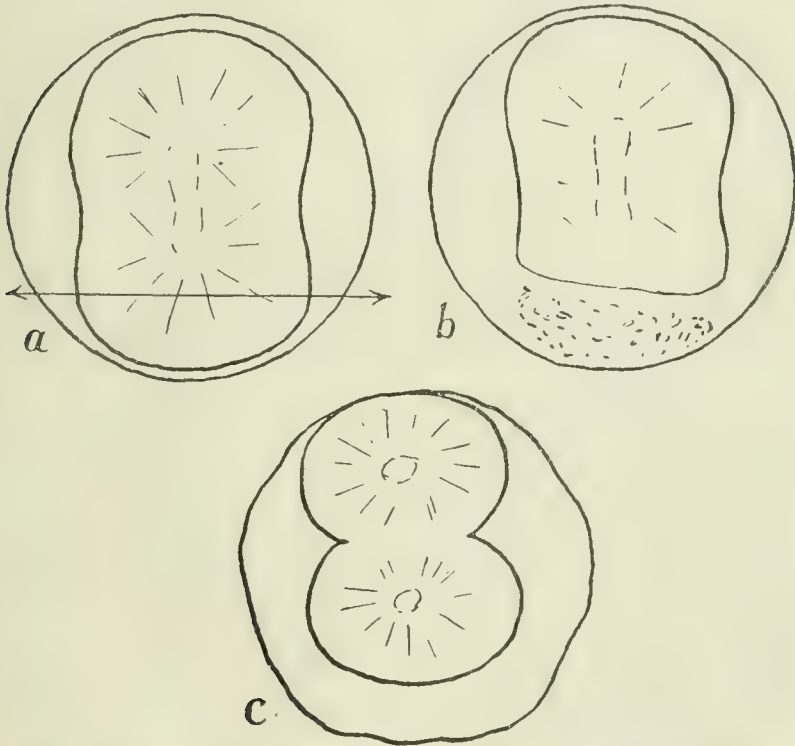
FIG. 7. Effect of cutting off a piece from one pole of an *Arbacia* ovum in the amphiaster stage. *a*, direction of cut. *b*, the piece cut off cytolized. The original shape of the remainder of the egg persisted for some time as the ovum of *Arbacia* is less pliable than that of *Asterias*. *c*, the reappearance of a new amphiaster resulting in the formation of two equal blastomeres.

which separates each piece into a larger nucleated and a smaller non-nucleated body.

The operated eggs were kept under observation until the gastrula stage, indicating that the operation had not destroyed the capacity of the egg for further development.

The following experiments are supplementary to the second. In all of them the results obtained are explicable on the basis of the existence, during cleavage, of reversible changes in the consistency of the cytoplasm.
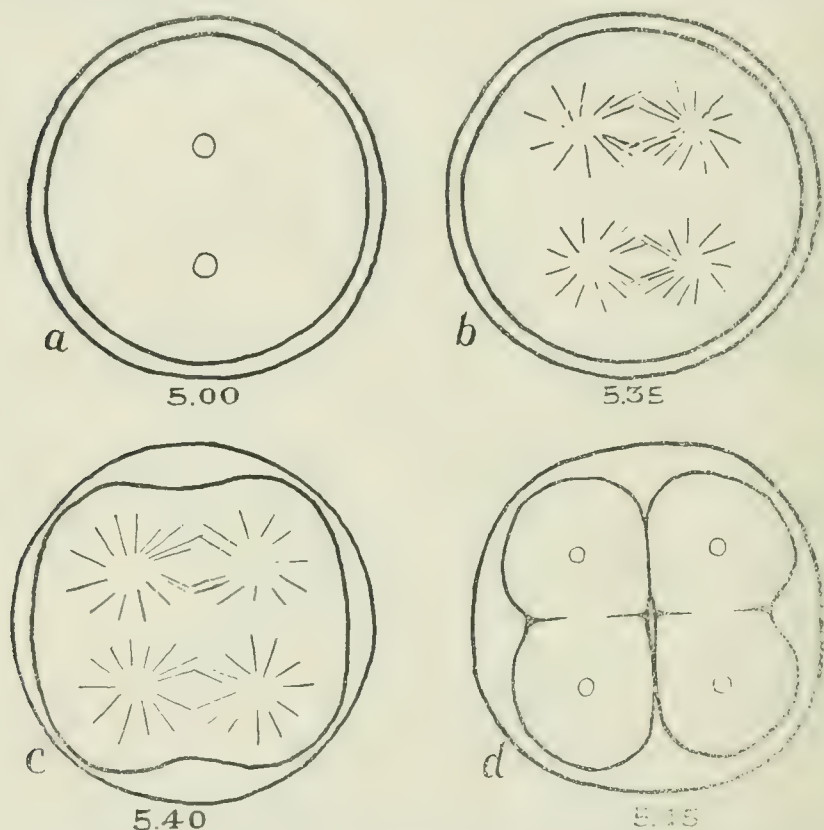
FIG. 8. Development of an *Asterias* ovum manipulated with a needle so as to suppress the first cleavage furrow. *a*, 5.00 p.m., disappearance of the amphiaster and obliteration of the cleavage furrow. *b*, 5.35 p.m., appearance of two amphiasters. *c*, 5.40 p.m., change in shape of the ovum with appearance of second cleavage furrow ahead of the first. *d*, 5.45 p.m., ovum cleaving into four blastomeres. (The ovum developed into a normal embryo.)

Experiment 3.—(Fig. 8.)   In this case the first segmentation furrow was prevented from forming by tearing at the equator whenever it made its appearance. The progressive changes within the egg were

undisturbed. As soon as the amphiaster disappeared there was no longer a tendency for the furrow to form (Fig. 8 *a*). The two nuclei now lay in a fluid cytoplasm. Within half an hour after the suppression of the first segmentation furrow, an amphiaster developed about each nucleus preparatory to the next division. The two amphiasters lay side by side but remained distinct from one another, no connecting radiations being formed (Fig. 8 *b*). The formation of the two amphiasters resulted in the transformation of the egg substance into four semirigid bodies, the four asters. Cleavage furrows now extended into the fluid regions between the asters and divided the egg almost simultaneously into four blastomeres. The furrow corresponding to the second cleavage started to form and cut through the egg about a minute ahead of that of the first (Fig. 8 *c* and *d*).

This experiment may throw light on the nature of the segmentation in ova in which several nuclear divisions follow one another with no outward manifestation of the segmentation of the egg. After a certain period the ovum breaks up simultaneously into as many blastomeres as there are nuclei. This is the normal method in certain *Actinozoa* and can be artificially produced in many eggs by exposing them to various reagents, notably hypertonic solutions.[6,7]

The solidification associated with the aster formation divides the egg cytoplasm into a number of bodies each surrounding a nucleus. Between successive divisions the cytoplasm reverts to a more fluid state but its viscid nature may suffice in preventing the merging of neighboring areas. After a varying number of nuclear divisions with accompanying solidification periods furrows suddenly appear between these bodies and the ovum tends to break up at once into separate blastomeres. A differentiation of this type may possibly have taken

[6] Loeb, J., Investigations in physiological morphology. III. Experiments on cleavage, *J. Morph.*, 1892–93, vii, 253. Norman, W. W., Segmentation of the nucleus without segmentation of the protoplasm, *Arch. Entwcklngsmechn. Organ.*, 1896, iii, 106. Wilson, E. B., Experimental studies in cytology. I, *ibid.*, 1901, xii, 529. Lillie, R. S., Fusion of blastomeres and nuclear division without cell division in solutions of non-electrolytes, *Biol. Bull.*, 1902–03, iv, 164.

[7] Wilson, E. B., Experimental studies in cytology, II and III, *Arch. Entwcklngsmechn. Organ.*, 1902, xiii, 353.

place in the unsegmented *Chætopterus* embryos experimentally produced by Lillie.[8]

Experiment 4.-- (Fig. 9.)   Fig. 9 *a* to *e* depicts the case of an egg
with the cleavage furrow just beginning in which the diagonal cut
was incomplete so that the two pieces remained connected at one
end of the cut.   The original furrow persisted for a time during which
it deepened considerably.   30 minutes after the cut had been made
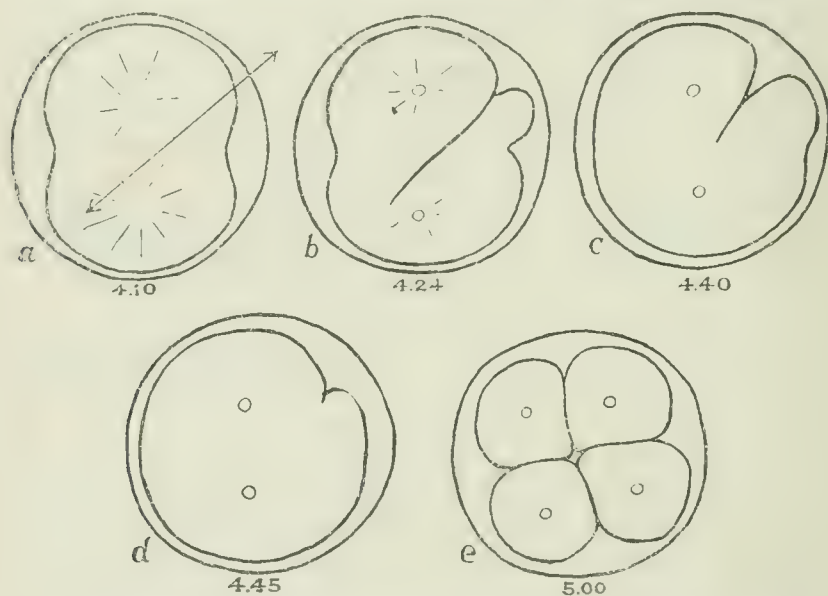
FIG. 9. Effect on an *Asterias* ovum of a deep cut which did not persist. *a*,
operation performed at 4.10 p.m. *b*, *c*, and *d* show the egg respectively at 4.24,
4.40, and 4.45 p.m. Both the cut and the cleavage furrow disappear together
with a reversal of the ovum from a semisolid to a more fluid state. *e*, 5.00 p.m.,
the ovum has divided into four normal blastomeres. (The ovum developed into
a normal embryo.)

no sign of astral radiations were present and both the original segmentation furrow and the cut produced by the needle were being
obliterated (Fig. 9 *c* and *d*).   At 5 p.m. the egg had divided into
four apparently normal blastomeres (Fig. 9 *e*) and was only slightly

[8] Lillie, F. R., Observations and experiments concerning the elementary phenomena of embryonic development in *Chætopterus*, *J. Exp. Zool.*, 1906, iii, 153.

bchind the normal controls. By the next morning it had developed into a swimming blastula not to be distinguished from the normal controls.

The obliteration of the cut and of the furrow is consequent to a reversal of the egg cytoplasm from a semirigid to a more fluid state. The film projecting into the egg gradually merges into the liquid
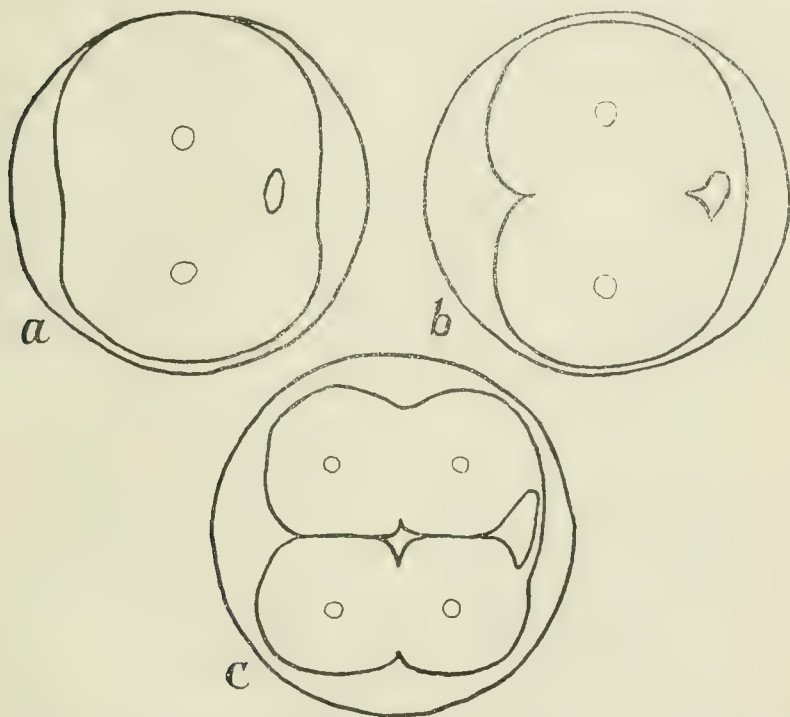
FIG. 10. Successive stages of an *Asterias* ovum showing persistence of a puncture made below the first cleavage furrow as it is beginning to form.

cytoplasm surrounding it and surface tension forces finally overcome the deformation of the egg. The egg now proceeded to divide into four blastomeres as in Experiment 3.

Experiment 5.—(Fig. 10.) This experiment demonstrates a peculiar property of the equatorial region during the formation of the cleavage furrow. A tear was made through the egg below the segmentation furrow (Fig. 10 *a*). The hole produced by the tear

remained open. The cleavage furrow continued its course beneath
the hole leaving an outer margin as a bridge of protoplasm which
connects the two blastomeres (Fig. 10 *b, c*). After several divisions of
the egg the bridge thinned down in its middle until it broke through
and the resulting strands were gradually drawn into the blastomeres
from which they had projected.
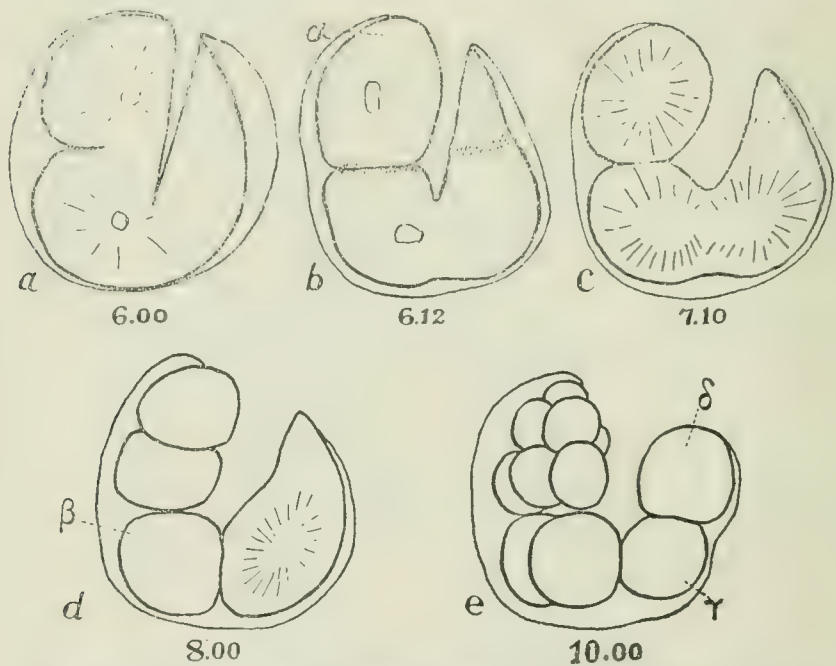
FIG. 11. Effect on an *Arbacia* ovum of a deep cut which persisted. For des-
cription of the results see text. Pigment granules collect in plane of original
furrow.

Experiment 6.—(Fig. 11.) This experiment was performed on an
Arbacia egg. An incomplete cut was made almost perpendicular to
the cleavage furrow but to one side of the daughter nuclei. The furrow
on the side away from the daughter nuclei became obliterated (Fig.
11 *a*). On the other side it continued its original course resulting in
the pinching off of the nucleated Blastomere α (Fig. 11 *b*). The
nucleus in the remainder of the egg shifted its position only slightly and

the amphiaster (Fig. 11 *c*), forming about it, resulted in a second un-
equal cleavage with the formation of Blastomere β (Fig. 11 *d*). The
projecting piece of the egg above the obliterated furrow remained
quiescent during these divisions and not until after the third unequal
cleavage resulting in the formation of Blastomere γ in Fig. 11 *e*, did
it become incorporated in Blastomere δ.

In this experiment the cut was probably made in the egg when the
process for the first cleavage was too far advanced for the egg to
retrace its course. The gash was therefore not obliterated and a
very peculiar condition resulted in a succession of advances of the
cleavage process about the gash. Blastomere α, being the earliest
formed, segmented ahead of its fellows (Fig. 11 *d*). Blastomere β
came next (Fig. 11 *e*). Unfortunately before Blastomeres γ and δ
divided the egg died.

It is significant that Blastomere δ is larger than γ as evidently the
former finally incorporated the hitherto inactive part of the egg that
lay above that part of the original first cleavage furrow which lay on
the right side of the gash (Fig. 11 *b*).

III. *Concerning the Mechanism of Cell Division.*

The changes in shape that an echinoderm egg undergoes during
cleavage can be in part understood on the assumption that the astral
formation is a solidifying process. It has long been known that at
the time of cleavage the eggs of echinoderms, many worms, mammals,
etc., become elongated,[9] the cleavage furrow forming in a plane at
right angles to the long axis of the egg. As the furrow deepens, each
resulting blastomere tends to assume the shape of a sphere (Fig. 12 *a*).

Nobody, however, has thus far been able to explain the cause of
this elongation. The observations recorded in this paper may ex-
plain this phenomenon. The two spheres of solidification grow at
the expense of all but possibly a small peripheral part of the fluid
egg substance. The combined diameters of the two fully formed
semisolid spheres are greater than the original diameter of the egg,

[9] Hertwig, O., Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung
des thierischen Eies, *Morph. Jahrb.*, 1876, i, 347. Gurwitsch, A., Morphologie
und Biologie der Zelle, Jena, 1904.

and hence the egg must elongate. After elongation the surface of the egg seems to tear in the plane separating the two semisolid spheres. The periphery of the two asters of the amphiaster stage never becomes so firm as their interior. This may account for the observation of von Erlanger,[10] confirmed by Spek,[11] who described peripheral currents in the rapidly dividing nematode egg. In this egg peripheral currents flow from the two poles toward the equator and from there inward to the center of the egg. Spek suggests that such currents exist in all dividing eggs, and that they are easily visible in the nematode egg because of the great rapidity with which it segments. Conklin[12] described an inward flow of granules at the equator of the dividing *Crepid-*
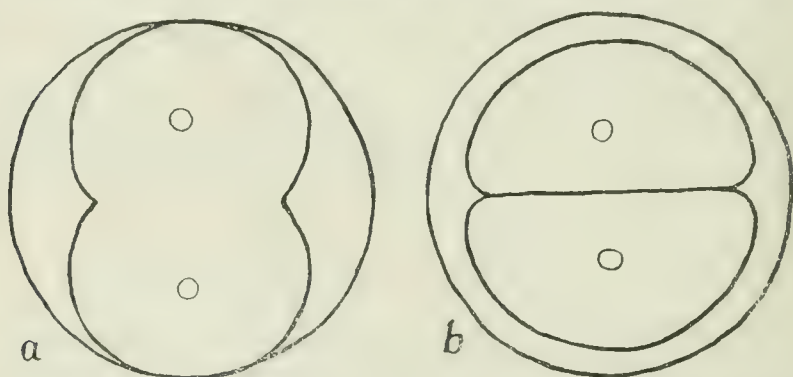
FIG. 12. Change in shape of an *Asterias* ovum (*a*) before and (*b*) after completion of the first cleavage furrow.

ula egg, and I[3] have observed a similar current, although a very slow one, in the sand-dollar egg.

Immediately after cleavage both of the two blastomeres are more or less spherical; but later, when they become more fluid, they are pressed against each other so as to be flattened at the plane of contact.

[10] von Erlanger, R., Beobachtungen über die Befruchtung und ersten Teilungen an den lebenden Eiern kleiner Nematoden, *Biol. Centr.*, 1897, xvii, 152, 339.

[11] Spek, J., Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung, *Arch. Entwcklngsmechn. Organ.*, 1918, xliv, 5.

[12] Conklin, E. G., Protoplasmic movement as a factor of differentiation, *Marine Biol. Lab.*, *Biol. Lect.*, 1899, 69.

Wilson,[7] in producing binucleate eggs by artificially obliterating the first cleavage furrow, noted that when this was caused by shaking, the resulting binucleate eggs retain the elongated shape (Fig. 13) characteristic of the egg in cleavage. During the ensuing pause (corresponding to the completion of the first cleavage and when
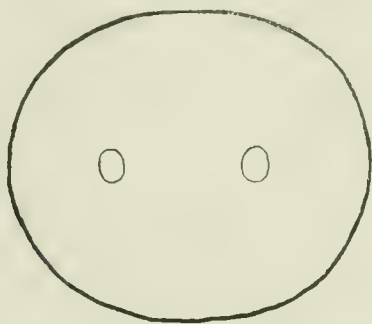
FIG. 13. Copy of Fig. 58 from Wilson[7] of *Toxopneustes* ovum immediately after shaking which caused obliteration of the first cleavage furrow.
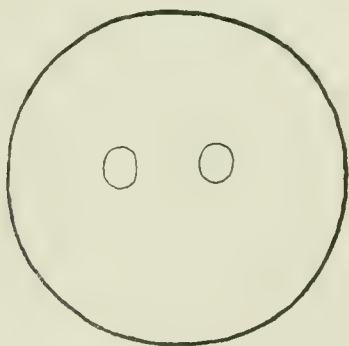
FIG. 14. Copy of Fig. 34 from Wilson[7] of *Toxopneustes* ovum in which obliteration of the first cleavage furrow was produced by exposure to ether.

the astral radiations fade out preparatory to formation of a new amphiaster system) the egg becomes more nearly spherical. Evidently the shaking does not necessarily produce a reversal of the semisolid astral system to the more fluid state. As soon, however, as this occurs (in the ensuing pause) the egg resumes its spherical shape.

Wilson noted that the suppression of the cleavage furrow can also be produced by placing eggs, during their anaphase stage, in a 2.5 per cent ether solution. The astral radiations disappear and the resulting binucleate egg at once resumes the shape of a sphere (Fig. 14). This phenomenon may be comparable to the experiments illustrated in Figs. 6, 8, and 9 where the obliteration of the astral radiations follows a precocious reversal of the cytoplasm to the more fluid state. The suppression of the furrow in these cases seems to be primarily effected by the change in the physical state of the egg substance which, on reverting to a more fluid state, merges into a single spherical mass.

<div align="center">CONCLUSIONS.</div>

1. The development of the amphiaster is associated with the formation of two semisolid masses within the more fluid egg substance.

2. The elongation of the egg during cleavage is possibly produced as a consequence of the mutual pressure of these two growing semisolid masses.

3. The division of the egg into two blastomeres consists essentially in a growth, within the egg, of two masses of material at the expense of the surrounding cytoplasm. When all the cytoplasm of the egg is incorporated in these two masses cleavage occurs.

4. After a certain period of time the semisolid masses revert to a more fluid state. In the eggs studied this normally occurs after the cleavage furrow has completed the separation of the two blastomeres. The formation of the furrow, however, may be prevented in various ways, upon which the egg reverts to a single spherical semifluid mass containing two nuclei.

5. An egg mutilated during its semisolid state (amphiaster stage) may or may not revert to a more fluid state. If the more solid state is maintained, the cleavage furrow persists and proceeds till cleavage is completed. If the mutilation causes the egg to revert to the more fluid state the furrow becomes obliterated and a new cleavage plane is subsequently adopted.

6. The nuclei of eggs in the semifluid state are able to alter their positions. In semifluid mutilated eggs the nuclei tend to move to positions which may assure symmetry in aster formation and cleavage.

A Report on Results Obtained from the

Microdissection of Certain Cells

by

ROBERT CHAMBERS

A Report on Results Obtained from the Microdissection of Certain Cells.

By ROBERT CHAMBERS, CORNELL UNIVERSITY MEDICAL COLLEGE,
NEW YORK CITY.

Presented by J. Playfair McMurrich, F.R.S.C.

(Read May Meeting, 1918.)

INTRODUCTION.

Cytological research has hitherto been confined largely to observation through the microscope 'at a distance' as it might be said. It has been clear to investigators that such a method may lead to erroneous conclusions and it is true that a great deal of confusion has resulted from misinterpretations of optical appearances and the description of artifacts as if they existed normally in the cell. This confusion is largely responsible for the fact that the true significance of cell anatomy is in danger of being ignored by many physiologists.

As long ago as 1859, Doctor H. D. Schmidt of Philadelphia, attempted to dissect cells by means of a 'microscopic dissector,' consisting of a base to be fastened on the stage of a microscope with a number of clamps to hold instruments, each clamp possessing three movements controlled by screws. A lever fastened in one of the clamps holds the tissue in place. Fine scissors, knives or steel needles are fastened in the other clamps. By turning the various screws, the instruments can be brought into place and be operated with remarkable accuracy. Doctor Schmidt worked with the tissue, the instruments and the lower lens of the objective immersed in water or diluted alcohol.

The principle introduced by Schmidt, viz., the use of screws to control movements of instruments lying in the focus of a microscope objective seems to have been for a long time lost sight of. It was revived in 1907 and elaborated in 1914 by M. A. Barber, lately of the University of Kansas, in his construction of an instrument to manipulate micro-pipettes. With this instrument Barber was able to isolate single micro-organisms and to inoculate living cells with bacteria. Barber's instrument was soon applied to the dissection of cells (Kite and Chambers '12) and a new field of endeavor was opened for the study of the structure of protoplasm and cell mechanics.

The Instrument.

The apparatus used in cell dissection is shown in the accompanying figure. The moist chamber, which is open at one end and with sides from 8 to 12 mm. high, is placed on the microscope so that it may be moved about with the mechanical stage. The chamber is roofed over with a specially cleaned coverslip, on the under surface of which, the specimen is mounted in a hanging drop of Ringer's or lymph fluid and held in place by surface tension. The dissecting needle is made by drawing out one end of a piece of hard glass tubing which is then bent at right angles, two or three millimeters from the pointed tip. The needle-holder, a mechanism allowing of three movements, is clamped to one side of the miscroscope stage, and the needle is adjusted so that it projects into the moist chamber with its tip pointing up into the hanging drop. By proper adjustment the cell to be dissected and the point of the needle can be brought into the same focal field. The three movements of the needle permitted by the needle-holder and the two movements of the moist chamber by the mechanical stage give the experimenter ample opportunity to carry on dissection under the highest magnification of the microscope. The dissecting needle-points can be made stiff and yet so fine that their size bears about the same relation to that of a human red blood corpuscle as an ordinary knitting needle does to the palm of the hand.

Through the courtesy of the Biological Board of Canada I was given in July 1917 the opportunity of continuing some microdissection work at the Atlantic Biological Station, St. Andrews, N.B. I have to thank Dr. Clara C. Benson for allowing me to take some material from lobsters she was using for experimental purposes.

Experimental.

The ganglion cells of the Lobster. The ventral nerve cord was laid bare and pieces of a nerve ganglion excised and placed on a thin coverslip in a drop of lobster blood serum. This liquid is expressed during a preliminary clotting of the blood and does not itself clot for a considerable length of time. The nerve cells are carefully isolated by teasing with needles under an ordinary dissecting microscope. The coverslip is then inverted and placed on the moist chamber so that the nerve cells lie in a hanging drop ready for microdissection. The cell bodies lie among the closely interlaced nerve and neuroglia fibers. When the fibers are torn away, the cell body may be isolated with ease.

The cell cytoplasm is very viscid in consistency and allows of considerable tearing without disintegrating. Highly refractive spindle-

shaped bodies, the mitochondria, imbedded in the cytoplasm are very prominent. The cytoplasm is very extensile and exhibits a certain amount of rigidity throughout its substance. It can be pulled out into long, viscous threads and the imbedded mitchondria are drawn in the direction of the pull.
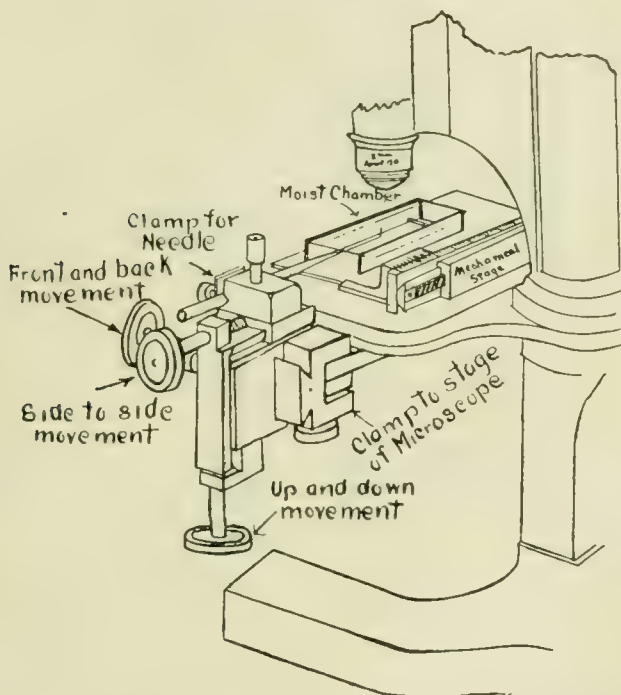
Figure 1.   Barber's Three-Movement Pipette Holder, Glass Needle and Moist Chamber arranged to illustrate method of dissecting cells in a hanging drop under the highest magnification of the microscope.   (Substage of microscope omitted in drawing.)

On exerting a pull on the cytoplasm in a direction away from the nucleus, a triangular space appears in front of the nucleus and persists for a few minutes.   This is due to the viscosity of the cytoplasm which prevents an even flow of material around the nucleus as should occur if the cytoplasm were liquid.

There is a limit to the amount of mechanical injury which the cytoplasm can bear without completely changing its normal properties.   When this limit is passed the viscid plasma sets into a coagulated, non-viscous mass which may be broken into non-glutinous pieces.

The cell nucleus is an optically hyaline sphere occupying about one-fourth of the cell. Within the hyaline substance of the nucleus lies a small body, the nucleolus, which is visible because of its high refractivity.

The extremely sensitive nature of the nucleus is evidenced by the fact that, on the slightest mechanical injury, certain changes occur which cause the nucleolus to fade completely from view. The nucleus may be pushed about in the cell without apparent injury. If the surface be torn the contents flow out and the nucleus disappears. If care be taken the nucleus may be cut in two, each portion at once assuming the shape of a sphere. This indicates a high power of extensibility and regeneration in the surface film. On pulling the nucleus out of the cell the nucleus immediately begins to swell and fades from view.

The Egg Cell of the Flounder. The immature egg of the flounder of about half a millimeter or less in diameter was selected for this study. The nucleus is a liquid sphere similar to that of the nerve cell. It, however, possesses a more persistent surface film or nuclear membrane. This may be caught by the tip of the needle and a considerable strand pulled out. The nucleus is easily cut into several pieces which immediately round up. On touching one another the portions fuse indicating the absence of a morphologically persistent nuclear membrane.

Considerable injury is necessary to bring about dissolution of the nucleus and the surface film is the last to disappear. Injury apparently causes this film to set into a definite membrane, so that when torn it often wrinkles and a fluid (apparently the nuclear sap) collects between the cytoplasm and the partially collapsed membrane.

The flounder egg is surrounded by a closely fitting tough egg membrane. This rather interferes with an adequate comprehension of the consistency of the cytoplasm, especially of that on the cell surface. Results obtained from studies made in other marine ova are more satisfactory.

The egg cell of Asterias. Work done at St. Andrews on *Asterias* confirms the views already published (Chambers '17[a], 17[b]) on the cell protoplasm of Echinoderm ova. The protoplasm consists of a hyaline fluid matrix in which are imbedded granules of various sizes. The fluid offers no perceptible resistance to the needle and an indication of its very slight viscosity lies in the fact that, when the needle is moved through the fluid, the only granules displaced are those in the immediate vicinity of the needle. The protoplasm coagulates with ease. Mere compression will sometimes cause an egg to coagulate into a solid mass.

The surface layer of the egg cell is dense in consistency when compared with the cell interior into which it merges insensibly. In the unfertilized egg, the cell granules are imbedded in it up to the very line of division between the egg and surrounding medium. With the needle the surface may be pulled out into long strands without otherwise disturbing the contour of the cell. On being released the strands tend to curl and retract slowly till they disappear. If a more rapid tear be made, and if the cell be under compression, the spot torn bulges out as the internal cytoplasm presses on the weakened surface. The surface layer of the swelling protuberance is very easily broken, upon which the interior may pour out. The cytoplasm then either disintegrates entirely in the surrounding water or, if remaining normal, reestablishes a film on its surface. When left undisturbed the new surface film gradually strengthens into a definite ectoplasmic layer and the protuberance slowly retracts until the original contour of the egg is reestablished. If the point of attachment of the protuberance be small, the protuberance may be pinched off to form a spherule of cytoplasm which to all appearances is normal.

.In summary, we may say that the surface layer is a highly extensile, contractile and viscous gel capable of constant repair. Its establishment and maintenance is a property essential to protoplasm. With the film intact the mass of protoplasm maintains itself and the life of the cell is assured. When the film is destroyed the cytoplasm flows out, the cell granules swell and disappear, the whole mass completely disorganizes and disappears in solution in the surrounding water.

The egg cell of Solaster. The Solaster egg is very large when compared with other Echinoderm eggs, being well over 1 mm. in diameter. This is partly due to the fact that it is heavily laden with yolk. The nucleus, however, is also very large, so large in fact that it is visible to the naked eye and can be easily isolated with needles under an ordinary dissecting microscope. Its enveloping surface film exhibits a distinct resistance to compression. Tearing the surface allows the fluid contents to escape and the nuclear wall collapses. The *Solaster* egg appears to be the only case on record of a Metazoon cell of which the nucleus is large enough to be actually handled and dissected with ordinary needles.

Conclusion

In conclusion, one may make the following statements with regard to the consistency of the living cells which were dissected:

1. The cytoplasm of an egg cell consists of a semi-liquid interior enclosed in a jelly-like and highly viscous surface layer. The surface

layer is very extensile and contractile and is readily regenerated upon injury. Tearing of this surface, if unrepaired, results in the pouring out of the internal cytoplasm and dissolution.

2. The cytoplasm of the nerve cell exhibits, throughout its substance, the properties of the surface layer of the egg cell, viz., it consists of a highly viscous, extensile, jelly-like hyaline substance.

3. The resting nucleus of all the cells studied is a liquid sphere, the external surface of which may form a more or less temporarily rigid membrane.

4. The production and maintenance of a limiting membrane appears to be one of the properties essential to protoplasm.

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A Report on Cross Fertilization Experiments,

(Asterias x Solaster)

ROBERT CHAMBERS AND BESSIE MOSSOP

Presented by J. P. McMurrich, Ph.D., F.R.S.C.

(Read May Meeting, 1918)

During the first two weeks of August, 1917, a number of adult Solaster endeca (Forbes) were obtained by dredging over a rocky reef in the vicinity of Joe's Point near the Biological Station, St. Andrews, N.B. At this time mature *Asterias forbesii* (Desor) were to be had at St. Andrews. The extent of the breeding season of Asterias in the vicinity of St. Andrews may be estimated from the occurrence of Bipinnaria in the plankton of Passamaquoddy Bay, which is being investigated by Professor J. P. McMurrich. During 1916 the first Bipinnaria observed occurred on July 20th. They were present in all subsequent tows till August 31st. In 1917, none appeared till August 8th, after which date they appeared throughout the remainder of the month.

The Asteriidæ and the Solasteridæ are comparatively closely related families, both belonging to the order Cryptozonia in the Asteroidea. The Solaster possesses a heavily yolk-laden ovum (1 mm or over in diameter) which undergoes a somewhat incomplete metamorphosis, the free swimming larva not having a completely formed alimentary tract. The Asterias, on the other hand, undergoes complete metamorphosis, the larval form being a typical Bipinnaria.

Because of this and because of the fact that the spermatozoa of the two species are very much alike, although the ova are very dissimilar in size, the possibility suggested itself that interesting results may arise from attempts at cross-fertilizing these two species.

Among the specimens of Solaster procured, the females contained large numbers of apparently mature ova. Repeated attempts at fertilizing them with spermatozoa of their own species as well as with those of Asterias proved unsuccessful. The breeding season of Solaster, according to Gemmill,[1] is normally in March or early April, at least for those on the British Coast. This may account for our failure with the Solaster eggs so late as July and August. On the

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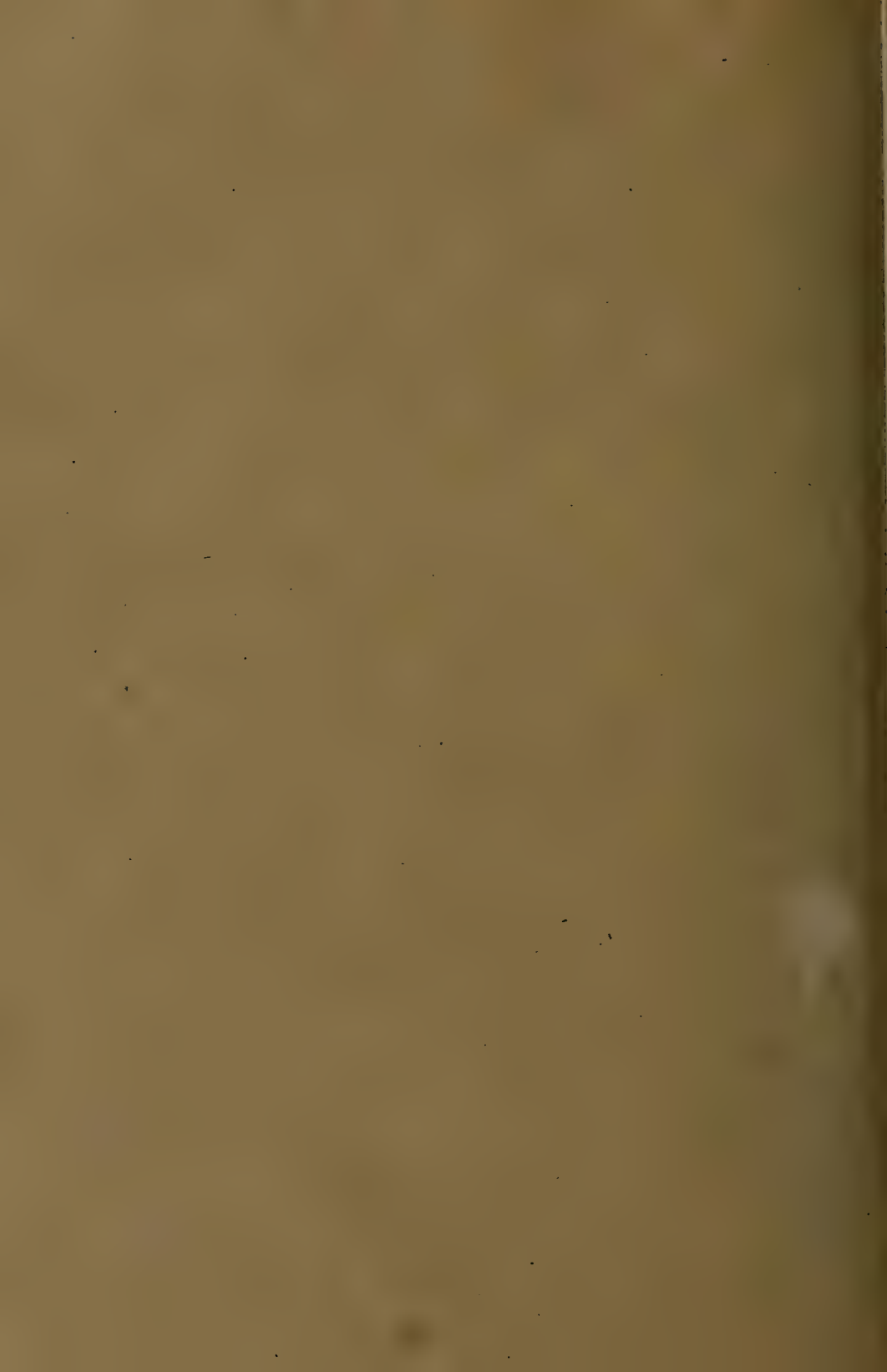The Asteriidæ and the Solasteridæ are comparatively closely related families, both belonging to the order Cryptozonia in the Asteroidea. The Solaster possesses a heavily yolk-laden ovum (1 mm or over in diameter) which undergoes a somewhat incomplete metamorphosis, the free swimming larva not having a completely formed alimentary tract. The Asterias, on the other hand, undergoes complete metamorphosis, the larval form being a typical Bipinnaria.

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other hand, the breeding season of Solaster may be much later at St. Andrews than in Great Britain (as is the case with a number of other forms) and the sperm may have ripened earlier than the ova, as was observed in the case of Asterias in 1917.

The Asterias sperm and ova were normal and ripe. The ova matured within 30-40 minutes after being placed in sea water. As the spermatozoa were rather sluggish, a few drops of ammonia were added to the water. Sperm thus treated became very motile, and when added to the ova of its own species induced a development of from 90 to 100 per cent.

On July 22, 1917, at least two weeks before Bipinnaria were found in the plankton, male Asterias were procured whose testes were swollen and large and had every appearance of being mature. The spermatozoa, however, when introduced into sea-water remained motionless. On adding enough ammonia to the water to raise the NH_3 concentration to $0 \cdot 0055$ grams in 100 c.c., the sperm became motile and capable of fertilization. A lowering of the concentration to $0 \cdot 0045$ grams in 100 c.c. stopped all movement. Fresh sea water was found to contain normally $0 \cdot 002$ per cent NH_3. Later in the season (early August) spermatozoa were active in normal sea water. It is known that increase in the hydrogen ion concentration will inhibit the movement of spermatozoa and cilia. Possibly the testis is acid owing to the presence of a relatively great amount of CO_2 produced in the active metabolism of the developing sperm. The mature sperm would thus be existing in an anæsthetized condition. The addition of ammonia neutralizes the inhibiting effect of the CO_2 and renders the mature sperm motile. In a fully ripened testis, the developing process has largely ceased and the acidity has had a chance to be dissipated. For such a testis the alkalinity of normal sea water is sufficient to activate the spermatozoa. The Solaster testes, according to this assumption, were not fully ripe, since the spermatozoa of Solaster were quite motionless in sea water and were activated only by being placed in alkaline sea water.

Solaster spermatozoa, which had been thus activated, were poured into bowls containing mature Asterias ova. In one and one half hours fertilization membranes appeared in about 20 per cent of the eggs. Their polar bodies were all *outside* the fertilization membrane. In the case of the control (Asterias sperm x Asterias ova) the polar bodies were all *inside* the fertilization membrane.

The difference in the position of the polar bodies is due the difference in time of the initiation of fertilization. In the cross-fertilized ova this is delayed well beyond an hour during which the polar bodies are extruded. When the membrane forms it

lifts up from the surface of the ovum and pushes the polar bodies ahead of it. In the self-fertilized ova the membrane forms within a few minutes, the polar bodies are produced later and, therefore, come to lie within the membrane.

Development proceeded regularly in the cross-fertilized ova, but more slowly than in the self-fertilized Asterias ova. Six hours after mixing the sperm with the ova the Solaster sperm x Asterias ova had developed into the 16-cell stage while the Asterias sperm x Asterias ova had developed into the 32-cell stage and some even into later stages. Except for this delay in rate and for the very much decreased percentage of developing eggs, no difference was discernible on comparing the living self and cross-fertilized Bipinnaria.

To exclude the possibility that the alkalinized water alone might have caused the development, Asterias ova were placed in alkaline water without sperm. None developed. Also, to exclude the possibility of Asterias sperm being present with the Asterias ova before introducing the Solaster sperm, all the water used was carefully heated to boiling point and then cooled. The starfish used were thoroughly rinsed in such water before removing the gonads.

We know that fertilization involves two sharply separated processes; first, an impetus which activates the hitherto quiescent egg so that it will segment and undergo embryonic development; and second, a fusion of the male pronucleus, introduced into the egg by the spermatozoon, with the female pronucleus, a part of the original nucleus of the egg. The second process involves the inheritance, in the offspring, of the paternal and maternal characters.

The first process has been independently produced in the laboratory by a variety of so called "parthenogenetic" agents. The Asterias ova are very easily induced to this sort of development. The application of heat and mere shaking occasionally suffices to start cortical changes resulting in the throwing off of a fertilization membrane followed by segmentation. It is possible that the Solaster sperm may have acted on the Asterias eggs only in so far as to induce them to parthenogenetic development. This would explain the purely maternal appearance of the larvæ resulting from the cross. Further investigation of this problem is being conducted.

REGENERATION OF BONE

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INTRODUCTION

The transplantation of bone is one of the very important branches of reparative and conservative surgery. The cardinal principles governing the successful transplantation of bone were laid down by Ollier, but there still are divergent views both as to the best technic for bone transplantation and the vital processes by means of which the reproduction of bone takes place.

The reproduction or regeneration of bone is the basic phenomenon upon which a successful transplantation depends, and it is in the hope of shedding more light upon this process that the experiments recorded below were performed by the authors.

A summary of the normal development, growth, and structure of bone along with a brief review of the literature on bone transplantation will be given before proceeding to the results of our work.

There are two types of bone found in man, cartilage bone which develops on a cartilaginous basis, and membrane bone, which develops directly from connective tissue without an intervening cartilaginous stage. The mode of development of cartilage bones only, and not of membrane bones, will be described here, because most of the skeleton is composed of bones of this type and most of the experimental research on transplantation has been done with cartilage bones. Previous experiments have yielded divergent results both as to the fate of the transplanted bone and the regenerative power of its various constituents.

THE DEVELOPMENT, GROWTH, AND STRUCTURE OF CARTILAGE BONE [1]

A description of the development of one of the long bones will serve as an illustration of the embryonic development of cartilage bones.

The site which is later occupied by a mature, fully developed bone is first filled in by embryonic connective tissue with closely packed cells. This tissue is transformed into cartilage. The cartilage is non-vascular, but is surrounded by a vascular, fibrous membrane, the perichondrium, which develops into periosteum.

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[1] This description is taken in the main from Cunningham's "Text-book of Anatomy," Fourth Edition; and Quain's "Anatomy," vol. ii, Part I, "A Text-book of Microscopic Anatomy," by E. A. Schafer.

Next, two changes occur simultaneously: (1) The cells at the centre of the cartilage become enlarged and flattened and pile up in longitudinal columns radiating from the centre towards the ends, and the matrix becomes hardened by a granular calcareous deposit. (2) The cells of the inner lining of the perichondrium assume a flattened or cuboidal shape and become osteoblasts, constituting *the osteogenic* or *cambium layer,* and form a bony layer on the surface of the cartilage.

Bone is formed from osteoblasts by the deposition of inorganic material about each cell. The osteoblasts are thereby included in small spaces or lacunæ and are then called young bone cells. These cells are not surrounded by a solid calcified wall, but small channels called canaliculi, which arise from the lacunæ, radiate irregularly through the surrounding matrix, and anastomose with canaliculi from neighboring lacunæ. The cells within these spaces also send filamentous processes into these canaliculi, thereby giving the cells a spidery appearance.

The next step is a migration of the subperiosteal vascular and osteoblastic tissue into the centre of the cartilage, followed by an absorption of the calcified cartilage and by formation of marrow spaces. These spaces are filled by jelly-like embryonic marrow and are lined by osteoblasts. This lining of osteoblasts is known as endosteum, but it should be noted that it arises from periosteum. Bone is deposited around these marrow spaces by an advancing line of osteoblasts. The osteoblasts follow and surround the blood-vessels and deposit bone in layers about them. As layer after layer of bone accumulates about the vascular structures, ramifying, communicating trabeculæ of bone are formed, and the large spaces between them become narrowed into intercommunicating channels. While this process is taking place layers of bone are also being deposited beneath the periosteum, but this cortical deposit is penetrated by numerous blood-vessels which communicate with those in the medulla.

At about this time large cells, which are usually multinuclear, appear along the edges of the bone which has been formed. Their action is the absorption of bone and they are termed osteoclasts. In performing their function they excavate hemispherical pits known as foveolæ of Howship.

Bone absorption is necessary for the growth of bone. The absorption occurs most actively in the centre, or medulla, of the bone, though to some extent it also occurs in the cortical portion. By this means the marrow spaces which have, in the meantime, been filled with cancellous bone are reformed as the secondary marrow space. The cortical bone is being removed from the centre, and while this is occurring bone is being deposited in lamellæ upon the outer cortical surface, thus bringing about lateral growth or increase in width. Growth at the ends occurs by an advancing deposit of bone in the growing cartilage.

At about this stage additional centres of ossification develop in each end of the bone. These are called epiphysial centres and are situated between the end, or epiphysis, and the shaft, or diaphysis. The epiphysial line is formed by blood-vessels or vascular loops advancing into the epiphysial carti-

lage and carrying with them endosteum or osteoblasts. This osteogenetic tissue then becomes completely separated from the osseous shaft by a portion of cartilage which remains. This cartilage persists in some places until after puberty, and continually grows, and by being continuously replaced by bone causes the longitudinal growth of bone.

The intercommunicating channels, referred to as arising in the marrow spaces and in the bone deposited by the periosteum, are lined by osteoblasts and deposit concentrically at their periphery successive lamellæ of bone. By this process the channels become reduced to very narrow canals, called Haversian canals. These Haversian canals therefore contain blood-vessels and are lined by osteoblasts. The systems of concentric bone lamellæ are joined together by irregular systems of lamellæ. Upon the surface of the shaft, completely around all these systems, additional concentric lamellæ are deposited beneath the periosteum by the cambium, or osteogenic layer of periosteum.

Eventually the marrow spaces become converted into a central canal filled with mature marrow [2] and containing only a few trabeculæ of bone. Around this central canal is a layer of interlacing bone trabeculæ forming cancellous bone, and all of these trabeculæ are covered completely by a layer or layers of osteoblasts. Outside of this is found the compacta, or cortex, the Haversian systems and lamellæ of which have been described above. The surface is covered by the osteogenic or cambium layer of osteoblasts, which can be considered as lying between the cortex and the fibrous periosteum, or, one might say by way of comparison, that the cambium layer lies between the tree and its bark. It is this similarity which has given rise to the name cambium layer of the periosteum.

The blood supply of the bone is furnished (1) by numerous minute nutrient arteries (mentioned above) which penetrate the cortex from the periosteum through the Haversian canals; (2) by one or more larger nutrient arteries which penetrate the cortex usually at about the centre of the shaft and enter the medullary canal.

The important points to be emphasized are:

(1) All cartilage bone is produced by cells arising from the osteoblasts lining the periosteum and is deposited in preformed cartilage, the latter being absorbed.

(2) The endosteum is formed of osteoblasts which arise from those lining the periosteum, and osteoblasts also extend from the endosteum and the osteoblastic (cambium) layer of the periosteum into the Haversian canals and line them.

(3) Cartilage which is about to be ossified undergoes certain changes, among which is an enlargement and flattening out of its cells, with their arrangement into columns at right angles to the plane of bone growth.

(4) Bone cells (not osteoblasts) are enclosed in bony lacunæ which intercommunicate by means of canaliculi.

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[2] The development of marrow will not be discussed, as it has nothing to do with the growth of bone.

(5) None of the bone cells are in immediate contact with blood-vessels or capillaries and their only nourishment must be plasma obtained through the canaliculi.

(6) The above described method of bone growth is known as growth by absorption.

In addition, several other things of importance may be mentioned. Young lacunar cells which have just been formed from osteoblasts may divide and form a very limited amount of bone. When bone develops in this manner immediately in apposition to older bone, the older bone may be absorbed and replaced by this young bone. The method by which bone is absorbed in this instance is not known, but it is not accomplished by the aid of osteoclasts. Apparently it occurs in some direct manner which appears somewhat like solution of the bony structure. This type of bone growth or bone substitution is called creeping replacement.

Most bone, however, arises directly from osteoblasts. *Fully developed bone cells, in the accepted sense of this term, that is, cells within well calcified lacunæ, have never been shown by microscopical observation to have divided and formed new bone.* Undoubtedly most normal bone is formed by the endosteum and cambium layer of the periosteum and only to a less degree by osteoblasts lining the Haversian canals.

REVIEW OF LITERATURE

The earliest experimental work bearing upon bone transplantation was done by that great master of bone surgery, Ollier, in 1867. He did not make a histological study of his transplants, nor did he have the aseptic and antiseptic methods of operating that make the work of to-day so sure of successful issue. His conclusions on bone transplantation were all based upon macroscopical observation, and up to the point where microscopic study is necessary they have been found absolutely true. Briefly stated, they are as follows:

1. For the transplantation of bone there is a fundamental difference in the use of autogenous, periosteum-covered grafts, on the one hand, and every other kind of bone material, on the other.

2. Only the former manifests an increase of thickness after a rapid fibrovascular connection with its bed, and it is the increase of thickness which is a sure indication of continued vitality of the transplanted bone.

A true graft of bone (*i.e.,* a graft that retains its vitality) is possible only after transplantation of living, autogenous periosteum-covered bone, and this is in virtue of its living periosteal covering. This latter, after transplantation, remains alive, *and thereby maintains the life of the transplanted bone.* It is, therefore, the most important factor in bone transplantation.

3. Every other kind of bone when transplanted dies, if it is not already dead at the time of its implantation. All of these varieties of transplants become foreign bodies, and either remain intact and encapsulated at the site of implantation, or they are resorbed, the latter process often being hastened by the blood-vessels which penetrate into them. If such material is im-

planted in a bed that is itself bone-producing, it is possible, under favorable condition, for it to be replaced by new bone formed from this bony bed.

4. Where it is desirable to restore bony continuity after the removal or destruction of bone, one must make use of living, autogenous, periosteum-covered bone grafts.

These conclusions were accepted and remained classical until the work of Radzimowsky, 1881, and Bonome, 1885. Both these experimenters contended in contradistinction to Ollier that all transplanted bone tissue dies even when it is autogenous and covered by living periosteum, but that the periosteum lives. As proof of their contention they instanced the death of the bone cells as shown by the change of the latter in morphology and staining qualities.

Radzimowsky demonstrated in the cranial and long bones of birds and mammals that bony union between bone fragments and adjacent bony tissue easily takes place irrespective of whether the periosteum is preserved or not, over such fragments. Such bony union, however, may be considered no evidence of enduring life in the fragments of bone, and further, the presence of blood-vessels in such fragments is no evidence of life therein, inasmuch as dead bone may also be permeated by blood-vessels from its vicinity. Microscopic examination of such bone fragments showed that they were dead, for the bone cells were dead. On the other hand, he demonstrated that the periosteum lived and produced new bone. Therefore, he concluded that when living periosteum-covered bone is transplanted, the bone tissue proper dies, but the periosteum lives and produces new bone that is deposited not only on to the surface of the transplanted dead bone, but also into its lacunæ and enlarged Haversian canals.

Bonome, working with rats, reached conclusions similar to those of Radzimowsky, and also showed that when fracture of the bone occurred the bone in the immediate vicinity died, as was shown by the staining qualities of the bone cells. As to the ultimate fate of the dead bone he concluded that this is resorbed and replaced by new bone which is formed from the osteogenetic layer of the periosteum.

The work of these two experimenters established, with the aid of microscopic study, the first great advance after the pioneer investigations.

No new advances or contributions to the subject of bone transplantation were made until Barth, 1893, reported the results of his experiments. He likewise found that when autogenous, living, periosteum-covered bone is transplanted the bone tissue dies, and in addition he makes the important assertion that he could not convince himself that the periosteum or marrow fared any better than the bone tissue proper. He deduced from his experiments, which were performed on the skull, that the transplanted periosteum also died, and was replaced by the growth of periosteum from adjacent bone which extends over and covered the graft. He concluded, therefore, that inasmuch as all the parts of a living periosteum-covered bone graft died, it is immaterial whether we use, for transplantation purposes, bone covered or uncovered by periosteum, or decalcified or macerated bone. He thought

that the transplant was a sort of splint, to be gradually replaced by new bone formed from the surrounding bony tissue. Briefly stated, the conclusions of Barth were as follows:

1. Fragments of any kind of bone like other foreign bodies can be implanted into the living tissues.

2. All varieties of bone material are similar in the process of their implantation and of their bony replacement.

3. When living bone with periosteum is transplanted, all of its integral parts die.

4. Therefore, all varieties of bony substance are foreign bodies at the outset, or become so, and are *gradually replaced by new formed bone from the adjacent bone-producing tissue.*

These conclusions, radically different as they were from those of Ollier and his followers, were accepted, and for the next decade surgeons gave up altogether bone grafting and used macerated or decalcified bone or other foreign material to fill gaps in the continuity of the skeletal system. The lack of success of these procedures was brought out in the German surgical congress of 1902, when the subject of bone transplantation was brought up for discussion. It was the general opinion at this meeting that though the experimental conclusions of Barth might be true, the same did not hold in practice. The opinion expressed seemed to be unanimous—that the best results were obtained when living, autogenous, periosteum-covered transplants were used. Even Barth had to concede this in the light of his subsequent experience.

In order to gain more information on the subject Axhausen took up the work anew, and by his experiments and histological examinations placed the whole subject on a firm basis. His conclusions were as follows:

1. The conclusions of Barth as to the relative equal value of all varieties of bony material for transplantation cannot be upheld.

2. The first law of Ollier, namely, that there is a fundamental difference, as regards transplantation, between the use of living, autogenous periosteum-covered bone, and every other kind of material, is true in other animals as well as in the human subject.

3. The difference lies not, as Ollier thought, in the survival of the life of the transplanted bone tissue proper, for most of this dies, only a few cells persisting, and is replaced by new bone, but exists in the periosteum which survives.

4. This surviving periosteum produces the new bone. When the transplantation is made into a bone-producing bed the new bone formation from the periosteum is not marked, for this bed is alone sufficiently able to fill the gap in its substance. But in the case of transplantation into a defect of the long bones this periosteal new bone formation, together with that from the marrow, are the only means for filling the gap.

5. The survival of the bone-producing periosteum established a rapid and intimate vascular connection between the transplant and its bed, and in virtue of its bone resorbing and bone forming power this surviving periosteum

forms an intimate connection with the underlying transplanted dead bone. This is in marked difference to the loose fixation of the transplant in the tissues when bone uncovered by periosteum is used.

6. The survival of the marrow has no dependence upon whether the graft is covered or uncovered by periosteum.

These experimental conclusions were in accord with the empirical ones reached by practical surgeons, and since these classical labors of Axhausen the surgical world has accepted, until recently, the fundamental law of Ollier, namely, that for a real bone graft we must use an autogenous portion of bone covered by living periosteum.

The truth of this law of Ollier has been disputed by William MacEwen in a monograph entitled " The Growth of Bone," published in 1912.

MacEwen claims that periosteum is merely a limiting membrane, which has not the capacity to form bone, but serves only to confine the bone within bounds and prevents its overproduction. He believes that bone has the power to reproduce itself, if it receives proper blood supply, and is the only tissue from which new bone can grow.

There are two important criticisms which can be made of MacEwen's work:

1. In spite of his large number of bone transplantation experiments there is an almost complete absence of microscopical study of the grafts. Therefore, evidence, which is only revealed by the microscope, that the *bone cells* of the transplant are alive and that regeneration has originated from them and them alone, is lacking.

2. He concludes that transplanted bone is alive if it has contiguous connective tissue adherent to it and gives with the X-ray a shadow almost as dense as that of the original living bone. This, however, has been disproved by Küttner, who showed that this occurred in homogeneous human transplants of large portions of bone, such as the head and upper third of the femur, and still on microscopical examination all of the bone cells were dead and no osseous regeneration of any sort had taken place from the transplant.

Since MacEwen's monograph several important contributions have appeared, in the main contradicting his conclusions.

Mayer and Wehner, from a carefully planned series of experiments of transplants of periosteum, subperiosteal resections, cap implantations, and bone transplants, conclude that all of the results " combine to emphasize the osteogenetic function of the specific osteoblastic cells (of periosteum, endosteum, and lining Haversian canals) and the inability of the adult bone cells to form new osseous growth.

Their cap experiments are especially conclusive.

They note the part played by the osteoblasts lining the Haversian canals in regeneration of bone, but do not assign to these cells the importance they probably deserve. They demonstrate, however, that it is the outgrowth of these cells from the Haversian canals which forms new bone about free transplants of cortex devoid of endosteum and periosteum, and that this is

not due to the metaplasia of the surrounding connective tissue according to Baschkirzew and Petrow.

Mayer and Wehner also have shown that in the replacement of old bone by new bone the process described by Marchand and Barth as "creeping replacement" is of equal, if not greater, importance than the well recognized one of absorption and substitution.

Phemister's investigation adds additional experimental evidence to that of Mayer and Wehner. In some very well selected experiments he demonstrated that in artificial or acquired fracture in the middle of a transplant of a portion of the entire shaft of a long bone, new growth of bone occurs at the fracture, whereas the bone between this fracture and the ends of the transplant (which are in contact with the original shaft) is dead. This new bone arises mainly from endosteum and periosteum. Phemister believes, however, that a few bone cells of the compacta do proliferate and form new bone. A careful perusal of his protocols does not reveal any definite evidence of this. It would seem that here he is dealing with osteoblasts of the Haversian canals as noted by Mayer and Wehner and not with bone cells. The healing of these fractures in the transplant, of course, completely disproves the idea that bone reproduction occurs only through "osteoconductivity," which was held originally by Barth and at present by Murphy and by Davis and Hunnicutt.

Smith also reached the conclusions from his experiments that mature bone cells are end products and that osteogenesis is limited to the osteoblasts.

OUTLINE OF EXPERIMENTS

The object of the experiments to be reported was to determine the fate of the various component tissues which make up bone, when these were transplanted either singly or in different combinations, and also to find out under what circumstances these transplants produced new bone, and which element or elements were capable of generating bone.

The following experiments were performed on twenty-two full-grown cats; the transplants were autogenous, and the material was taken from the tibia. In all cases primary union was secured at the wound over the tibia and at the site of transplantation. A few of the transplants were placed on the surface of, and within the substance of, the spleen, or subcutaneously. Most of them, however, were placed upon the costal cartilages, after these were either scraped bare of perichondrium or else after removing a wedge from the cartilage or cutting away its outer half. An attempt was made at first to lift up the perichondrium and insert the transplant between it and the cartilage. The perichondrium was found so intimately bound down that it was impossible to do this without tearing it severely, so this method had to be abandoned. The transplants were held in place by two black silk ligatures which were placed at the two ends and around the cartilage. With a sharp, full curved needle, the ligature could be passed around the cartilage without entering the pleural cavity. In order to determine the nature of the material transplanted, pieces of tissue were removed from each transplant as soon

as it was secured, placed in 10 per cent. formalin and examined micro-
scopically. A series of control experiments was performed by injuring
the cartilage in a number of ways, in order to determine if any impetus or
tendency to form osseous tissue could be given cartilage by manipulations
similar to those necessary in using it as a site for transplantation.

Cartilage was chosen as the site of most of these experiments for several
reasons. It has been shown that tissues differ in the degree of their capacity
for serving as a satisfactory medium or soil for the implantation upon them
of other types of tissue. Certain structures, such as the spleen, serve in this
capacity very poorly, and in fact the spleen seems to have a destructive action
on grafts placed within it. Subcutaneous or intramuscular situations have
been the ones most often and most successfully used in transplantation experi-
ments. There are also certain special affinities which exist between tissues,
as, for instance, with testes and ovaries. Stockard has shown that these
organs serve well as a base for transplantation of grafts taken from each
other, whereas grafts from either do very poorly on other tissues. Conse-
quently because of the intimate relationship which exists between bone and
cartilage, both during the process of growth and in the stage of full develop-
ment, it was thought that it would be interesting to note in what manner
cartilage would serve as a base for transplants of different elements of fully
developed bone, and whether it still retained its ability to readjust its own
elements in the peculiar manner which occurs in the laying down and growth
of enchondral bone.

Also one of the requisites for the successful transplantation of tissue is
that the graft should be rapidly vascularized. For this reason transplants
are usually made subcutaneously, intramuscularly, or into parenchymatous
organs. Cartilage, however, is nonvascular, and it was thought that trans-
plantations upon this would prove a severe test of the regenerating power
of the transplanted bone, which could not get its vascular supply from the
cartilage but would have to obtain it from only one side which would lie in con-
tact with the connective tissue. In addition, because of the freedom of
the cartilage from blood-vessels, if the bone graft did grow in the direction
of the cartilage, it would furnish a good opportunity to observe just which
element or elements of the graft proliferated.

The material for the transplants was obtained in the following manner:

The anterior surface of the right tibia was exposed and all tendons and
muscular attachments cut away, care being taken not to injure the periosteum.
The periosteum was obtained from the entire surface exposed, by outlining
a long quadrilateral piece with the scalpel, then lifting it up at one end with
a fine forceps, and teasing it away from the bone with the handle of the
scalpel. It always peeled away very easily and no macroscopic bone came
away with it. The question as to the nature of the cambium (osteogenetic)
layer, or portion of this layer, which was lifted off with the periosteum is
discussed later. Next, with a gouge chisel, thin layers of cortex, from 1 to 3

mm. thick, were removed, care being taken not to enter the medullary cavity. It was assumed that a portion at least of the cambium layer remained upon the cortex, and for convenience the pieces of the cortex taken in this manner are called cortex plus cambium. To obtain pieces of cortex alone, the surface was first thoroughly scraped with a heavy scalpel, and then the pieces were removed with a gouge. Cortex covered by periosteum was obtained by outlining with a scalpel an area of periosteum about .5 x 1 cm. Then the cortex beneath this, with the periosteum still adherent, was removed with a gouge chisel. Care was taken not to enter the medullary cavity so as not to include endosteum.

The transplants called cortex plus endosteum are simply pieces of cortex, where the gouge was allowed to enter the medullary cavity and remove in addition a portion of the medulla.

Portions of these different types of transplants which served us as controls showed uniform microscopical pictures, and need not be described repeatedly under the different experiments, but can be taken up collectively here.

Periosteum Control-Serial Sections.—Sections are made up of the usual connective and fibrous tissue, but along the edge corresponding to the cambium layer, the connective tissue is rather compact and the cells are arranged parallel to the surface. A portion of the surface which faced the cortex is lined by cells which resemble the adjacent connective-tissue cells, but at the same time present an appearance somewhat similar to endothelium. In other places, these surface cells are slightly larger and more rounded and seem to differentiate themselves from the connective-tissue cells immediately beneath. This special layer of cells which constitutes the cambium layer is found only over portions of the surface, occupying about half of its extent. The remainder of the surface is made up of bare connective tissue.

Cortex Control.—This is made up of typical dense cortical bone. The Haversian canals are small, most of them the size of capillaries. (Much smaller than in any of the transplants described below.) The edges are everywhere devoid of connective-tissue cells or any cells similar to the cambium layer of the periosteum described above. Also the edge to which the periosteum was attached, and where the cambium was scraped away, is slightly uneven, as though the surface of the bone had been scraped away.

Cortex plus Cambium Control.—The edge from which the periosteum has been removed is smooth, and in places the cortex is bare, whereas in other places there is adherent to the cortex a small amount of loose areolar tissue. At these latter places, the cells immediately next to the cortex are drawn out and flattened, and have somewhat the appearance of endothelial cells, and are similar to those of the cambium layer found lining the periosteum described above. Immediately beneath this layer of cells the superficial cortex does not exactly resemble the deeper portions, staining somewhat differently, taking a slight diffuse hæmatoxylin stain, and its lacunar cells are closer together. The nuclei of the lacunar cells immediately beneath the cambium layer are drawn out and stained densely, and are identical with

those of the cambium; whereas the nuclei of cells further away are round or oval in outline. A number of nutrient vessels spring from the cambium layer and enter the cortex at an angle, and their canals are sparsely lined by the same type of cells which are immediately adjacent to the cortex and which form the cambium layer.

Cortex plus Periosteum Control.—Shows cortex covered by periosteum, these structures being identical with those described above. The cambium or osteogenic layer is similar to that described under cortex plus cambium, only it is thicker and made up of more of the same type of cells. In one series of sections, whereas the lacunar cells immediately beneath the cambium are similar to the cells of the cambium layer, after a distance of one or two cells into the cortex the lacunar spaces are larger and their cells are also larger and more oval and more vesicular, so that these cells, taken altogether, have the appearance of young lacunar cells. (These are somewhat similar to those seen in the new-formed bone in the transplants to be described below.)

Cortex plus Cambium plus Endosteum Control.—These transplants are made up of cortex and cambium identical with those described above, and in addition a portion of the medulla of the bone made up of marrow spaces filled with marrow and subdivided by trabeculæ of bone. The marrow spaces are lined by flattened cells somewhat endothelial in type, identical with those forming the cambium layer. These cells are seen dipping down into the Haversian canals and lining them.

GENERAL DISCUSSION OF RESULTS

Experiments with Transplants of Periosteum.—Twenty-five periosteal transplants upon costal cartilage were examined at periods from 7 to 389 days. All showed active bone formation. One subcutaneous transplant after 77 days showed bone, one on the surface of the spleen showed bone after 82 days, but one in the depth of the spleen for the same period showed nothing but a scar. This is in accordance with the experiments of others with different tissues, and shows the tendency of spleen to absorb foreign tissues.

The process of growth of bone from these periosteal transplants has therefore been studied at such intervals that a definite conception may be formed of the manner of its occurrence.

In the youngest transplants (7 days) there is found in the midst of granulation tissue, an extremely young osteoid tissue which is in contact with the osteogenic layer of the periosteum. The next stage is found after about 28 days, where young bone is seen as branching columns, covered by a continuous line of osteoblasts. Here the lacunar cells are just about formed and are large, with large, oval vesicular nuclei. From this stage on, there occurs in older transplants well formed bone, with all of the characteristics of bone. The lacunæ are fully developed and are grouped concentrically around Haversian canals, and also irregularly disposed between these concentric systems. At a distance from the periosteum instead of canals are found larger spaces which are irregular in shape and are first filled with blood-vessels and delicate granulation tissue, and lined by osteoblasts, and

are later occupied by genuine hæmatopoietic marrow (sometimes admixed with fatty marrow), composed of myeloblasts, myelocytes, megalokaryocytes, megaloblasts, etc. When this new-formed bone is in contact with cartilage it almost invariably invades it in the same manner as enchondral bone invades its cartilaginous matrix, in the normal development of bone, *i.e.*, in the manner described in the protocols as epiphyseal line formation. It would seem from this that cartilage is an excellent medium to accommodate the growth of bone, and that even though it is nonvascular, bone will grow into it and carry its nourishment along with it.

The older the transplants the more calcified they are and the more the lacunar cells assume the appearance of adult bone cells, their spaces and nuclei being smaller and more elongated, and the latter also staining darker.

There is one observation of importance concerning the young lacunar cells which are not yet in a bony matrix but are away from any layer of osteoblasts. The same process of amitotic division and growth is found as was described by Mayer and Wehner. It seems definite that these young cells proliferate in this manner and so account for some of the production of bone. They are found crowded together in areas, in the midst of well developed bone. Here the lacunar spaces are somewhat larger than elsewhere and in some of the areas are in the midst of a matrix which partakes of the properties of both bone and cartilage, for with the hæmatoxylin and eosin stain it has the appearance of bone but stains bluish, like cartilage. Nuclei which are much elongated, indented, or figure-eight in shape, are relatively common in these lacunar spaces, but two nuclei in a single space are seldom seen. Young lacunar cells which lie close to one another or are barely separated by a very thin partition and which appear to have just divided are relatively frequent. Therefore, taking all these facts into consideration, it must be recognized that this is one manner of bone growth. This growth of bone is from young, immature lacunar cells which have just developed from osteoblasts and whose lacunæ are not yet formed by calcified osseous material. It is this type of growth which occurs in bone transplants and in the development of bone and causes the " creeping replacement " of Marchand and Barth. We have never observed this or any other manner of growth proceeding from adult bone cells.

The source of origin of the hæmatopoietic marrow and its method of development is another point of interest and needs further study for its elucidation. The transition has gradually progressed from (1) Haversian canal containing a blood-vessel with a wall formed of a single layer of endothelium and a layer of osteoblasts between the vessel and bony wall, (2) a larger space containing in addition delicate connective tissue, and, finally, to (3) a completely developed marrow. Whether this process of the development of marrow has to do with the histocyte or the endothelium or the osteoblasts is a problem for further investigation.

The manner in which cartilage serves as a medium for bone is remarkable in that it is identical with the embryologic development of bone in cartilage. The reason why the cartilage cells arrange themselves in columns

at right angles to the line of advancing bone has never been explained. This method might serve as a means to attack the problem.

In the older transplants bone absorption is taking place as well as bone growth, for osteoclasts are found at various places along the periphery of the new bone and also of the marrow spaces.

Many of these transplants could not be identified macroscopically as bone, and certainly most of them would not have thrown a shadow with the X-ray. Therefore, it follows that in all experiments of this kind the end result must be carefully controlled by microscopical examination, and where this is not done the conclusions arrived at are based on incomplete evidence.

It is inconceivable that after the careful microscopic examination with negative findings of control pieces of periosteum any bone cells could have been adherent to these transplants. Therefore, the conclusion must be drawn that the bone grew from osteoblasts which form the osteogenic layer of the periosteum and did not arise from bone cells. The fact that the control pieces of periosteum were lined only in places by osteoblasts and not over their entire extent explains why bone did not always spring from the entire surface of the periosteum.

Transplants (of Cortex minus Cambium, Cortex plus Cambium, Cortex plus Cambium plus Endosteum, Cortex plus Periosteum).—Although some of these transplants were in place in the cats as long as 250 days, and were comparatively small pieces, not more than 2 x 3 x 10 mm., none were completely absorbed in that time. Many of them which showed microscopically either well formed bone or growth of bone were too small to have cast a shadow with the X-ray.

The bone or cortex of all the different types of transplants showed the same process, and the nature of this would explain the discordant results which have been reported about transplantations and the osteogenic power of bone.

The transplants removed after the shorter intervals show that most, but not all, of the bone cells are dead and therefore unstained. Those cells which are stained, however, are situated near sources of nourishment (by diffusion), such as the edge of the transplant, the periosteum, or Haversian canals. After a somewhat longer interval there are fewer stained bone cells, but even these have small, irregular, darkly-stained nuclei. In the oldest transplants the tracing of these cells is interfered with by other processes which are going on, but always a certain number of these cells are seen, which are undoubtedly the original cells transplanted, and can be differentiated from the young bone cells present. Therefore it must be as Axhausen contended that, whereas many, if not most, of the bone cells transplanted die, some persist and live. Although carefully examined, none of these adult cells which persist were ever found in any place in the transplant either dividing or giving rise to new bone.

The blood-vessels in most of the Haversian canals evidently, very soon after transplantation, join with surrounding blood-vessels, for their walls are made up of living cells which stain normally, and they contain blood-

cells which are normally stained and alive. The cells of the vessel walls must have been kept alive in the meantime by the blood which was in them or by plasma which diffused in from the tissue next to the transplant. Some canals are empty or contain abnormally-stained blood-vessels and blood-cells which are dead. Very soon after transplantation the Haversian canals enlarge and are found lined by osteoblasts, though a few osteoclasts are also found at this stage. But the absorption of the bone around the canals seems to occur largely in a direct manner and not by the aid of the osteoclasts.

Almost simultaneously with the enlargement of these canals is found a formation at their periphery of a ring of young bone composed of young bone cells. Soon this ring is several cells thick and is found progressing into the bone originally transplanted, the canal becomes progressingly larger. These young cells then proceed more or less irregularly, and, where the canal is near the surface of the transplant, they gain the surface and spread out over it.

This process of growth from the osteoblasts lining the Haversian canals is a very active one and is seen replacing all, or almost all, of the bony transplants and spreading out beyond. These young lacunar cells are also seen dividing just as they were in the bone formed from periosteal transplants. As there are no osteoclasts between them and the original bone which is being absorbed, the absorption must be accomplished by them, perhaps through a biochemical action, and they replace the old bone by " creeping replacement." It is conceivable that they might slip into the empty lacunar spaces of the original bone, but this was never observed.

The transplant, cortex without periosteum, removed after an interval of 53 days (see Fig. 7) is an excellent illustration of this type of bone growth. It also shows that bone cells of the original cortex transplanted, even though they are well nourished by being in apposition with living granulation tissue, do not grow, whereas osteoblasts lining the Haversian canals do.

In order to follow these two types of cells carefully, full grown and not young cats were used in these experiments so as to be able to observe the behavior of adult bone cells and not confuse the study with young bone. Consequently the results of this study carry with them the firm conviction that adult, differentiated bone cells are sufficiently specialized, in the same manner that nerve cells are, to be unable to reproduce themselves.

In the oldest transplants practically none of the original bone is left, but its place is taken by this new formed bone. Osteoclasts are also found here and there along the edges of the transplant, and at the periphery of enlarged Haversian canals.

As the Haversian canals enlarge they first form spaces, thinning out the intervening bone into trabeculæ, and later forming larger marrow spaces filled with hæmatopoietic marrow.

The new bone arising from the Haversian canals grows from the edges of the cortex transplant just as vigorously as does that arising from the cambium layer of periosteal transplants, and invades cartilage in the same manner with epiphysial line formation.
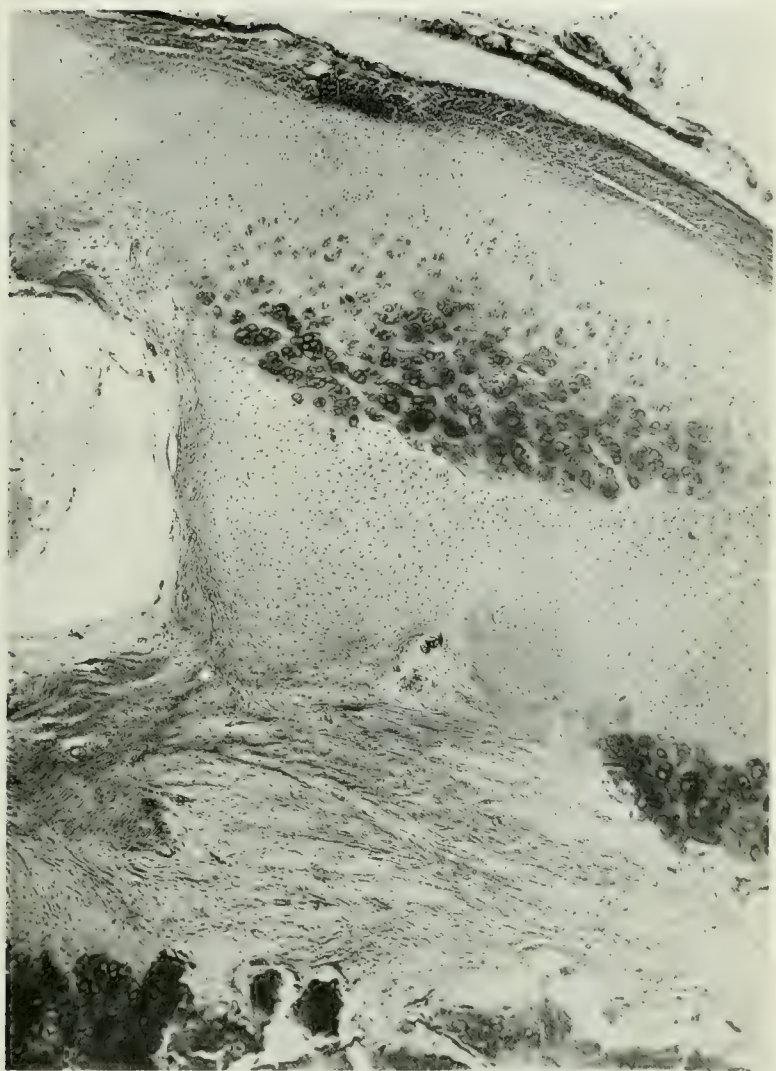
FIG. 1.—Control: Regeneration of cartilage only, after slivers of cartilage were lifted up and tied back again in place (X 60)
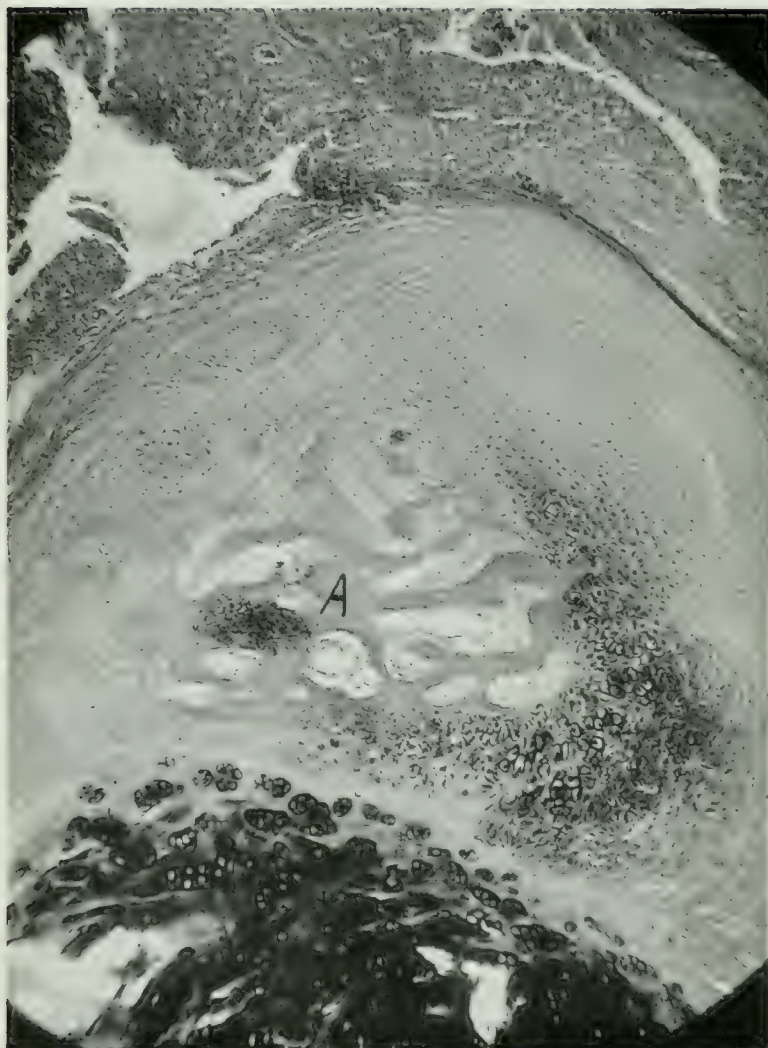
FIG. 2.—26-day transplant of periosteum upon the surface of cartilage denuded of perichondrium; showing the growth of osteoid tissue from the periosteum (A) (× 60).

FIG. 3.—53-day transplant of periosteum upon surface of cartilage denuded of perichondrium; showing growth of young bone from the periosteum (A) (× 60).
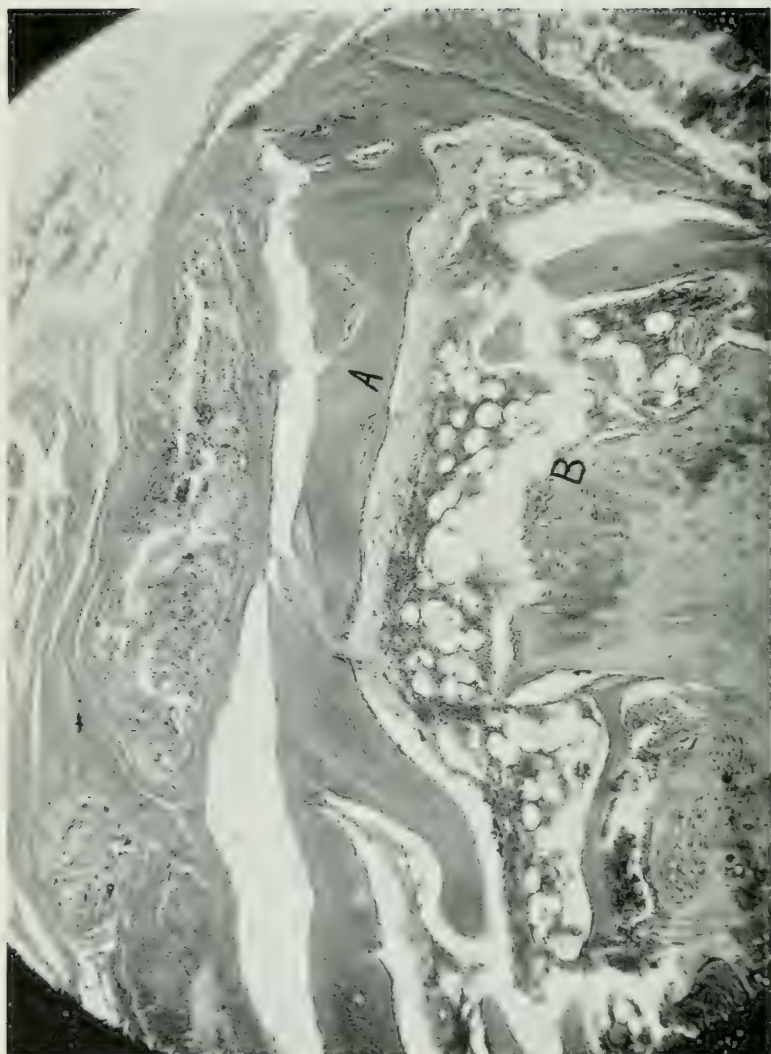
FIG. 4.—245-day transplant of periosteum upon surface of cartilage denuded of perichondrium; showing bone (A) with epiphyseal line formation (B) growing from periosteum. × 50.
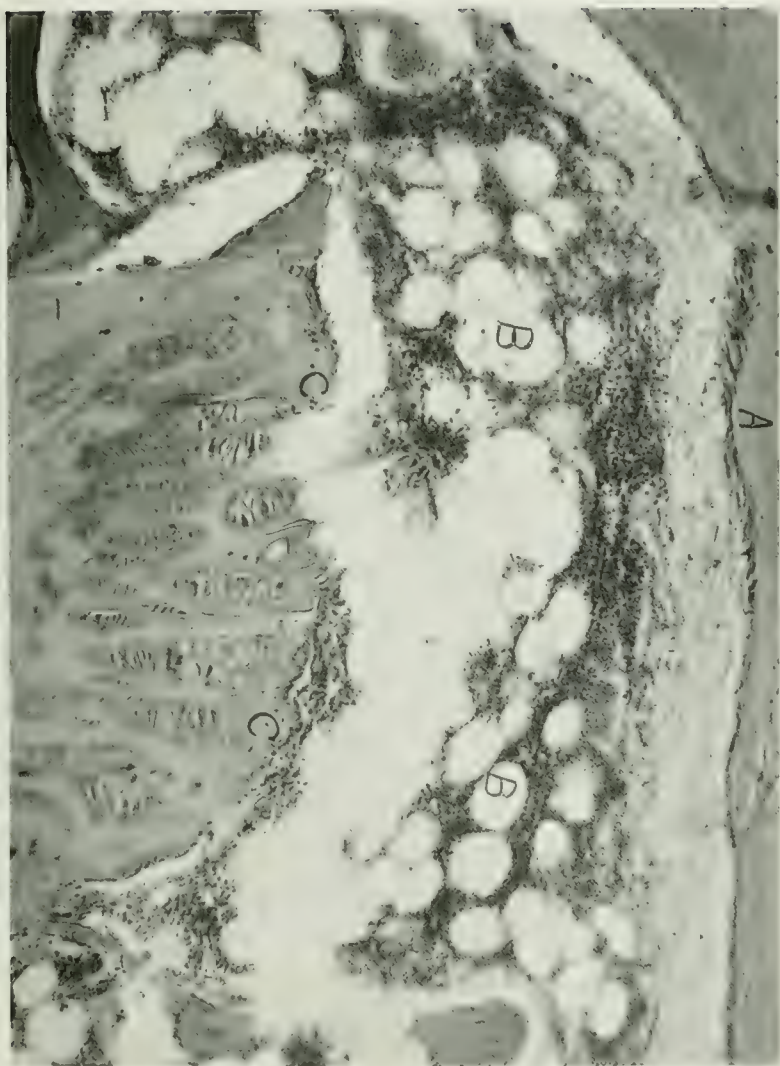
Fig. 5.—High power of Fig. 4. A, bone; B, marrow; C, epiphyseal line formation (× 100).
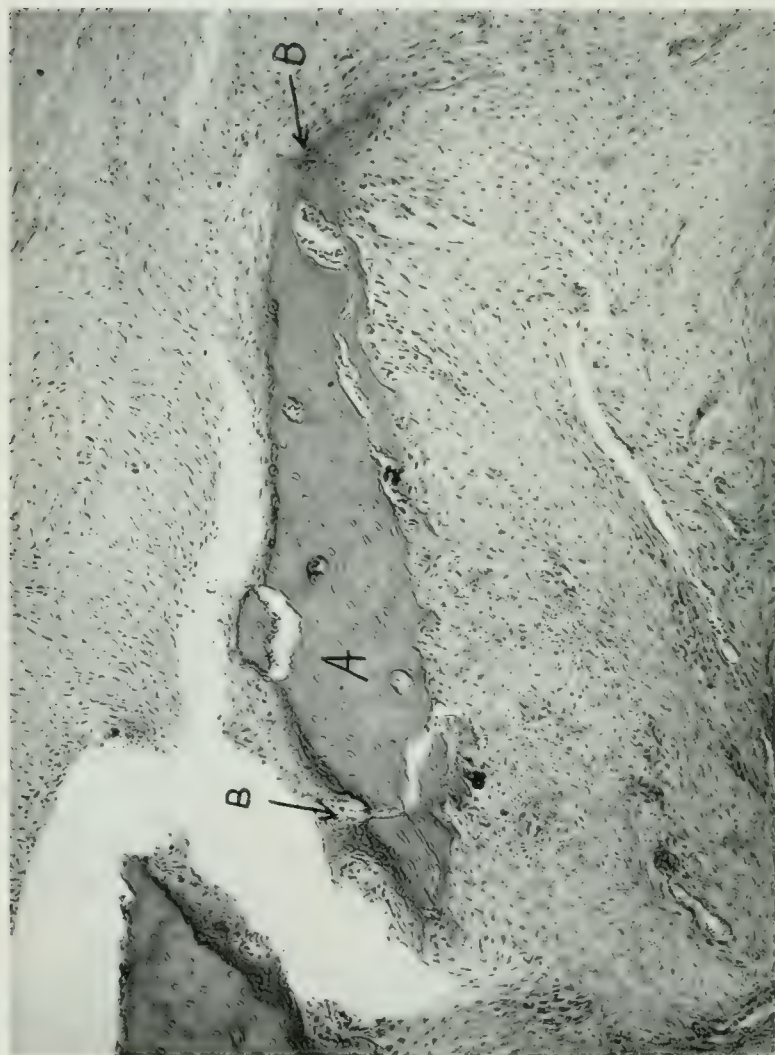
FIG. 6.—28-day transplant of bone cortex without periosteum. Transplant is upon surface of cartilage, but the cartilage is not shown in the photograph. The transplanted bone is dead (A) and the lacunar spaces are empty. There is growth of bone around some of the Haversian canals (B) (× 60).

Fig. 7. Osseous transplant of bone cortex without periosteum. Transplant is upon surface of cartilage. There is proliferation of bone from around Haversian canals (A) and spreading out upon the surface of the bone transplant B (× 100).
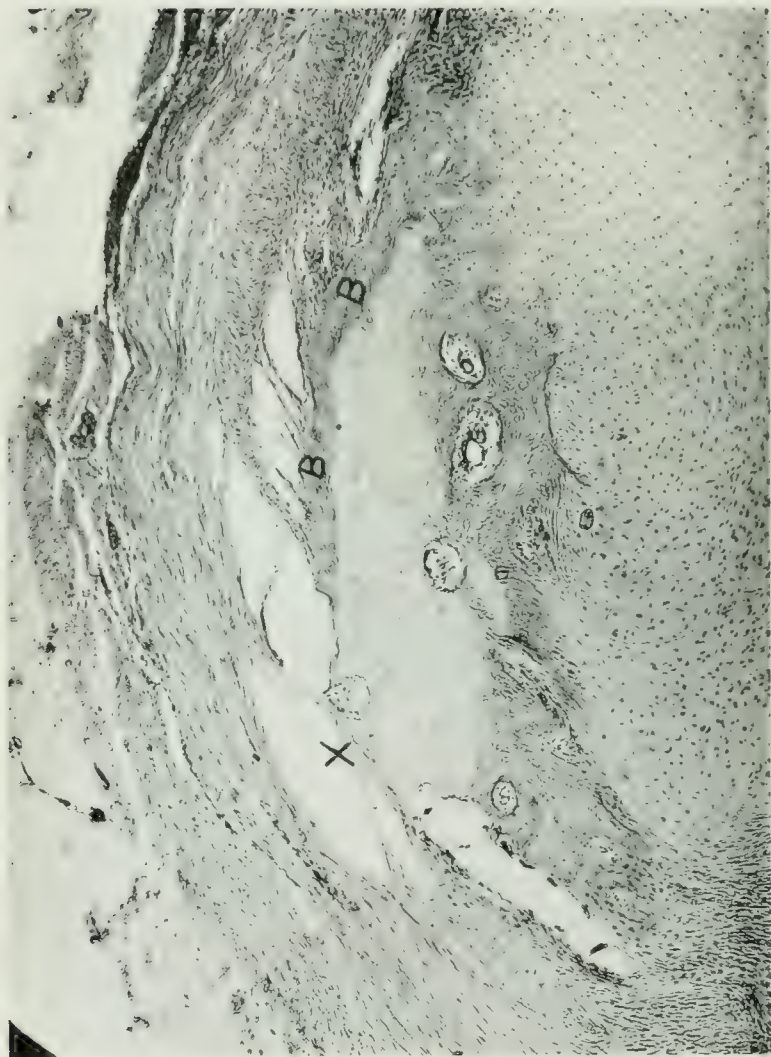
FIG. 8. 55-day transplant, same as Fig. 7 and showing the same. At X, where there is no proliferation of bone, the surface of the transplant of bone is adherent to the surrounding connective tissue just as it is at B, where there is proliferation of bone. Consequently, if the growth of bone occurred from adult bone cells the conditions of growth, such as vascular nourishment, are as good at X as at B. Since growth has not occurred at X, this section would indicate that adult bone cells are incapable of growth and proliferation (× 60).
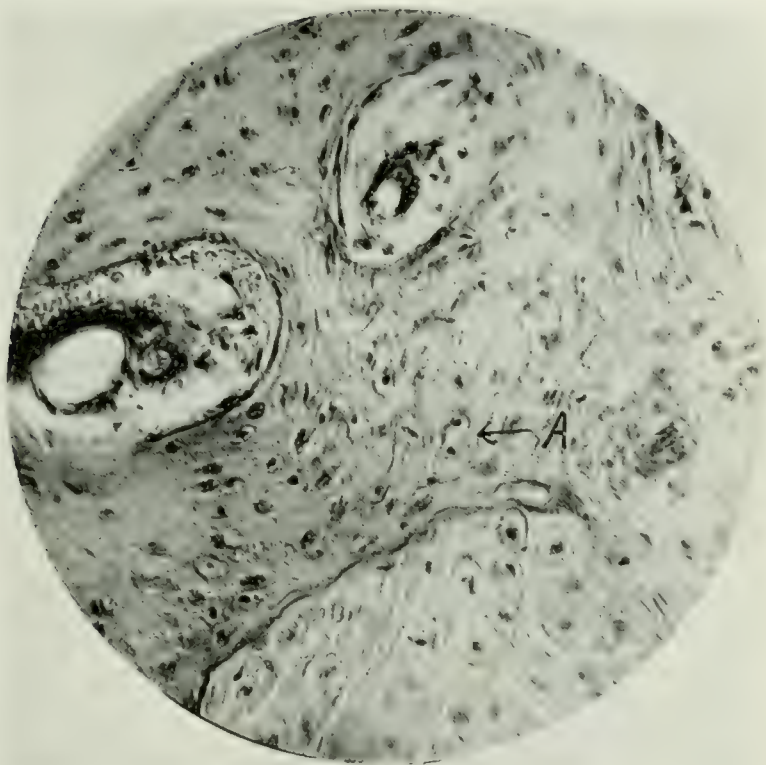
FIG. 9.—High power of same as Fig. 8, showing double nuclei in a young lacunar cell (A) (× 200).

FIG. 10.—35-day transplant of cortex plus periosteum upon surface of cartilage denuded of perichondrium. This shows proliferation of bone (*B*) between periosteum (*D*) and an area of dead transplanted bone (*E*). There are present other areas of dead bone and also bone proliferation around Haversian canals (*F*) (× 100).

Those transplants which are covered by periosteum show a growth of bone between it and the cortex which is more advanced than that proceeding from the Haversian canals and so undoubtedly is formed from the intact cambium layer.

Where the transplants are made up of cortex plus cambium, the growth from the Haversian canals, which breaks through on to the surface, is as active as the growth from the cambium layer.

The subcutaneous transplants show this same group of processes. One series of transplants into the spleen shows also the same findings, but another series left for even a longer interval showed less growth of the transplants, but persistence of them. This must be attributed to either a difference in action of similar transplants in different animals, or to a difference in the action of the spleen in different animals.

The few transplants which included endosteum, though not enough to allow any definite conclusions to be formed, showed an even greater growth from the endosteum than from any other transplants, even including periosteum.

It must also be noted that the transplants of cortex plus periosteum retained their vitality longer than those devoid of periosteum and more of their bone cells persisted and less of their bone was absorbed.

To summarize: Especial emphasis must be placed on the activity of the osteoblasts lining the Haversian canals in forming new bone. These cells are always transplanted along with bone and consequently play a considerable rôle in bone formation under these circumstances. This point has been noted by Mayer and Wehner, but its significance and importance has not yet been fully emphasized. Many workers have reported new growth of bone as occurring from freely transplanted pieces of cortical bone. As these pieces have been devoid of periosteum, cambium layer, and endosteum, the new bone production has been considered as arising from the bone cells. Most of these specimens have not been carefully examined microscopically, and consequently the exact source of osteogenesis was not determined.

We believe, therefore, that it has never been shown conclusively that an adult bone cell can divide and produce new bone cells and new bone—and by an adult bone cell is meant a fully developed bone cell within a lacuna formed of completely calcified osseous tissue. The adult bone cell must be carefully differentiated from the osteoblast within a lacuna surrounded by uncalcified matrix. This latter is not a bone cell in the accurate sense of the term, but has frequently been erroneously so called. This young cell is the active one in creeping replacement. Mayer and Wehner indicate that they have also reached this conclusion.

The study of this point in the literature is difficult because of a confusion arising from a loose use of the term bone cell. An instance of this can be noticed in the excellent recent article by Phemister, where he describes bone formation from the osteoblasts lining the Haversian canals

and apparently considers the osteoblasts to be bone cells. Of course, in regenerating bone where replacement of the osseous tissue is occurring by both lacunar absorption and substitution and by creeping replacement, the young bone cells are in apposition with the old cells and differentiation is at times difficult. Still, we believe that this differentiation is possible by careful microscopical study. The identification of periosteum, endosteum, and osteoblasts lining Haversian canals, and the bone which arises from them, is in reality easily accomplished. It has been definitely proved that osteoblasts in all of these locations can produce bone and that those in the Haversian canals can assume considerable osteoproductive activity when they are properly nourished. This accounts for their activity in transplants of small fragments of bone where the revascularization of the Haversian canals takes place rapidly and the lining osteoblasts are kept alive till this occurs by plasma from surrounding tissues. In large transplants more time is required to establish a new source of nourishment for these particular osteoblasts and their osteoproductivity is correspondingly reduced. Nevertheless, in transplants of cortex devoid of periosteum and endosteum these cells are responsible for whatever new intrinsic bone formation takes place. It naturally follows that any factor which aids in the nourishment of these cells also aids bone growth in the transplant. Since the periosteum covering the cortex is of material assistance in this nourishment, it can readily be seen how transplants of cortex covered by intact living periosteum are more probable of success, aside from the osteogenic function of the cambium layer, than are transplants devoid of periosteum.

CONCLUSIONS

1. Periosteum, devoid of adherent bone cells when transplanted into foreign tissues, produces bone.

2. Endosteum and osteoblasts lining Haversian canals in bone transplants produce bone very actively.

3. The cambium layer when adherent to transplanted cortex produces bone.

4. Some bone cells in the transplants are able to persist for almost a year, but most of the bone is absorbed.

5. Fully developed adult bone cells, although they may remain alive for a considerable time, do not reproduce themselves and form bone.

6. Very young lacunar cells (frequently erroneously called bone cells) can reproduce themselves and form bone.

7. Transplanted bone is absorbed not only by osteoclasts, but also by a direct action (biochemical?) of growing, young bone, and the transplanted bone is replaced either by a creeping forward of the new bone or a gradual extension or expansion of the new bone into the transplant.

8. Marrow spaces and hæmatopoietic marrow are formed in the bone which develops from transplanted periosteum. The source of these hæmatopoietic cells was not determined.

9. Bone, when it grows into cartilage, does so in the same manner characteristic of the normal embryonic development of enchondral bone, including also epiphysial line formation.

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CONTENTS

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DEVELOPMENTAL RATE AND STRUCTURAL EXPRESSION: AN EXPERIMENTAL STUDY OF TWINS, 'DOUBLE MONSTERS' AND SINGLE DEFORMITIES, AND THE INTERACTION AMONG EMBRYONIC ORGANS DURING THEIR ORIGIN AND DEVELOPMENT[1]

CHARLES R. STOCKARD

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THIRTY-TWO TEXT FIGURES AND SIX PLATES

CONTENTS

moment will produce a double monster or identical twins and at
another moment slowing by the same method will give rise to
the cyclopean defect. In fact, the same thing which causes
the double monster may later in development induce one of its
heads to be cyclopean.

Thus there is no longer any ground for considering certain
defects as specific responses to particular treatments. And there
is as little reason for further descriptions of individual monsters,
since all belong to the same class and the individual differences
simply result from the different moments during which the devel-
opmental interruptions have acted.

The important consideration then arises as to what internal
and external factors may tend to introduce the developmental
arrests. Does one growing part in any way inhibit the activity
of other developing organs? We shall devote a section to a con-
sideration of the interaction among the developing and growing
organs within the embryo. The study of the growth influences of
one embryonic organ on another is one of the most important
problems in the analysis of structure.

Finally, the interaction among growing parts and the inhibit-
ing effects of one rapidly proliferating region over other regions
will be very briefly considered in connection with abnormal and
malignant growths.

2. THE SPECIFIC RATE OF DEVELOPMENT IN A GIVEN SPECIES

It is a generally known fact that the eggs of different species
do not progress at the same rate of development even during
comparable stages. The lengths of time between fertilization
and the first cleavage and the rates at which the early cleavages
follow one another may differ decidedly among the eggs of
even closely related forms. These differences in developmental
rate are probably fundamentally connected with differences in
chemical structure of the egg substances, and in particular with
the different rates of oxidation of certain stuffs. It is a well-
known chemical fact that very slight differences in composition
between substances may cause very great differences in their
oxidation capacities.

The efforts on the part of numerous embryologists to associate the differences in rate of cleavage and time required to attain certain stages of development with the size of the egg, the amount and position of the yolk substances, or even the types of cleavage have not been satisfactory. Certain meroblastic eggs develop much faster than certain holoblastic ones, while other holoblastic eggs have a rate of cleavage far more rapid than the meroblastic types. All of the so-called laws of cleavage rates based on morphological differences among egg types have been found to fail so decidedly when applied in general that one is forced to seek more deep-seated causes for the differences in developmental rate.

At the present time we can only state that such causes probably reside in the differences in chemical make-up of the several species of eggs. The rate of development certainly depends, particularly during later stages, on the amount of food available, but the supply of oxygen and the degree of temperature at which development is taking place have a far more striking influence on the rate. Cessation of development also occurs much more promptly from absence of oxygen or sudden changes in temperature than from any other natural modifications which happen in the environment. These facts point decidedly to the rate of development as being dependent upon kind and rate of chemical change, most particularly upon rate of oxidation. The egg probably has a definite coefficient of metabolism dependent upon the interaction of its specific chemical structure and the given environment in which it normally develops. The rate of development results from both the internal qualities of the egg and the nature of the surrounding environment.

The present extremely crude state of our knowledge of the chemistry of development will permit of no more satisfactory statements of the principles underlying differences in developmental rate than those which have been attempted above. The inadequacy of such statements is as keenly appreciated by the writer as by the critical reader, but this inadequacy concerns chiefly the absence of the details involved, while the statements in general I believe are correct.

Although there is a definitely normal rate of development for
a given egg, this rate is frequently subject to wide variations, usu-
ally as a result of variations in the surrounding conditions. The
two chief, or most frequent, modifying causes are a change in
oxygen supply or a change in temperature. An acceleration of
the usual rate only takes place to a limited degree under natural
conditions and but slight increases in developmental rate have
been experimentally obtained. On the other hand, a very wide
range of decrease in developmental rate is readily brought about.
Slight changes in the surrounding temperature or reduction in the
oxygen supply will readily tend to slow the rate of development
to a marked degree. Finally, the entire progress of development
is frequently stopped in nature by removing the supply of oxy-
gen or by sufficiently lowering the surrounding temperature, as
will be discussed in subsequent sections.

3. CONTINUOUS AND DISCONTINUOUS MODES OF DEVELOPMENT

Although, as stated in the foregoing section, each egg has a
more or less characteristic rate of development, this rate is not
uniform throughout the different developmental stages. All eggs
develop with rythmical changes in rate, going alternately faster
and slower from stage to stage. Certain stages are passed very
rapidly, almost suddenly, while others are slowly attained in a
tedious manner, yet the process of development is as a whole con-
tinuous. That is, development begins with fertilization which
is soon followed by cleavage, and then continues without inter-
ruption until a free living larva or young embryo is formed.
This then proceeds to grow and change until the adult structure
is attained. Such a continuous mode of development is most
common, indeed so common, that it is often carelessly consid-
ered to be universal, while a discontinuous mode is looked upon
as something very strange or unusual and not as a phenomenon
extremely important in an understanding of the more common
continuous type of development.

The continuous mode is found among the great majority of those
animals in which the eggs develop in a uniform or homogeneous
environment, such as the sea-water. The general conditions of

moisture, oxygen supply, and temperature are comparatively uniform, and although the eggs may develop faster or slower under slightly different conditions of temperature, etc., yet the variations in the medium are rarely sufficient to inhibit or stop development entirely, and when they are the eggs usually die.

On leaving the sea the fresh-water and land-living invertebrates and vertebrates show most varied and complex methods and arrangements for insuring an environment of sufficient uniformity to permit an uninterrupted development. Many forms, as is also the case in certain sea-living animals, have evolved a method for the development of the embryo within the body of the mother. Such an internal environment tends to control very effectively the conditions of moisture and in mammals also the temperature, but at times, as we shall see beyond, the oxygen supply is not properly adjusted and the continuity of development may be interrupted or interfered with on this account.

The land-living animals have not always succeeded in obtaining an ideal developmental environment, and there are many examples of a discontinuous mode of development as a result of environmental breaks in the strictest sense. That is, the egg begins to develop and attains a certain stage, when a more or less sudden change or break in the environment occurs and development stops completely and may remain at a standstill for various lengths of time—days or possibly weeks. Another alteration in the environment then occurs which again permits development to start and continue until the fully formed animal is obtained. Such a discontinuous mode of development is universal among one great class of vertebrates, the birds. Among the birds development, as far as studied, is invariably interrupted when about the stage of gastrulation, at which time the egg is laid or passed out of the warm body of the mother. The fall in temperature causes development to stop and the egg remains in the gastrular stage until incubated by the heat of the parent's body or until artificially incubated at a similar temperature.

The means of interrupting development seem to reside entirely outside the egg itself, they are properties of the environment. As far as is known, all eggs having once begun to develop will pro-

ceed in a continuous manner from stage to stage until the larva or free living embryo is formed, the environment permitting. Stops in development take place through lack of oxygen, unfavorable temperature, insufficient moisture, or shortage of available nutriment. but the egg itself is wound or set for development so as to continue through if possible. Thus experiments on discontinuous development must apply as methods various means for modifying the environment, and the results will depend upon the power of the egg to adjust itself to or withstand these changes. Being unable to meet the situation, abnormal or unusual developmental productions may arise.

The question then presents itself as to whether the development of any egg may be interrupted for definite lengths of time and later be allowed to finish or proceed. What would be the consequences of such interruption in the case of a normally continuous mode of development? Would the effects of the manner of development be the same following interruptions at different stages, or would the effects vary depending upon the stage of development at which the interruption occurred? In other words, are there indifferent and critical moments of developmental interruption? Would a complete stop in development have an effect similar to a decided slowing of the rate, or would the one be more effective than the other? The experiments recorded in the following sections were devised in order to answer these and other queries.

4. EXPERIMENTALLY CHANGING A CONTINUOUS INTO A DISCONTINUOUS MODE OF DEVELOPMENT

a. The method of experiment

The continuous mode of embryonic development is the more common type in nature. We are, therefore, warranted to some extent in assuming that the discontinuous mode is nature's experimental modification of the continuous. What methods of modification has nature employed that may be artificially imitated? The simplest. commonest, and most evident natural method is change in temperature which causes the interruption of development in the eggs of all birds.

Changing the temperature of the environment and, therefore, of the egg, is the method employed in most of the present experiments in order to interrupt or make discontinuous a normally continuous development.

There are several definite natural cases of discontinuous development among mammals, the significance of which will be considered in another section of this paper. But in the present connection we may be certain that nature has here employed another method than temperature change in causing the interruption. The temperature of the maternal body in which the mammalian embryo is developing is sufficiently uniform never to interrupt the progress of the egg. For reasons to be more fully cited beyond, changes in the supply of oxygen would seem to be the most probable cause of interrupted development in the rare cases of this phenomenon among mammals. Lack of oxygen or excess of CO_2 has also been resorted to in the present experiments as a means of interrupting or retarding the rate of a normally continuous development.

Neither of the two methods is new. A number of experimenters have studied the influence of temperature changes on the manner of development of different eggs. The effects of abnormally high and low incubator temperature on the development of the hen's egg have been recorded by Dareste and many others, most recently by Miss Alsop ('19). The development of amphibian eggs under unusual temperature conditions has been considered by O. Hertwig ('96), King ('04), and others. The influences of low temperatures on the development of the fish's egg have been investigated by Loeb ('16) and Kellicott ('16).

These studies on temperature, however, are of interest in the present connection only in so far as they almost all show how readily abnormal development of the embryo may be induced by unfavorable temperature conditions. The attempted explanations of the deformities which were given in only a few cases, as by Kellicott, entirely disregard or dismiss the real point of fundamental importance; that is, the induced change in the rate of development resulting from the modified temperature. Kellicott

attempted to refute the slow rate as a cause of structural modi-
fication in discussing my assumption of arrested development.
The present experiments differ from the previous temperature
experiments in that they were undertaken with an almost
completely different problem in view. The former experiments
will be considered only as they bear on the specific questions in
the discussion to follow.

Numerous studies on the behavior of eggs deprived of oxygen
as well as in the presence of various reducing and anaesthetic
substances have been conducted. All of these oxygen studies
have little or no bearing on the immediate problems and are not
treated in this connection.

The material used in the present experiments were the eggs
of the common minnow Fundulus heteroclitus. I have studied
and experimented with these eggs for a number of years and am
familiar with a great many common deformities which they may
be induced to present. The exact method of experimentation
with temperature change was as follows: the eggs were taken
from the female and fertilized in a 'dry bowl.' About fifteen
minutes later they were rinsed free of foreign material with sea-
water and left standing under water. The first cleavage takes
place after about two hours, varying a little with the season and
the temperature. The next cleavage follows after another hour,
and development proceeds in a continuous fashion from then on
until the fully formed fish hatches from the egg membrane and
swims freely about within from eleven to eighteen or twenty
days, depending again upon the season and temperature. There
is a wide variation in the rate of development of these eggs, yet
under all usual conditions after development once starts it is
continuous.

The eggs were placed during different stages of development in
compartments of a refrigerator at temperatures of 5°, 7° and 9°C.
and left for varying lengths of time, from one to five days. At
the lowest temperature development was almost if not completely
stopped, while in the other two compartments it was slowed
down to from one-twentieth to one-fiftieth of the normal rate.
The responses shown in the manner of development are so differ-

ent in eggs stopped or slowed at different stages that the exact time of treatment will be considered in connection with the different effects obtained. The difference in effects between slowing and actually stopping development will also be considered.

Other eggs were crowded close together in bunches and developed in bowls at room temperature. The eggs near the center of the masses or bunches obtained much less oxygen and were in a higher concentration of CO_2 than the more superficial ones. These were slowed in their rate of development. Sea-water was boiled so as to drive out most of the air and afterward kept stagnant. Egg masses were developed in this water and the inner eggs of the mass were almost completely stopped in many cases. In all such arrangements the rate of development was so retarded that many abnormal and deformed embryos resulted.

These in general are the methods employed; the different times of application and the results will be discussed in the particular cases below.

b. *Stopping or retarding the progress of development at stages of apparent indifference to such interruption*

In order to successfully change a continuous into a discontinuous mode of development, without producing ill effects on the resulting embryos, it becomes necessary to locate certain indifferent periods during embryonic development at which the interruption may be induced. Certain of these indifferent periods are those moments at which the interruptions of development occur in nature. Should the stoppage naturally take place during a sensitive period, the species would readily be eliminated on account of the high proportion of abnormal embryos which would result.

When the eggs of Fundulus are placed in low temperatures after having passed through the earliest active stages of development, cleavage, gastrulation, the formation of the germring and early appearance of the embryonic shield, they may be stopped for several days, or caused to develop at an extremely slow rate, without marked injury to the resulting embryos. In fact, when such eggs are returned to room temperature after

being in the refrigerator for three or four days, they may often resume development at such a fast rate, probably as a result of the stimulation of raising the temperature, that they may hatch only a day or so later than control embryos. The percentage of such eggs that do hatch may also be equally as high as that from the control.

These statements may be illustrated best by a somewhat detailed consideration of the records from experiments. A large number of experiments have been performed and are recorded in my notes, but only a few of these may here be selected as typical examples of the series in general.

Experiment 905. A group of eggs, 23 hours after fertilization, with high segmentation caps just beginning to flatten on the yolk-sphere, were carefully selected, being certain that every one was developing, and arranged as follows.

Lot C_1 was placed in the refrigerator at 5°C., C_2 at 6°C., C_3 at 8°C., C_4 at 9°C., and C_5 was placed in the top compartment of the refrigerator which ranged from 9.5° to 10°C.

When 27 hours old, the control group showed the germ-disc somewhat further flattened on the yolk-sphere, but there was no visible indication of a germ-ring and the disc had not begun to descend over the yolk. This experiment was being conducted during the early June season, and normal development at this time was unusually slow.

At 27 hours old, three other lots were placed in the refrigerator as follows, D_1 at 5°C., D_2 at 6°C., and D_3 at 8°C.

When 48 hours old, the control showed the germ-ring about one-fourth over the yolk-sphere with the embryonic shield clearly forming. The C and D series had become arrested and were still in much the same condition as when placed in the low temperatues on the previous day.

The control at 3 days, or 72 hours old, showed the embryos well formed, though the germ-rings were not yet entirely over the yolk-sphere (fig. 1).

Lot C_1, having been 49 hours at 5°C., was still in high segmentation stages much the same condition as when placed in the refrigerator (fig. 2). These were now returned to room temperature.

Lot C_2 showed much the same condition as C_1 and were also removed from the refrigerator.

Lot C_3 seemed as completely stopped as the other two and was returned to room temperature.

The members of the C_4 group were also in about the same stage as when placed in the refrigerator, though their temperature was 9°C. These remained in the refrigerator.

The C_5 lot in about 10°C. had developed slowly, the caps had flattened and the embryonic shield had just become visible, though the

germ-ring had scarcely begun its descent over the yolk (fig. 3). These also remained in the refrigerator.

Lot D_1 after 45 hours at 5°C., was still in about the same stage of development as when placed in the low temperature at 27 hours old. These are now placed at room temperature.

Lot D_2 was in a closely similar condition to D_1, but remained at the reduced temperature.

Lot D_3 had also failed to make noticeable progress during the 45 hours at 8°C., but was allowed to remain at this temperature.
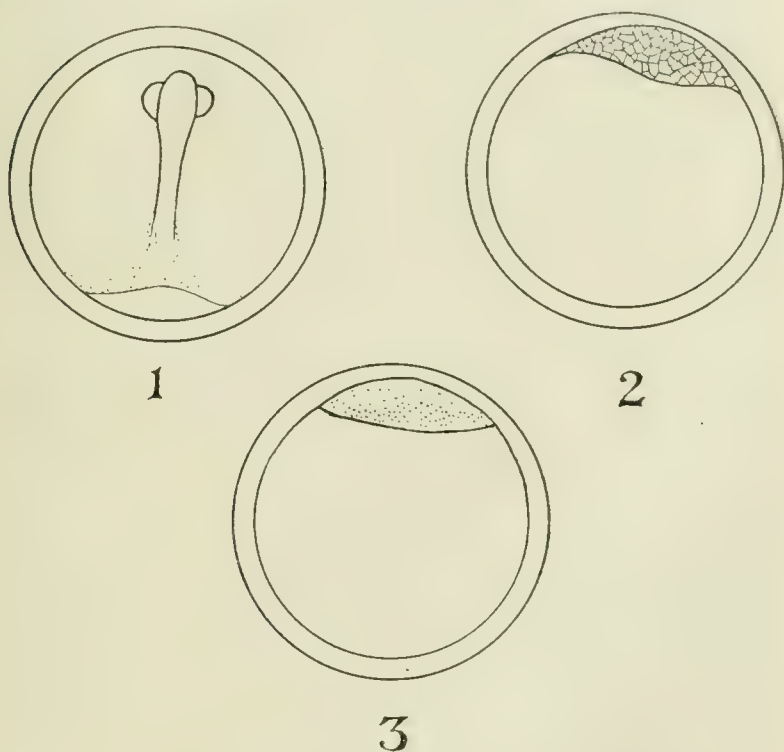
Fig. 1 A control embryo 72 hours old, the body is well outlined and the germ-ring almost completely over the yolk.

Fig. 2 An egg 72 hours old that had spent the last 49 hours at a temperature of 5°C. Development had been practically stopped in this high segmentation stage.

Fig. 3 A specimen 72 hours old that had been during the last 49 hours at a temperature of 10°C. Development had progressed slowly, the germ-disc being flattened and the embryonic shield, indicated by stippling, has just become visible.

When four days old, the control embryos were fully formed with prominent optic vesicles, hearts were formed, but not yet pulsating. Thus they were not more than up to a midsummer 72-hour stage, since the heart beat had generally begun about this time. However, all of these embryos were normal and well, as is shown by their later records, even though the cool season had thrown them about 24 hours behind within four days.

Lot C_1, now having been at room temperature for 24 hours, were all going very well. The germ-rings varied in position from one-quarter to one-third over the yolk-spheres. Only a few had failed to resume development and the eggs in general were about up to the condition of the present control when they were 50 hours old. These C_1 eggs had now actually developed at room temperature for about 47 hours, the first 23 hours after fertilization and the fourth day.

Lot C_2 was also after similar periods of experience in a uniformly good condition with the germ-rings all about one-third over the yolk-spheres. Thus subjecting to low temperature after 23 hours of development is decidedly less injurious than similar treatment during the early cleavage stages, as will be seen from the records beyond.

In lot C_3 the germ-rings had all descended about half way over the yolk-sphere.

The D series showed somewhat the same response. Lot D_1, after 24 hours at room temperature, were developing normally with the germ-rings from one-half to two-thirds over the yolk-spheres and the embryos well formed. Thus stopping for 48 hours after 27 hours of development, when the segmentation caps were flattened over the top of the yolk, showed no ill effects on their present development except to render them almost exactly two days behind the developmental stage of the control.

The control at 5 days old had a vigorous heart beat, but the circulation was just beginning to be well established.

Lot C_1, almost all of the embryos were full length, the optic out-pushings were just beginning, but not fully formed, thus about in the condition shown by the present control at 72 hours. These were still about two days behind the control, or had practically lost the time spent in the refrigerator. There were a few with the germ-rings not entirely covering the yolk and with the body of the embryo short and poorly formed at the caudal end.

Lot C_2 were about in the same condition as C_1.

Lot C_3 seemed on the average a little further along, though closely similar to the two foregoing lots.

Lot C_4, now four days in the refrigerator at 9°C., seemed in good condition, with the germ-rings well formed and descended about one-half over the yolk. These specimens had thus continued their development at this temperature, although very slowly, and had advanced about 12 hours in development within the 4 days. They were now returned to room temperature.

Lot C_5 at about 10°C. for four days, were possibly a little further along then C_4, though in general they showed a similar condition. These were also returned to room temperature.

Lot D_1 contained full-length embryos, some with the optic processes already formed and others without. These specimens were about one and a half days behind the control or about in the stage of the two and a half day control.

Lot D_2, after four days at a temperature of 7°C., introduced after 27 hours of normal development, were still in about the same stage as when placed in the refrigerator. The segmentation caps were flat with early germ-rings forming and the embryonic shields just beginning. The descent of the germ-ring has been considerably prevented. All of the specimens were living and seemed well. They were now returned to room temperature.

Lot D_3, all seemed in good condition with germ-rings from one-quarter to one-half over the yolk-sphere and with well-formed embryonic shields. Thus this slightly higher temperature of 8°C. had given the D_3 group a considerable advantage in progress over the D_2 lot. These were now also returned to room temperature.

When six days old, the black and red chromatophores were fully expanded on the yolk and embryonic bodies of the control specimens. The embryos were now occasionally twitching and moving their bodies.

Lot C_1, after being out of the refrigerator for 3 days, had embryos comparable to about a usual midsummer 70-hour stage, or about the condition of the present control when 4 days old. The heart beat had not begun.

Lot C_2, embryos were also in a stage just prior to the heart beat, and the C_3 group was about the same.

Lot C_4 were now out of the refrigerator for one day after having been at a temperature of 9°C. for 4 days. The embryos were well formed and the blastopore was about closing, so they had made a considerable advance from the condition of the previous day when the germ-rings were only one-half way over the yolk. The C_5 group are still further advanced with the optic outpushings prominently shown.

Lot D_1 now showed chromatophores both on the yolks and on the embryos' bodies, yet no heart beat could be detected in any of those examined.

Lot D_2, when one day at room temperature after being at 7°C. for four days, showed the germ-rings two-thirds over the yolk-sphere, with the embryonic axis well formed in the shield.

Lot D_3 contained long embryos with the optic outpushings just beginning, so these were still ahead of D_2.

At seven days the control embryos were actively moving and the yolk vessels were now clearly mapped out by the pigmented arrangement.

Lot C_1, now out of the refrigerator for 4 days, showed many embryos with good circulations and pigment migration, some had a

heart beat, but had not established a circulation and others had not yet developed a heart beat.

Lot C_2 showed a good circulation in almost all.

Lot C_3 presented a majority with good circulation, there were, however, many with imperfect circulation or no circulation, although the heart was pulsating.

When 9 days old, the control presented a perfectly normal condition.

Lot C_1 showed practically every specimen normal and strong, apparently just as good as the control, though somewhat behind.

Lot C_2 were in equally as good a condition.

Lot C_3 was much the same as the other two groups.

Lot C_4 also seemed to contain all normal embryos.

Lot C_5 were further advanced than C_4, since they had continued to develop slowly while in the refrigerator at the higher temperature of about 10°C. They had, therefore, developed slowly for 4 days, and after having been out for 4 days were practically perfect in their development.

Lot D_1 were all normal at 9 days old and as perfect as the control except for the fact of being behind in developmental time due to the few days stand-still spent in the refrigerator. Thus development can be discontinued for 3, 4, or 5 days at the stages used in this experiment (27 hours old, just after gastrulation has started) with no subsequent ill effects on the development and structure of the early embryos.

Lot D_2 contained specimens further behind in development than the D_1 group, since they remained in the cold longer, but all appeared perfectly normal at this time.

Lot D_3 were all normal.

At 12 days old, the control seemed about in the condition to hatch.

The C series which had been subjected to developmental interruptions after being 23 hours old now presented perfectly normal conditions. In lot C_1 three specimens had not developed and sixty were normal This is as good a record as is usually found under ordinary conditions. Lot C_2 contained about 100 specimens, which were all living and normal. Lot C_3 had about the same number in similar conditions. Lot C_4 also contained about 100 normal specimens, so that the numbers examined were sufficiently large to furnish a very reliable index of the reactions.

Lot C_5 contained a few more than 100 normal specimens and a single individual that was abnormally small, yet even this one was sufficiently normal to have a free blood circulation.

Lot D_1, which was put in the refrigerator 27 hours after fertilization, contained six specimens that did not develop out of a total of seventy-five eggs. The other sixty-nine specimens were normal. The D_2 lot were all normal, and so was the D_3 group, yet all were behind the control in their developmental stage corresponding to about the length of time they had spent in the refrigerator.

When 19 days old the control were almost all hatched actively free swimming young fish. The few yet unhatched seemed normal and ready to hatch at any time.

Lot C_1 contained a majority hatched and all seemed normal.

In lot C_2 there were not quite as many hatched, but all were in good condition.

Lot C_3 were about the same in hatching record, so there was little effect to be noticed at this time resulting from the two days spent in the refrigerator following their first 23 hours of development.

Lot C_4 had remained longer in the refrigerator, 4 days, and at this time none had hatched, though they seemed fully ready. In lot C_5 also none had hatched.

Lot D_1 contained a majority hatched, almost as large a proportion as the control. These had remained in the cold only two days. Lots D_2 and D_3 had remained in cold for 4 days, and only one specimen in the two groups had hatched. All appear normal and ready to hatch.

When 20 days old, the first one in C_4 had hatched. In lots D_2 and D_3 many had now hatched, so these are not very much later than the control in spite of their 4 days' arrest.

In lot C_5 none had yet hatched, although during the next 24 hours many of them did hatch.

When 22 days old, a few of the control were still unhatched, though they were normal. Lot C_1 had 12 unhatched and 50 hatched. Lot C_2 contained 18 unhatched and about 80 hatched. Lot C_3 had 29 un- . hatched, one with a deformed body, and about 70 normal ones hatched. This record was about as good as a usual control.

About half of the C_4 lot had hatched, and all seemed normal, though they remained in the refrigerator twice as long as C_1, C_2, and C_3 had.

Lot C_5 also showed about half of the specimens hatched.

Lot D_1 had 7 unhatched and about 60 hatched, all of them seemed normal.

Lot D_2 contained 29 unhatched and about 40 hatched, all of which were normal.

Lot D_3 showed 20 unhatched and about 30 hatched.

When 25 days old, every egg in the control had hatched.

Lot C_1, only 4 were unhatched, one of these had abnormally small defective eyes and no blood circulation. So these are a little behind their particular control in quality at this stage, but very little, and probably their disadvantage is of no significance, since such a single specimen might occur in any group of eggs.

Lot C_2, every specimen hatched. In lot C_3 only 3 failed to hatch. One of these was grossly deformed and the other two had slightly abnormal eyes. So this group is somewhat inferior when compared with the control record.

Lot C_4 contained 12 specimens still unhatched. One hatched specimen was bent and unable to swim. One of the 12 unhatched was abnormal, so this record also was a little worse than the perfect control.

Lot C_5 contained 10 unhatched, one of which was abnormal, the others were all normal.

Lot D_1, only one unhatched, all seem fine.

Lot D_2 contained 2 unhatched, and lot D_3 had 3 unhatched, though all of these seemed normal.

This experiment shows very clearly that stopping or arresting the development of Fundulus eggs after about twenty-four hours of development, when gastrulation has definitely begun, produces very slight or no ill effects on such specimens up to the time of hatching and becoming free swimming little fish. Whether during later stages of growth these fish might show some disadvantages following the developmental interruption we have not attempted to determine. It is probable, however, that these specimens were interrupted in their development during a particularly passive period and that no later disadvantages would accrue. This would seem further probable since it is at just such a stage in development that the eggs of birds are normally interrupted, and clearly without ill effects on the group.

These experiments not only show that *stopping* development at this stage, just after gastrulation has started, is not noticeably injurious in effect on the development of the young fish, but further, that after gastrulation has commenced the rate of development of the embryo may be *slowed to a most extreme degree*, as occurred in the upper temperatures of the refrigerator, without serious injury to the structure of the young fish.

To further establish the correctness of the above results, we may record one other similar experiment in brief detail.

Experiment 906. $B_{4 \cdot 1}$. A group of eggs when 24 hours old containing all normal fine specimens were placed at a temperature of 5°C., and later compared with a selected control from the same parents.

At 46 hours old, the control were developing rapidly, with the germ-rings almost completely over the yolk and the embryos well formed.

The $B_{4 \cdot 2}$ lot now in cold for 22 hours showed the same condition as when placed in the refrigerator except that the segmentation cavities were distended so that a vesicle appeared below each disc. These eggs were now moved to an upper compartment of the refrigerator to allow them to develop slowly at a temperature of about 9°C.

When four days old in the 9°C. temperature they were developing slowly but normally, with the germ-rings about one-half over the yolk-spheres and with embryonic shields in which the axis of the embryo was beginning to form.

At five days old these eggs were still developing remarkably well although very slowly. The germ-rings were a little further over the yolk. They were now returned to room temperature after having spent 4 days in the refrigerator, 24 hours at 5°C. and 3 days at 9°C.

One day later, all of the eggs were developing and almost every one presented a well-formed embryo normal in appearance.

When ten days old, all were living with a fine circulation of the blood and otherwise apparently normal.

When 17 days old, 18 of these embryos had hatched and 24 were unhatched.

After 24 days, 12 were still unhatched, one of these being very abnormal. All of the embryos had seemed normal when ten days old, but at this time it was readily seen that the 12 unhatched specimens were really far behind the control. While showing no gross deformities they were smaller and not so well developed as the control.

Although these early arrests do not give marked effects on the very young fish, it is certainly possible that many later symptoms might develop if their existence was observed through longer periods of time.

When 29 days old, 4 embryos were still unhatched, one had died and 3 seemed normal and ready to hatch. Thus the record of this group for the length of time it was followed does not compare unfavorably with the ordinary control records of Fundulus embryos up to a comparable period. As might be expected, however, eggs after being 24 hours old which were stopped or retarded in development for 4 days are not able to hatch on schedule time with the control; but are several days late in reaching the hatching stage.

Such results will be found to differ entirely from those considered beyond as obtained when the eggs are stopped during more critical developmental stages or at times when rapid cell proliferation and developmental changes are occurring. Therefore, it may be stated in general that certain indifferent moments in development do exist during which time the rate of development may be slowed to almost stopping, or development may be actually stopped, and later resumed at a normal rate without causing structural anomalies or unusual conditions in the resulting young fish.

It is also shown by the above experiment that development may be stopped at certain indifferent periods, in a temperature of 5°C. and then resumed at an extremely slow rate in 9°C. for several days, and later increased to a normally rapid rate at room temperature without injury.

Thus it is not always necessary that development be promptly resumed at a normal rate in order to avoid structural defects.

The next experiment is cited to show the behavior of eggs arrested in still later periods of general indifference.

Experiment 907.— Eggs with germ-rings one-quarter to one-third over the yolk sphere and with embryonic shields well formed, a stage acquired after 48 hours of development during the early cool June season, were placed in the refrigerator in two groups, E_2 at 6°C. and E_3 at 8°C.

After 24 hours in the refrigerator they had advanced only slightly beyond the condition of the day before. The E_3 group had advanced somewhat more than the E_2 lot particularly in the formation of the embryonic line, or axis, in the shield.

When 5 days old, and after having been in the refrigerator for 3 days, the E_2 group at 6°C. have advanced the germ-ring to about two-thirds over the yolk sphere. They were thus not as completely stopped by this temperature of 6°C. as were eggs placed in the same temperature during early cleavage stages, as will be seen beyond. These eggs were now, after 3 days of extremely slow development, returned to room temperature.

The E_3 lot at this time showed the germ-ring almost completely over the yolk-sphere, and the embryonic body was well formed in the majority of the eggs. These specimens at a slightly higher temperature had developed somewhat further than those above. They were now also returned to room temperature.

After being at room temperature for 24 hours, the rate of development had greatly increased in both lots. The E_2 group now showed long embryos with the optic outpushings well begun in many. The E_3 lot showed optic outgrowths well formed in all, and were thus a little ahead of the E_2 ones in development.

At 9 days old, the specimens in both lots seemed behind the control to the extent of their 3-day stay in the refrigerator.

When 12 days old, they were closely examined for slight anomalies. The E_2 lot showed one abnormally small embryo with no blood circulation, 4 had stopped, and did not develop after removal from the refrigerator, and 45 specimens seemed to be in normal condition.

The E_3 lot all appeared to be normal except that they were about 3 days behind the control in their development.

Thus subjecting the embryos to a severe reduction in developmental rate after they were 48 hours old had only slight, if any, detrimental effect on their ability to resume a normal developmental rate and to form apparently normal young embryos. Very probably, however, minor effects are produced which would be indicated in the later structural or physiological history of the specimens could they be studied through a longer season of their existence.

At 19 days old, when a large majority of the control had hatched and were free swimming, none of the E_2 or E_3 lots had hatched. But when 21 days old, a number were hatched in both lots.

When 22 days old, the E_2 group contained 25 hatched and 20 un-hatched. Three of the latter were abnormal with no blood circulation, two being small and inactive, and the third was grossly deformed. The E_3 group had 25 hatched and 11 unhatched, all of which seemed normal in structure.

At 25 days old, 4 of the E_2 group were still unhatched, but all of the E_3 lot had hatched. They were kept until 34 days old, at which time many had died on account of the difficulty in feeding them, but the 4 specimens in lot E_2 never succeeded in hatching.

When these records of late arrests are compared with those from eggs arrested during early cleavage stages, one will be struck with the low mortality following removal from the refrigerator in the case of the former. The complete absence of double monsters, ophthalmic deformities, etc., among the specimens arrested during late stages also contrasts with the common occurrence of such conditions among specimens arrested during cleavage stages. The general nature of the circulatory disturbances, etc., which do occur after late arrests is also characteristic. A contrast is further noted by considering this experiment in comparison with the specimens described above which were introduced into the cold after one day of development—there again the advantage in subsequent development is on the side of those specimens caused to develop very slowly during the later developmental stages. But of the specimens almost completely stopped in development, those stopped very soon after gastrulation seem to have an advantage over specimens stopped when one day older, or further advanced in development. The stage immediately following the first rapid changes of gastrulation would seem to be an extremely indifferent period.

Two other sample experiments will be reviewed in brief to illustrate the gross reaction following still later developmental interruptions. It must be realized that in all of these experiments we are at present simply recording the outward gross appearance and behavior of the specimens. A closer microscopic examination of the young fish in section might show a considerable depression in the development or expression of certain internal organs, for example, the conditions in the branchial regions, digestive glands, etc., while observation of the living specimen had given no indication of its inner defective condition.

Experiment 908. Specimens 72 hours, or three days old, with the optic cups already invaginated and formed, but just before the beginning of a heart beat, were carefully selected, so that every individual was normal and good, and arranged in two groups. Group F_1, consisting of 62 vigorous specimens, were placed in the refrigerator at 5°C. and group F_2, containing 36 normal embryos, were subjected to a temperature of 8°C.

When 6 days old and after being 3 days in the refrigerator the F_1 lot were in much the same condition as when put in the cold, the hearts had not begun to beat and the general structural appearance had not changed. The F_3 lot were a little further advanced, but there was still no heart-beat. The control embryos at this time have, of course, a vigorous circulation of the blood, they are well pigmented and the yolk vessels are mapped out by the chromatophores.

At 8 days old, the F_1 group were still in the same condition as when put in the 5°C. temperature 5 days before. There was no heart beat and the embryos appeared as if about 3 days old. They were now returned to room temperature.

The F_3 lot, after 5 days at 8°C., were further advanced, their hearts were pulsating feebly and very slowly, blood-cells were formed on the yolk-sacs and masses of blood were frequently observed in the tail regions. These embryos were also now returned to room temperature.

After being at room temperature for 3 days, with a total age of eleven days, the F_1 lot seem recovered and are developing well, though about 4 or 5 days behind the control. All of this lot were living. The F_3 lot were also all alive and in apparently perfect condition.

When 18 days old, almost all of the control embryos had hatched. The F_1 lot all seemed normal, but none had hatched, and the same was true of the F_3 group. Two days later, however, many had hatched in both lots. Thus they were 3 or 4 days later than the control in hatching, which was a little less than the time they had spent at low temperature.

Finally, when 27 days old, none of the embryos in the two lots had died, which indicates that they were all unusually good specimens. Every one of the 36 in the F_3 group hatched, and but 2 in the F_1 group failed to hatch, although these appeared normal in structure.

A complete stop or an arrest in developmental rate of as much as five days after the optic cups are already formed and just before the beginning of a heart beat does not exert an injurious effect upon any organ that would prevent the normal development of the body form or the capacity to hatch and swim freely.

Experiment 909. Embryos 6 days old, with fully vigorous blood circulation over the yolk-sac and within the embryonic body, with chromatophores fully migrated and expanded, and with their bodies moving and twitching, were placed in a temperature of 7°C. After

24 hours the hearts were still beating, but much slower than the control, and they had fallen about 20 hours behind the control in development.

After 3 days in the cold these embryos had fallen far behind the control in size and development. The heart was beating slowly and the blood was circulating in all.

Two days later, when the embryos were 11 days old, they were still in about the 6-day condition, although all were living at a slow rate during the 5 days in the refrigerator.

When 13 days old, and after being 7 days in the low temperature, the embryos were all alive. They had a slow heart beat and a circulation which in many was so sluggish as to allow large sinuses in the yolk-sac to remain distended with blood, although the circulation within the embryonic body was complete. At this time they were returned to room temperature, and after 24 hours the heart beat had regained a normal rate and the blood was circulating freely and fast in each of the specimens. All seemed fully recovered from the depression caused by the low temperature.

At 19 days old, almost all of the control embryos had hatched, but none of these that had spent 7 days at 7°C. were yet up to the point of hatching.

At 22 days old, still none were hatched. But when 23 days old, 16 had hatched and 38 were unhatched. They were thus 5 days behind the control in beginning to hatch as a result of their 7 days of slow development at the low temperature.

On the 25th day only 2 were still unhatched, and finally, on the 27th day, these two had not hatched, although they seem normal in structure.

There is, therefore, no evidence that any harm was done by subjecting advanced embryos with blood freely circulating to low temperatures. Although under the cold conditions the heart rate was greatly reduced and the circulation rendered extremely sluggish for a period of seven days. On return to normal temperature recovery was rather prompt and seemed on superficial examination to be complete.

A number of similar experiments to those reviewed above are recorded in my notes, and in all cases the results are in close accord. If we consider them entirely from a standpoint of the external evidence of injury produced, a fair comparison may be made with the results of further experiments in which the eggs were stopped and arrested at other developmental periods or moments. It will be readily shown that periods very close to some of those used above are decidedly dangerous moments at

which to stop or interrupt the progress of development. From
such experiments one seems justified in classing these moments
in development as indifferent at which arrests may be induced
without causing subsequent high mortality among the embryos
and without a considerable percentage of gross structural de-
formities resulting. The eggs treated in the above experiments
were all stopped at comparatively indifferent moments in the
course of development so far as their gross structure and behavior
up to the newly hatched free swimming stage of life would indi-
cate. In the section following a review of experiments with
decidedly different results will be considered.

*c. Stopping or retarding the progress of development at stages of
critical susceptibility to developmental interruption*

From facts we know of development in nature, as well as, from
the experiments discussed in the preceding section, it becomes
evident that the course of embryonic development need not nec-
essarily progress in a continuous manner, but may be stopped
entirely for a considerable length of time or may be decidedly
reduced in rate without necessarily injuring the end result. On
the other hand, it is equally well known in a general way, and
even more widely believed, that when a developing egg is injured
in such a manner as to cause its development to stop, it is usu-
ally incapable of resuming development at all, or if it does start
again to develop it will only continue for a short time and often
in a very abnormal fashion.

These two apparently contradictory statements are equally
true. This is due to the fact that the way in which a developing
egg responds after having had the progress of its development
stopped or arrested by any unfavorable condition depends
entirely upon the stage in development at which the interruption
occurred. In the first case stated above, the interruption is
introduced at a stage in development when no unusually rapid
changes are taking place, a comparatively quiescent moment
during which all parts are developing, but during which no par-
ticular or important part is going at an excessively high rate.
Such a time we may term a 'moment of indifference.'

In the second case, the interruption occurs at a time when certain important developmental steps are in rapid progress or are just ready to enter upon rapid changes, a moment when a particular part is developing at a rate much in excess of the rate of the other parts in general. Gastrulation is an important developmental step which apparently cannot be readily interrupted without serious effects on subsequent development. Many of the chief embryonic organs seem also to arise with initial moments of extremely high activity, processes of budding or rapid proliferation and growing out. During these moments a given organ may be thought of as developing at a rate entirely in excess of the general developmental rate of the embryo. Such moments of supremacy for the various organs occur at different times during development. As is well known, a certain organ arises much earlier or later in the embryo than certain others. When these primary developmental changes are on the verge of taking place or when an important organ is entering its initial stage of rapid proliferation or budding, a serious interruption of the developmental progress often causes decided injuries to this particular organ, while only slight or no ill effects may be suffered by the embryo in general. Such particularly sensitive periods during development I have termed the 'critical moments.'

That we may analyze the responses of embryos in which developmental interruptions have been introduced during some of these critical moments, resource may again be had to the records of the experiments. Here also a large number of experiments have been performed, but we shall only attempt a review of certain typical examples from the entire series.

Experiment 901, B Series. Eggs were fertilized at 11 A.M., and three hours later, immediately before the first cleavage, they were divided into four lots, one for control and three others which were placed in a refrigerator at temperatures of 5°, 7°, and 9°C.

When 24 hours old, the control had reached a high segmentation stage, the germ-discs in only a few had flattened down on the yolk sphere, but in none had the cap begun to descend over the yolk or to form the germ-ring. The night had been unusually cool and the control was thus developing far more slowly than the normal summer average rate. At 24 hours old, the germ-ring is usually well formed and has descended about one-third to one-half way over the

yolk-sphere. The inhibition resulting from the cool nights of the early season very probably accounts for the almost uniform inferiority of embryos developed at this time as compared with those developing during early July, the height of the spawning season for thus locality.

Lots B_1 and B_2, in temperatures of 5° and 7°C., respectively, for 19 hours, were all in either 2- or 4-cell stages. They were thus almost completely stopped in development. The 2-cell stage was about reached when they were placed in the low temperatures, and probably some were dividing the second time before the surrounding water had cooled to the temperature of the refrigerator (all dishes contained 60 cc. of sea-water).

Lot B_3 at 9°C. contained after 19 hours fairly regular 16- and 32-cell stages. At this temperature cell division had been able to continue, although at a greatly reduced rate, accomplishing only three or four divisions in the 19 hours.

The control eggs 48 hours after fertilization showed the germ-ring only one-quarter over the yolk sphere, with the embryonic shield beginning to form (fig. 4), a stage that should be attained within 24 hours during the warmer part of the season.

Lot B_1, after 45 hours at 5°C., was in first-, second-, or third-cleavage stages. The arrangement of the cell groups was often very irregular and many cells contained large vacuoles. There were a very few almost typical 2- and 4-cell groups. In some of the '2-cells' a large central vacuole seemed to almost divide each of the cells (fig. 5).

These eggs at 5°C. have thus only in rare cases divided more than once during 48 hours. This lot was now removed from the refrigerator and returned to the room temperature after being 45 hours in the cold.

Lot B_2, at 7°C., was in much the same condition as lot B_1, except that some eggs had undergone one or two further cleavages. There were many irregular cleavage patterns and a few almost regular 16- or 32-cell stages. A number of the germ-discs consisted of irregular partly divided masses (fig. 6).

Lot B_3, at 9°C., had developed very slowly but fairly well, and now after 45 hours in the low temperature contained germ-discs composed of from 64 to about 128 cells. The cell arrangements and shapes of the discs were almost uniformly regular. Therefore, at this temperature development progresses, though very slowly, and none of the cell masses had yet begun to flatten down to cap the yolk-sphere.

When 3 days old, the control embryos were well formed, although the germ-ring was not yet entirely over the yolk-sphere, much the same stage as shown above in figure 1.

Lot B_1, after being at room temperature for 24 hours, had passed from the 2-, 4-, and 8-celled conditions and had reached a high segmentation stage. The discs had not fully flattened on the yolk-spheres, but were beginning to descend. There was no gross indication of germ-ring or embryonic-shield formation. Many eggs had promptly recovered their ability to develop on return to higher temperature and had progressed during the 24 hours about as far as the control had gone during the first 24 hours of their development.

Lot B₂ had now been for 70 hours at 7°C. These showed many irregular germ-discs, but some were fairly regular 16- and 32-cell stages. Their condition was thus much the same as on the day before and they had scarcely progressed at all during the 24 hours. These eggs were now returned to room temperature.
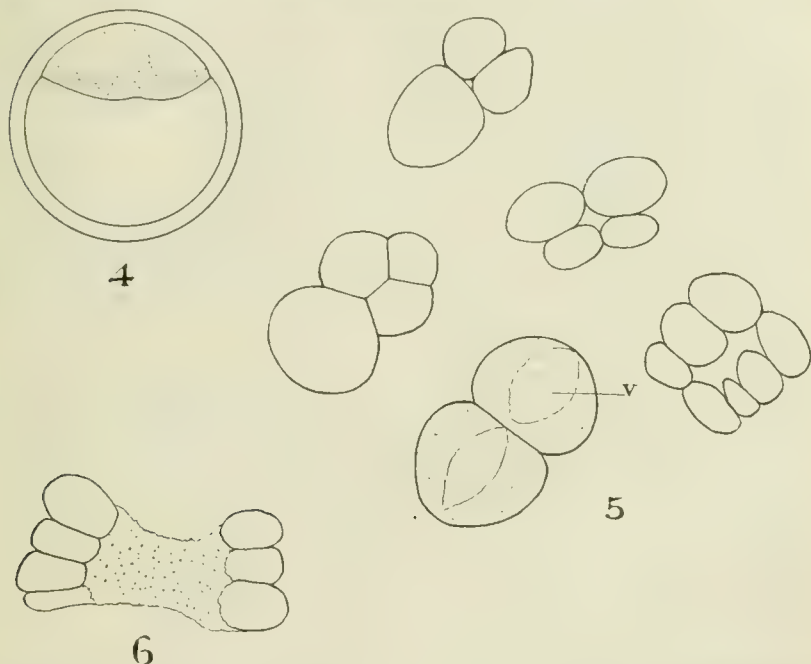
Fig. 4   A control embryo 48 hours old, the germ-ring only one-quarter over the yolk, far behind the usual stage on account of the cool season.

Fig. 5   A group of cleavage patterns 48 hours after fertilization and after 45 hours at a temperature of 5°C. Development is practically stopped. In many of the two-cell stages large vacuoles, V, occupy the entire center of the cells.

Fig. 6   An irregular partly undivided protoplasmic mass with blastomeres at its ends, 48 hours old after 45 hours at 7°C.

Lot B₃ still had, after 70 hours at 9°C., high segmentation discs about the 128-cell stage. The discs were normal in general appearance. Thus at this temperature development continues, but at an extremely slow rate. This lot was now also returned to room temperature.

When the eggs were 96 hours, four days old, the control embryos were fully formed with prominent optic vesicles, the embryonic heart was not yet visible, and there was no pulsation. These embryos were thus scarcely up to the midsummer 72-hour stage, since the embryonic heart beat is often fully established before such a time. The cool

weather of early June had caused this control to fall about 24 hours behind in the four days. Although such embryos appear to be normal, many of them are inferior in size and general appearance when compared with more rapidly developing specimens of the later warmer season. This advantage is no doubt due to the retarded development primarily resulting from the cooler temperature, and not to a poorer quality of the eggs, since the midsummer eggs will fare in a similar fashion when caused to develop at the same temperature. Such a retardation, however, is too slight to produce gross defeats in any average lot of eggs, yet the embryos very probably are somewhat below par as their physiological responses would indicate.

Lot B_1 had now been for 2 days, 48 hours, at room temperature after having spent 45 hours at 5°C. The germ-caps were about one-half over the yolk-sphere, the germ-rings and embryonic shields were well formed in most of them. They presented the condition of a midsummer 24-hour stage, or were about up to the condition of the present control at 48 or 50 hours. Thus during the 48 hours at room temperature these eggs had developed about as rapidly as did the control during their first 48 hours of development.

The embryonic shields with the embryo in outline appeared normal, although some were considerably behind others and a great many failed to resume development after being removed from the refrigerator.

The lot B_2, after 24 hours at room temperature following a stay of 70 hours at 7°C., showed disc-like caps flattened down, but no germ-rings were yet formed and the disc had not begun to descend over the yolk-sphere. Some caps were still high or mound-like and many were irregular, containing cells of different sizes (fig. 7). A large number of eggs failed to resume development and there were many discs with vacuoles in their centers, etc.

The mortality resulting from this exposure was, therefore, high and many embryos were rendered abnormal during these early stages.

The lot B_3, after 24 hours at room temperature, were in an even worse condition than those in B_2, although a single individual had a germ-ring one-fourth over the yolk-sphere and was thus the most advanced specimen of the two lots. The majority, however, presented high germ-discs with a peculiar vacuole occupying about half of the disc and distorting the position of the cells (fig. 8).

Vacuoles similar in appearance are frequently present in eggs slowed by other methods, such as solutions of LiCl, etc. But in this case the vacuole differs somewhat in not being a simply distended segmentation cavity.

It will be recalled that these eggs developed very slowly at 9°C. for 70 hours, so that they had progressed much beyond the lots B_1 and B_2 when removed from the cold. Yet after 24 hours at room temperature they were at a disadvantage rather than an advantage when compared with B_2 at this moment. The extremely slow progress during the 70 hours would seem to be more detrimental at this stage than the almost complete cessation of development in lot B_2. In later stages, however,

those eggs which have been subjected to the higher temperature will gain a decided advantage as compared with the lower-temperature groups.

At 5 days old, the control showed the heart beat just beginning, but no circulation. Lot B_1, after 3 days at room temperature, con-
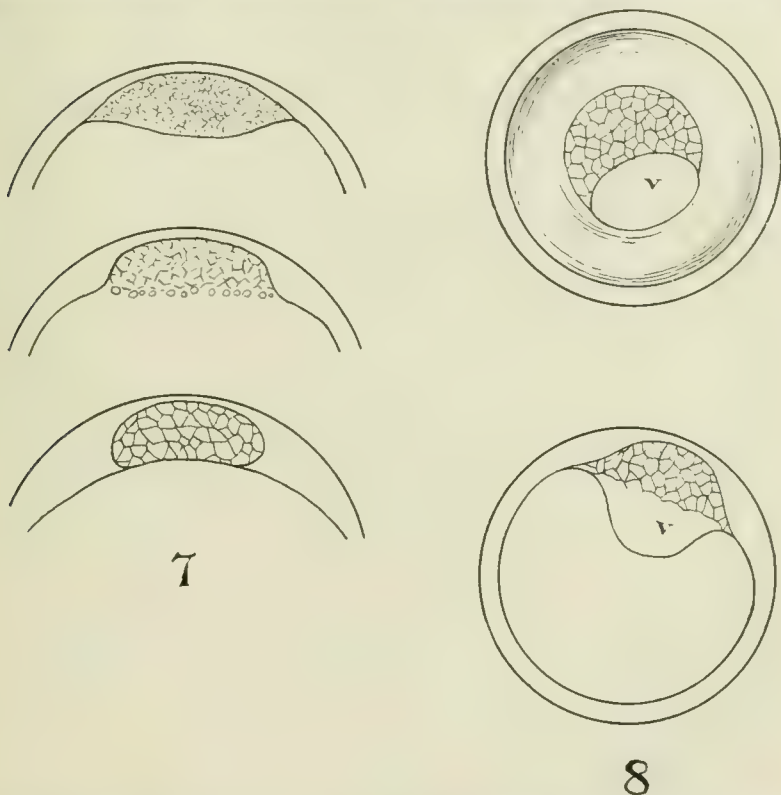
Fig. 7   Three specimens 4 days old, having been 24 hours at room temperature following a stay of 70 hours at 7°C. The upper outline shows a disc-like cap flattened down on the yolk-sphere; the middle one, a high segmentation cap; and the bottom specimen has a cell mass comparable to a normal 12-hour stage.

Fig. 8   Top and lateral views of 4-day specimens, having been 24 hours at room temperature following 70 hours at 8°C. These segmentation masses are very abnormal and are distorted by the presence of a huge vacuole, V.

tained short embryos on the surviving eggs, but the majority of eggs failed to develop at all after being removed from the cold. Lot B_2 had germ-rings only about one-half, or a little more, over the yolk-sphere. Thus the one day longer in the refrigerator had caused these to be far behind B_1.

The Lot B₃ had germ-rings also a little more than half over the yolk, though here again a great many were not developing at all.

The 6-day-old control presented black and red chromatophores fully expanded on the yolk-sac and the embryo. The circulation was completely established both within the embryonic body and on the yolk-sac. The embryos had begun twitching and moving their bodies.

Lot B₁ had now been at room temperature for 4 days after having been arrested for 45 hours at a temperature of 5°C. The embryos were small with no circulation, almost all seemed abnormal at the head end and many were short; the tail region was not properly formed. They were thus far behind a usual 4-day embryo.

Lot B₂, after now developing at room temperature for 3 days, contained many small cyclopean and otherwise defective embryos, but the majority of eggs had stopped and did not develop beyond the condition shown by them after the 70-hour stay at 7°C.

Lot B₃ contained some fairly regular 3-day embryos, but with no circulation, and many of these were deformed.

Seven days after fertilization the blood-vessels of the control embryos were well mapped out by the alignment of pigment and the embryos themselves were vigorously active.

Lot B₁ contained at this time many well-formed embryos with good circulation, pigment migration, etc. Others had a sluggish and poorly established circulation, some showed a heart beat, but no circulation, and many more had stopped in development and the cells had wandered apart to lie over the yolk surface. Some eggs presented simply yolk-sacs with blood-spots scattered over them, but without an embryo. A few of the apparently well-formed embryos were abnormal in various ways.

Lot B₂ showed no circulation, many eggs did not develop, and almost all were readily seen to be abnormal. The lot B₃ also showed no circulation, but contained some well-formed embryos just about in condition for the heart beat to begin.

When 9 days old, the control contained all fine vigorous embryos.

Lot B₁ still showed those with only blood and pigment on the yolk-sac, with no embryonic body present. Others still had the cell-mass confined to the upper yolk-pole and there were a few abnormal embryos, some with and others without a circulation. The majority of the living specimens were now normal in appearance with a vigorous circulation, as if some degree of regulation and recovery had taken place

Lot B₂ contained many apparently normal embryos with a good circulation, while some were small and some were abnormal without a circulation. Some eggs showed the old mass of early cleavage cells at the upper yolk-pole still alive after 9 days, though not developing; the cell-masses were irregular and the individual cells spherical in form. Several yolk-sacs also contained blood-cells and a few pigment cells, although no embryo was present.

Lot B₃ contained a few eggs with early cell-masses similar to those in lot B₂. The large majority of the surviving individuals now seemed

normal with a good circulation; very few were slightly deformed with poor or no circulation.

The majority in all B lots were now normal in appearance with a good circulation. In the B_3 lot 47 seemed normal out of 61, so that 14, or about 25 per cent, were abnormal, and of these 6 showed the early cell-mass condition or were not developing. Thus only 8 embryos were smaller or slower than normal. Yet it must be recalled that many dead eggs had been removed during the first few days following return to room temperature. In the control, however, there were no abnormal ones and there had been no unusual mortality.

When 12 days old, the control were all normal and about in the condition to hatch.

In lot B_1 6 showed that development had stopped during an early stage, 4 showed yolk-sacs with blood and pigment but no embryos, 10 were deformed embryos with no circulation, 4 were also deformed, 2 being eyeless, but with a circulation. Of all the survivors in this lot 24 were affected and 45 were apparently normal at this time, thus over 34 per cent were bad.

In lot B_2 14 failed to develop beyond the cell-mass stage, 4 presented only yolk-sacs with blood-spots and pigment cells, 8 were abnormal with no circulation, and 3 were abnormal with a circulation, while 31 appeared to be normal. Thus 15 of those that continued to develop, or about 33 per cent, were abnormal and 25 per cent of the total number that lived were unable to resume development after their stay at 7°C.

In lot B_3 6 stopped development early, though continuing to live, 3 were deformed and possessed a circulation, 5 were deformed without a circulation, and 47 individuals were apparently normal. Here, then, only 14 per cent were deformed of those that developed. Such a record is twice as good as that attained by either of the other groups. Thus the 9°C. temperature, at which an extremely slow rate of development is possible, is not so injurious to the later development of those individuals which survive it as are the more severe temperatures of 5° and 7°C., which practically stopped the progress of development entirely.

The control when 15 days old had not yet begun to hatch, on account of the cool season. In lots B_1, B_2, and B_3 one or two more of the abnormal embryos in each had died and all of the individuals were behind the control in their developmental condition, though, as stated above, many in all groups now appeared normal.

When 19 days old, a large majority of the control were hatched and swimming about in a typically active fashion. In lot B_1 none had hatched and several more had died. In B_2 none had hatched and a few more also had died. In B_3 none had hatched, many still seemed normal, and many were deformed, showing distinctly typical eye anomalies, cyclopia, etc., and there were many types of head and caudal end deformities.

When 24 days old, in lot B_1 2 more had died and 3 had hatched, in B_2 2 had hatched, and in B_3 many had hatched.

The control at 22 days old showed 47 hatched and 18 unhatched, although all were normal. In lot B_1 5 had hatched, and 52 were unhatched, the majority were normal in appearance, but 13 were grossly deformed in the head region and possessed small ill-formed bodies. In lot B_2 4 had hatched and 36 were unhatched, of these 11 were grossly deformed and 25 seemed normal in structure. In lot B_3 15 were hatched and 39 were not, of the latter 7 were grossly deformed, one a typical cyclops and one a monophthalmia. Four others had slightly underdeveloped eyes in addition to the 7 actually deformed.

When 23 days old, only 2 of the control were still unhatched. In lot B_1 there were 35 hatched and 20 unhatched. Lot B_2 contained 27 hatched and 12 unhatched. In lot B_3 over 40 had hatched and only 9 were unhatched. One had died and 2 of those that had hatched showed their bodies so badly twisted that they were unable to swim. One of these had a badly deformed body and one eye was abnormally small with the lens protruding.

At 25 days old, every individual in the control lot had hatched. Lot B_1 had 16 unhatched and 4 of those that had hatched showed deformed bodies and could not swim in a straightforward manner. Thirty-seven of those hatched were normal in appearance, 3 of the unhatched had died. The following deformed conditions existed: One was a double-headed specimen, many had no eyes, monophthalmia, abnormally small eyes, short bodies, etc. Thus at this time after the great number of specimens had died there were still over 20 per cent deformed.

In lot B_2 11 were unhatched and 29 had hatched. Two of those hatched were so deformed and twisted as to be unable to swim. The 11 unhatched ones were all grossly deformed, so there were 13, or 33 per cent, of the total living specimens deformed at this time.

Lot B_3 showed 5 unhatched and about 40 hatched. Four of those hatched were so deformed as to be unable to swim in a normal fashion. One of these presents the peculiar condition of a heart beat, but no circulation in a hatched fish with a long normally shaped body. There was a large accumulation of blood-cells within the sinus venosus and the median vein in the region of the anus was filled with red corpuscles. This specimen could swim poorly from place to place, had fairly regular respiratory movements, and waved its fins without a circulation of its blood.

When 34 days old, the B_1 lot finally had 9 specimens which were unable to hatch, all of them were deformed.

Lot B_2 showed 6 unable to hatch, all deformed and without a blood circulation. In lot B_3 4 failed to hatch. It must be recognized that a great many specimens in each of the lots B_1, B_2, and B_3 had died during the preceding 20 days. The weaker and actually most defective individuals are eliminated as shown by the early mortality records.

The above 6 unhatched embryos in lot B_2 were kept in order to determine how long such specimens might be able to survive. When 52 days old, these specimens were still alive, although the yolk-sphere had become very small, being almost absorbed. The small monsters were practically at a stand-still as to their life processes and were not kept after this time.

These experiments are here considered in a general way without going into the details of the deformities concerned. They demonstrate the fact that a normally continuous development may be modified into a discontinuous one by stopping its course during a very early cleavage stage. The fact is also shown that this stoppage is followed by a too slow resumption of the developmental rate and results in about 33 per cent of gross anomalies among those specimens able to survive the treatment. The mortality induced by stopping at such periods is high, the majority of eggs in all cases dying after return to normal temperature. Great variation in ability to withstand such treatment is shown by these hardy Fundulus eggs. The weakest ones succumb without resuming development on removal from the cold. Stronger specimens may undergo a few further divisions and live for some time in a high segmentation stage without being able to continue or progress further in their development. Other eggs continue development, but in such extremely abnormal fashion as to fail completely to form the embryonic body and only differentiate certain tissues scattered irregularly over the yolk-sac. Still more hardy specimens succeed in forming the embryonic body, but many organs requiring a high degree of cell proliferation and growth for their development, such as the eyes, other brain diverticula, mandibular, hyoid, and branchial pouches, etc., are unable to form in a normal fashion, and numerous defects in these parts are to be found.

Finally, the most resistant or hardiest eggs withstand the stoppage due to the low temperature and are able to resume development at an almost normal, though slightly retarded rate. These individuals may seem typically normal in structure, and often develop into hatched free-swimming fish, yet even these not infrequently show some indication of a subnormal condition in having their bodies slightly twisted or bent, and in being unable

to swim in a perfect fashion. Very probably the best of these specimens would present various ill effects from their early arrest could they be kept and observed throughout a longer life period. There are only a few simple performances to be observed in the actions of a newly hatched fish. Whether they are later capable of feeding and digesting food, reproducing, and performing other functions in a normal fashion is unknown for such individuals. The probable later effects as well as the classification of the deformities following stoppage at various developmental moments will be more fully considered in the subsequent sections.

One other similar series of experiments may be briefly recorded to further make clear the results which follow various degrees of interference with the rate of development during its early stages. A careful consideration of these records also brings out some of the differences between the effects of completely stopping and of slowing to a decided degree. The significance of the very varied types of deformities which result from early interruptions will be considered in connection with the records in the following sections of the discussion.

Experiment 902, B, C series. Three hours after fertilization, when in the 2-cell stage, eggs were placed in the refrigerator in the following arrangement: B_1 and C_1 at 5°C., B_2 at 7°C., and B_3 at 9°C., with a control from the same groups of eggs kept at room temperature.

When 24 hours old, the control were all developing in a perfect manner, but again somewhat slower than the maximum midsummer rate. The germ-caps had flattened on the yolk, but there was neither germ-ring nor embryonic shield formation yet visible. The B_1 and C_1 lots had all divided once or twice before cooling down to the 5°C. temperature. Every egg in both vessels was alive and in the 2-, 4-, or 8-cell stage. In the C_1 lot almost all were 8 cells. In many the 8 cells were arranged into two groups of four (fig. 9).

Lot B_2 were, as a rule, in the same condition, all eggs being alive, the great majority in the 8-cell stage, with a few showing the 4-cell stage.

The B_3 lot, after 24 hours at 9°C., were practically all developing at a very slow rate and had reached about the 64- or 128-cell stage. They seemed normal and in good condition other than for their very slow progress.

Forty-six hours after fertilization, the control showed every egg developing, the germ-ring having grown almost completely over the yolk-sphere, the embryonic body was well formed, but the optic outpushing had not yet arisen.

In lots B_1 and C_1 the eggs had divided once during the last 24 hours and were now almost all in the 8- and 16-cell stages, while a few were irregular 32-cell stages. Much cellular disorganization had taken place and the cell groups were broken and irregular, often with large unsegmented protoplasmic masses.

Lot B_2 were in somewhat similar conditions, all showed more or less irregular 8- and 16-cell masses. Many also showed large unsegmented protoplasmic areas with a few cells around the periphery (fig. 10).

Lot B_3 at 9°C. were all developing somewhat faster than the above, and now presented well-arranged high-segmentation caps. They were normal in appearance up to this time.

When 4 days old, the control showed a perfect condition with not one egg having failed to develop. There was a vigorous heart beat and a
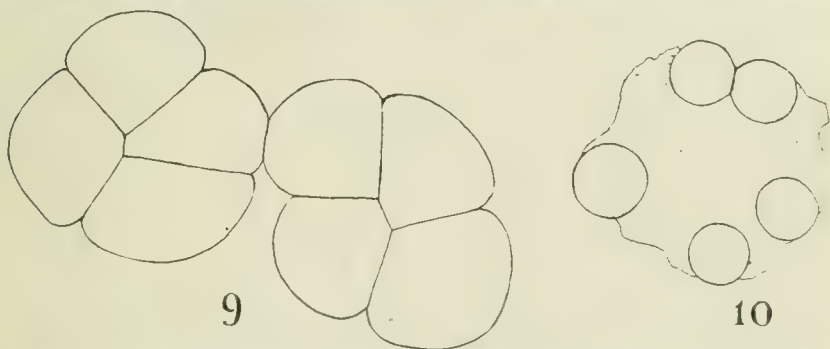
Fig. 9 A cell group 24 hours old, having been in a temperature of 5°C. since three hours after fertilization. The 8-cells are peculiarly arranged into two groups of 4 each, such specimens may give rise to ordinary single individuals.

Fig. 10 A large unsegmented protoplasmic mass with blastomeres around the periphery. A frequent specimen in lot B2, experiment 902, when 46 hours old after 43 hours at 7°C.

good circulation fully established. They were thus developing considerably faster at this time of the season than did the control of experiment 901, which was fertilized 10 days earlier. These 901 embryos had not developed a heart beat or established a circulation when 4 days old.

Lot B_1, after 4 days at 5°C., showed a few regular cleavage caps of about 64 cells. The majority, however, exhibited very irregular cleavage arrangements and some were almost amorphous protoplasmic masses, although all were translucent and alive. The eggs had, therefore, developed at an extremely slow rate, but had not completely stopped. These specimens were now placed at room temperature.

In lot C_1 the majority had, after 4 days at 5°C., rather regular 64- or 128-cell caps. This lot was from a different group of eggs than the B series, and its control was going in a manner exactly similar

to the B control. These eggs, however, may be individually more resistant. This lot was also now returned to room temperature.

Lot B_2, after 4 days at $7°C$., showed some eggs with regular cleavage caps of 64 cells and more, but the majority showed caps of irregular cell masses. These were now placed at room temperature.

Lot B_3, after 4 days at $9°C$., had all reached a high segmentation stage comparable to about the condition of the control at 18 or 20 hours old. All of these eggs had a distended bubble-like segmentation cavity similar to that described by me ('06) as resulting from treatments with LiCl solutions. Every egg was developing and furnished a particularly fine lot for an experimental test of this sort. These also were now placed at room temperature.

At 5 days old, the B and C controls were perfect with all embryos developing well. In lot B_1 the great majority had failed to resume development after being 24 hours at room temperature. The segmentation caps were breaking down and becoming disorganized. The few specimens that had resumed development showed the germ-ring formed and about one-half over the yolk-sphere.

The lot B_2 were in very nearly the same condition as B_1.

In B_3 the great majority were developing and the germ-rings were here also about half over the yolk-sphere.

Lot C_1 showed many stopped in development, but here the majority seemed well and showed the germ-ring about one-quarter over the yolk.

When 6 days old, the treated groups had been at room temperature for 2 days, the B_1 lot presented the following condition: Eight embryos had formed, there was one yolk-sac with scattered cells, and 33 eggs had died or failed entirely to resume development. All eggs in this lot had originally begun development and the control from the same group of eggs was perfect, thus the low temperature for 4 days had caused a very high mortality. Only about 22 per cent of the eggs resumed development.

In lot B_2 49 had formed embryos, 6 of these were short, lacking a complete formation of their caudal ends, the others were well-formed specimens with optic vesicles present. Fifty-eight did not resume development, although all had begun before being placed in the cold, thus there was a mortality of 54 per cent in this group.

In lot B_3 practically all formed embryos which now showed optic vesicles and body somites clearly formed. This lot was about as good as the control in respect to the number of eggs developing. Thus a 4 days' sojourn at $9°C$., with an extremely reduced developmental rate did not prevent the possibility of again resuming a development of normal rapidity. This extreme slowing at a slightly higher temperature is not nearly so fatal or injurious to later development as the almost complete stop caused by the lower temperatures of $5°$ and $7°C$.

Lot C_1 similarly treated at $5°C$., but consisting of eggs from another parental pair, contained at this time 21 embryos with optic vesicles forming, 7 short embryos with the germ-rings not completely over the

yolk, and 13 had died or failed to resume development. Therefore, in this lot 66 per cent were able to resume development, which is a somewhat better record than the B series. This difference may easily be due to individual variations between the two lots of eggs from the two different pairs of fish, yet both lots of eggs were unusually fine, as was shown by the perfection of the B control as well as the C.

When 10 days old the controls were going perfectly and seemed about at the point of hatching, having grown long with the tails curved around to cover the side of the heads, yet the yolk-spheres were still rather large.

In lot B_1 7 of the 9 living eggs showed embryos almost normal in appearance with good circulations, one was badly deformed and had a pulsating heart, but no circulation, while the one yolk-sac without an embryo had not progressed in development.

Lot B_2 showed 36 strong embryos with good circulation, though one of these was slow, with eyes abnormally close together. Four specimens were badly deformed, one with a circulation of the blood and three without. There were two yolk-sacs with blood and pigment cells present and two others did not develop. Thus 42 eggs were still alive, of which 7, or $16\frac{2}{3}$ per cent, were grossly deformed.

All eggs in lot B_3 seemed normal and well, although far behind the control.

In lot C_1 15 specimens seemed normal in structure, though two of these were slower than others in development. Ten specimens, or 40 per cent of the total, were deformed, 8 showed grossly malformed heads and bodies, one embryo being represented by an amorphous mound of tissue on the yolk-sac, and two other specimens had only deformed heads with a fair circulation of the blood. Thus in this lot where the mortality following removal from the cold was low, the percentage of deformed specimens is two and one-half times greater than from the B_2 lot that had suffered a high initial mortality.

When 16 days old, the majority of both control lots had hatched, though none of the inhibited ones had. When 17 days old, one in lot B_2 and 3 in lot B_2 had hatched, though none in B_1 and C_1.

At 18 days old, the controls still had a few unhatched.

In lots B_1 6 were hatched and 2 were not; in B_2 21 were hatched and 20 were unhatched; in B_3 33 were hatched and 27 were not; in C_1 13 were and 12 were not hatched.

When 24 days old, lot B_1 contained one badly abnormal specimen still unhatched. In lot B_2 17 were still unhatched, 5 of these were grossly deformed. In lot B_3 12 were unhatched, though seemingly normal in structure. These were all far behind the control in time and manner of hatching. In lot C_1 8 were deformed and unhatched, and one, slightly abnormal in gross appearance, partially succeeded in freeing itself from the egg membrane. Thus really 9 of these were deformed and unhatched.

When 29 days old, one individual in the B control had not hatched though the others had been free swimming for 10 days. This was the

only lack of perfection in this control of more than 50 individuals. In B_1 there was one unhatched monster. In B_2 10 were still unhatched, though 6 seemed normal and ready to hatch; therefore, the cold treatment greatly reduces the strength and delays the hatching moment of these embryos. In B_3 3 were unhatched. In C_1 8 were unhatched, 7 of these were deformed, one being a twin specimen and one almost normal.

This series of experiments further shows the possibility of almost stopping, or reducing to an extreme degree, the rate of development during the earliest cleavage stages and again resuming a more or less normal rate on the part of a few individuals. An almost complete stoppage at an early cleavage stage results in a very high mortality ranging from as great as 78 per cent and 54 per cent, down to 34 per cent. However, the reduction in rate brought about by a less severe temperature of 9°C. does not cause so great a mortality and does not prevent the resumption of development of almost normal rapidity.

It is clearly shown, however, that although certain specimens may resume a fairly normal developmental rate after such treatments, the early arrests have had injurious effects upon the quality of the resulting embryos. A considerable percentage of gross abnormalities occurs in all of the groups, and even those embryos which appear on close examination to be normal in structure are extremely slow in hatching and are not in all cases capable of typical swimming reactions and perfect behavior as young fish.

A point of particular importance is that in such a series as this which had been arrested during an early cleavage stage, the monsters resulting are not limited to any particular type, but exhibit, in a series of sufficient extent, almost all known types. There may occur double monsters of varying degrees, from separate twins, fused but with complete bodies and tails, to double bodies and single tails, and finally different degrees of double-headedness on single bodies. There are specimens exhibiting anophthalmia, monophthalmia, microphthalmia, cyclopia, and all types of malformed eyes. The brains may be slightly asymmetrical, irregular, tubular with no primary ventricles, or deformed in various ways. The mouth and branchial region may

exhibit almost any known defect. The fins may be poorly developed and the bodies ill-shaped and twisted. The tails may be short, bifed, and undeveloped due to a slow or arrested descent of the germ-ring. And finally there may be such minor defects as would escape observation until the hatched embryos were found to be unable to right themselves and swim. These are the defects to be seen on simple external examination, the internal structures are as frequently abnormal. The latter fact is borne out by numerous examinations of these monsters in sections. I have studied a great many of the sectioned specimens during the past number of years.

The reason for this great variety of monsters following arrests during cleavage stages is that the development of all organs or parts must subsequently take place and all may thus become arrested and deformed. When eggs are treated at later stages, as at the beginning of gastrulation, no double monsters will occur, their moment has passed, though the various brain, branchial, and other defects mentioned may exist. When treated after the embryonic axis is visible, it is most difficult to get any gross eye defects and so on.

Thus it may be said that the earlier the arrest the more numerous will be the type of defects found and the later the arrest the more limited the variety of deformities, since there are fewer organs to be affected during their rapidly proliferating primary stages.

The same treatment that causes a gross deformity when applied during an early stage, will during a later embryonic stage often give only a minor effect.

The further records of experiments will render these statements more fully certain. Here I wish simply to call attention to the great variety of gross deformities resulting from these early arrests. The contrasts in detail between these and the later treatments will be shown in the following pages.

I hasten, however, to caution any experimenter who may in the future find a double monster or cyclopean monster, for example, in a group of eggs arrested or treated during late developmental stages, not to assume that this is due to the late treatment or that it disproves the standpoint stated above. For

such an occurrence is simply accidental and due to the fact that the specimen was already arrested or defective in an early stage as might by chance happen in any normal lot of eggs. It is clearly true, as I shall show beyond, that only very early and carefully regulated treatment can artificially produce twins and double monsters, a phenomenon which must happen about the stage of gastrulation. Therefore, the treatment must be applied much before this time. Cyclopia may be induced by slightly later treatments, but only during a rather limited time, and quite early at that.

Other experiments will later be considered in order to illustrate the difference in response on the part of these eggs following treatments similar to those above, but applied as nearly as possible at certain particular developmental periods.

d. Differences in effect between greatly reducing the developmental rate and actually stopping temporarily the process

In the foregoing review of experiments attention was frequently called to the fact that in certain of the low temperatures employed an almost complete stop in development was actually obtained, while at the somewhat higher degrees the progress of development was reduced to an extremely slow rate, but not actually stopped. A more specific comparison between the effects resulting from actually stopping and greatly slowing the rate of development may now be made.

Three groups of Fundulus eggs when in the two-cell stage were placed in temperatures of 5°, 7°, and 9°C., respectively, as reviewed under experiment 901, B series. The first two temperatures were sufficiently low to almost completely stop development, so that after twenty-four hours of such exposure the eggs were still in the two- or four-cell stage. The group at 9°C., however, developed very slowly and attained either sixteen- or thirty-two-cell stages within the first twenty-four hours. In other words, at this temperature three or four cell divisions occur per day. When all had remained for three days in these low temperatures, they were removed from the refrigerator and the following results ensued:

The two groups that had been completely stopped in development suffered very high mortalities. In each a considerable majority of the eggs failed to resume development at room temperature, and during the early days of development very many of the survivors appeared abnormal in structure. These, however, later showed some ability to recover, but finally at an advanced stage about 33 per cent of them were still deformed. In contrast to this, the eggs that had developed slowly at 9°C. suffered only a low mortality on return to ordinary temperature and there was not nearly so high a percentage of abnormalities. At a late stage only 14 per cent were deformed as against over 33 per cent in the two other groups. The slowed group also hatched earlier and with a better record than the two stopped groups.

Similar differences in records between such groups of eggs were often even better shown, as is indicated in the results of experiment 902. In this case three lots of eggs in the two- and four-cell stages were placed at 5° and 7°C. for four days, after which interval they had divided four or five times and were all in about the sixty-four-cell stage. They were almost, though not actually stopped, accomplishing only one cleavage per day. On return to room temperature, one of the lots from 5°C. suffered a mortality of 78 per cent, only 22 per cent of these eggs being able to resume development, although every one was developing when first placed in the cold temperature. The lot from 7°C. showed a mortality of 54 per cent.

Another group of eggs from the same parents and accompanied by the same control were placed at 9°C. at the same time and for the same interval as the above lots. These eggs developed slowly at 9°C., so that after their four-day sojourn they presented high segmentation caps, similar to the condition of the control after eighteen or twenty hours of development at normal temperature. On return to room temperature, these slowly developing eggs resumed a normal rate and practically all formed embryos. Thus, in respect to the number of embryos that were developed, their record compared favorably with the control and contrasted acutely with the only 22 per cent which resumed development after the 5°C. interruption. The number of de-

formed embryos was decidedly less from the 9°C. slow lot than
in the groups from 5° to 7°C. which had been almost stopped
in their development. The 9°C. group also hatched earlier and
somewhat better than the other inhibited lots.

It is thus seen that during the early rather critical stages of
development an almost complete stop is much more severe in
effect than a decided slowing, on both the resumption of develop-
ment and its later progress. An egg developing very slowly but
still continuing the process during the early cleavage stages ap-
parently possesses sufficient powers of adjustment or regulation
to take up a much more rapid development either gradually or
rather abruptly. When, as a result of low temperature, devel-
opment actually stops during the cleavage or pregastrular stages
on raising the temperature, it is frequently stimulated to start
again, but the start is so irregular and so out of normal rhythm
that many specimens are unable to continue development.
These undergo a cellular disorganization followed by death. A
considerable percentage of the specimens that do succeed in re-
establishing development, still fail to obtain a proper adjustment
and balance of developmental activities among their parts.
Thus numerous arrested and defective organs are found. This
lack of developmental balance among the various parts and the
resulting defects are again not so common with eggs that have
maintained a continuous development, although for a time it
may have been slowed down to an extreme degree. In nature
development rarely or never stops during the active early cleav-
age stages, though slight temperature changes may frequently
cause considerable slowing. The natural interruptions usually
occur later, as among the birds, just after gastrulation has been
well established. The experiments in previous sections also con-
tain data bearing on the effects of stopping and slowing during
these later developmental moments.

Experiment 905 shows the record of two series of eggs both
stopped and slowed when twenty-three and twenty-seven hours
old, respectively. In both cases the germ-rings were about
formed and gastrulation was well on its way. The lots C_1,
C_2, and C_3 after being twenty-three hours old, or in gastrula-

tion, were almost completely stopped for two days. Their condition when returned to room temperature was about the same as when placed in the refrigerator. Development was very promptly resumed at room temperature and only a slight mortality resulted from the stopping. Only a few of the embryos showed slight defects, but they were behind the control in time of hatching, on account of the two days' arrest.

Lots C_4 and C_5 at twenty-three hours old were placed at temperatures of 9° and 10°C. in which they continued their development at very slow rates, so that after four days the germ-rings had descended over about one-half of the yolk-sphere. During these four days they had advanced in development to a stage usually attained in about twelve hours or were going approximately at a developmental speed of one-eighth of the control rate. On return to room temperature these lots quickly resumed the normal rate, suffered no mortality on account of the retardation, and developed into normal specimens which hatched somewhat later than the control. These slowed embryos possibly had some real advantage over the completely stopped groups C_1, C_2, and C_3, though it was only slight if any.

The D series stopped and retarded at twenty-seven hours old, when in gastrular stages, gave exactly similar records. There was no noticeable excess mortality and no later injurious effects. It may be generally stated that stopping or slowing the development of Fundulus eggs after the gastrular stage, with the temperatures here employed, have no appreciable effect upon the quality of the young fish up to the time of hatching. The moment after gastrulation is established seems generally to be a particularly passive stage at which neither stopping nor slowing the rate of development is followed by injurious results.

The question now arises whether after stopping development during the gastrular stage there is any difference in result if it recommences rapidly or slowly. When twenty-four hours old, eggs were stopped for one day by cooling to 5°C. They were then allowed to resume development very slowly by being brought into a temperature of 9°C. They developed at a very slow rate for three days and were then brought into the room temperature

and resumed a normal rate of development. Such a procedure introduced at this given developmental stage seemed to have no effect other than to throw the lot of eggs several days behind the control in their degree of development and time of hatching.

In order to determine the ability of the embryo to initiate certain functional reactions when developing at an extremely gradual rate, specimens three days old, just prior to the establishment of a heart beat, were placed in the refrigerator at 5° and 8°C. These temperatures do not seem to inhibit changes in the late embryo to the same extent as they do the early cleavage processes. The group in 5°C. still had no heart beat after being chilled for five days, so these specimens may be said to have been almost completely stopped. After five days the lot at 8°C., however, had developed a very slow and feeble heart beat. Thus these had definitely progressed at such a temperature and had established the functional activity of the heart muscle. Both groups on being returned to room temperature recovered completely and hatched in a normal fashion. Therefore, neither stopping for five days nor slowing to an extreme degree the development of these three-day-old embryos produces noticeable effects on their subsequent development and hatching ability.

If abnormal development is simply the result of developmental arrests, why should not eggs which have been decidedly slowed in their developmental rates by lowering their temperature give rise to monsters as frequently as do those eggs which have been actually stopped in development at critical stages? When eggs are treated with alcohol, other anaesthetics, or a great variety of chemical substances, their development is not necessarily entirely stopped in order to induce monstrous results. These specimens, however, act during later development in a manner much more comparable to that shown by eggs actually stopped by refrigeration than like specimens in which the developmental rate was simply greatly reduced. The explanation of this fact is probably as follows:

Specimens which are caused to proceed at a greatly reduced though continuous rate of development by simply lowering their temperature apparently adjust the developmental progress of

their several parts to the slow rate in such a manner as to maintain the normal differences in rate of activity among the several parts. The developmental rhythm of the parts is retained and the proper system of balance is unchanged. On resumption of the normal rate the parts all respond in their usual accord. After a complete interruption in development at a critical stage, on resuming the process those parts or organs that were formerly developing at a rate in excess of the parts in general are unable to start up again with their original excess or advantage and other parts have an opportunity to compete equally with them and may thus cause their reduced or arrested expression. That organ developing at the most rapid rate or having the highest degree of metabolism or oxidation at the time of the stop is less able to initiate its original rate when the moment of resuming development occurs than are those parts that were developing more slowly.

The production of abnormal development and malformation of organs by treating eggs with strange chemical materials is brought about in a similar manner to the abnormalities following stopping. The part or organ developing at the most rapid rate is inhibited more decidedly by the treatment than are less rapidly developing parts and is, therefore, most affected or modified in its development. For example, at certain stages, the formation of the optic outpushings from the neural tube is the most energetic process taking place in the embryo. Any injury to the egg at this time works to the particular disadvantage of this process and results in underdeveloped or deformed eyes. If the injurious element is then removed, all other parts may continue their development normally, since they were not sufficiently active at the time of injury to be affected in particular. In other words, all of the other parts were affected similarly and no one was any more inhibited than another.

The results of slowing and stopping development may be stated very concisely as follows: On slowing development, all parts and organs lower their rates in a somewhat relative fashion, the faster-going parts, even though more decidedly slowed, are still progressing at a faster rate than the slow-going parts. On

resuming a normal rate, the more rapidly developing parts still maintain their necessary supremacy.

On completely stopping development at a critical stage, that is, when certain parts are progressing at excessive rates, as compared with the rate in general, the rate of all parts is reduced to zero or equality. On resuming development from such a condition, the differential rates are not again established with sufficient promptness and certain parts or organs are suppressed, poorly expressed, or deformed in structure. On stopping development at an indifferent stage, that is, when important inequalities in developmental rate of the different parts are not occurring, it matters not if the entire rate be reduced to zero. On resuming development the parts all begin at about equal rates without the necessity of a prompt establishment of differences and no particular arrests or suppressions occur.

c. The types of arrests or deformities following a stop or slowing in the rate of development

Only a general statement of results from a few experiments have been given in the previous pages without going into particulars regarding the variety of deformities occurring. At this juncture I should like to enumerate in a very brief way the kinds of abnormalities which have occurred in all of the experiments where development has been stopped or slowed by a reduction in temperature.

In the first place, there were produced a number of double-headed, double-bodied, and twin individuals which will be fully considered in the following section. Along with these were single individuals with all varieties of eye defects, anophthalmia, microphthalmia, monophthalmia, cyclopia, etc. These defects were present in heads with either structurally normal or variously malformed brains. The mouth and branchial arrangements were frequently deformed. The otic vesicles were occasionally suppressed to various degrees or developed abnormally during the later stages. A number of specimens were short bodied, some with bifed caudal ends. The general body form and the shape

of fins showed frequent peculiarities.   Extreme cases arose in
which amorphous masses of embryonic tissue were present on
the yolk, but no definite embryo was formed.   There were simple
yolk-sacs with blood-cells and chromatophores scattered irregu-
larly through them.   Along with these variously defective indi-
viduals were almost invariably certain specimens which in gross
structural appearance were normal and succeeded in hatching
and swimming freely about.   Others were almost normal with a
pulsating heart, but without a circulation of the blood.   Further
more detailed conditions need not be mentioned.

This list of defects is sufficient to show that the types and actual
individual conditions resulting from a simple interruption of
development by reducing the temperature are all identical in
character with those induced by treating Fundulus eggs with
various chemical solutions (Stockard, '07, '09, '10, '15, etc.) dur-
ing their early developmental stages or actually with the results
of certain mechanical operations upon these (Lewis, '09) and
other eggs (Stockard, '13).   Furthermore, deformed hybrids
resulting from crosses between distantly related species also
present exactly the same structural peculiarities (Newman, '15).
And finally the progeny derived from male guinea-pigs that have
been chemically treated for long periods of time occasionally
exhibit exactly similar deformities of their eyes and other parts
(Stockard, '13; Stockard and Papanicolaou, '15 and '17).

It seems difficult to imagine that the deformities occurring
among the eggs that have been merely interrupted by being
placed in a refrigerator temperature could be interpreted as other
than simple arrests in development resulting from the slow prog-
ress which had taken place at certain critical times.   It seems
equally as certain that the comparable conditions following the
other experimental procedures have resulted from a similar cause,
simply a lowering of the developmental rates of certain parts at
critical moments in their origin or developmental history.   In
the several sections to follow I shall give much crucial evidence
bearing on such an interpretation.

5. EXPERIMENTAL PRODUCTION OF TWINS AND 'DOUBLE MON-
STERS' BY AN EARLY ARREST OF THE DEVELOPMENTAL RATE

One of the earliest accomplishments in experimental embry-
ology was the production of two embryos, or twins, from a single
egg (Driesch, '92; Wilson, '93; Morgan, '93; Zoja, '95; Loeb,
'95; Schultze, '95, and others). This phenomenon was first pro-
duced by separating the two primary blastomeres so that they
were no longer in their usual intimate relation, each then devel-
oped independently and produced a complete individual. In the
light of this striking experiment, the occurrence of twins and
double monsters under natural conditions was readily explained
as being the result of an undue separation of the two blastomeres
during the first cleavage. Such a separation might have been
caused in a mechanical way, the two cells being pressed or
squeezed apart, or something unusual in the chemical nature of
the environment may have reduced the normal degree of cohe-
sion between the first two blastomeres, allowing them to fall
abnormally far apart and finally to become entirely separated
from one another.

This clean-cut experimental production of twins and its ready
application and acceptance as an explanation of the modus oper-
andi for a well-known natural phenomenon, has undoubtedly
held back our real understanding of the phenomenon and strik-
ingly illustrates the dangers of directly interpreting occurrences
in nature on the basis of results from experiments.

Almost at once evidence began to accumulate which questioned
the general application of the separate blastomere explanation of
twin formation. Such evidence was not always appreciated in
this connection, but from our present point of knowledge its
bearing is more readily seen. The discovery was very soon
made (Wilson, '04; Conklin, '05, and others) that on separating
the primary blastomeres in certain species of eggs complete twin
embryos do not result. Yet there is no reason to believe that
in nature twins and double monsters do not at times arise from
the eggs of such species. Twin formations are certainly not due
to the separation of the first two blastomeres in these particular
species, since each of these blastomeres developing independently

gives rise to a partial and not an entire embryo. Such eggs have an early differentiation and localization of 'organ-forming stuffs' and these stuffs are unequally distributed to the blastomeres even at the first cleavage. The individual blastomeres are, therefore, not totipotent, but only capable in their later development of giving rise to certain parts of the embryo and not the whole. The eggs of a number of worms and molluscs present this very early localization of differential stuffs, yet in some of these various types of double individuals are not uncommon. These double individuals I believe, in the light of evidence contained in the literature along with that presented here, are the results of a simple process of budding.

Again it was shown by Enders and later by Spemann ('03) that double specimens not only resulted from the separation of blastomeres, but the late blastular and gastrular stages could mechanically be caused to develop into double instead of single individuals. The degree of duplicity depended somewhat upon the extent to which the eggs were constricted in a given plane. This was evidently a case of dividing or separating into two parts the growing region of a single individual and thereby establishing two new growing points instead of the original one. The division of a single growing bud into two may be illustrated on plant buds, embryonic animal limb buds, etc. The interpretation of the two separated regions as being the exact derivatives of the two original blastomeres, as Wilder has suggested, is in many cases entirely implausible.

Doubleness in nature is probably due to a modification of a budding process, and double monsters and actually identical twins, like all other abnormalities, may result from an arrest or inhibition in development. To state that twins and double individuals are induced by a developmental arrest seems at first thought almost absurd; for how could an arrest serve to give a formation structurally exceeding the normal in extent? One might accept developmental arrests as explanations for many deficiencies in structural expression, but such an explanation of excessive conditions or double-headed and twin individuals would scarcely be suggested. In the present consideration, however, it

will be very conclusively shown that double conditions and twinning in nature are the result of an unusual budding process produced by an early interruption of developmental rate, and are not connected with a separation of the primary blastomeres except under experimental procedure.

Before entering into the particular points of the present experiments, it may be well to explain in some detail the writer's conception of embryo formation and the general process of budding in plants and animals.

It has long been known that the notches around the border of certain plant leaves, such as Bryophyllum, have the power under certain conditions to bud and give rise to an entire new plant. It is observed, however, that the new shoots, as a rule, arise from only one or two notches instead of from many. Loeb ('16) has performed most elucidating experiments on the budding phenomena in these leaves. In the first place, although in nature only a few notches on any one leaf send out shoots at any one time, yet Loeb has shown that there is a potential ability present in every notch to form a shoot. This fact is demonstrated by cutting the leaf into parts in such a way as to isolate each notch. Following such an operation a tiny shoot grows from every one of the isolated notches. It becomes evident, therefore, that not only does each notch possess the potential ability to form a shoot, but under ordinary circumstances this shoot-forming ability is suppressed in most of the notches by ths growth of shoots from only one or a few notches.

It was further found that almost any notch on the leaf could be selected and forced to bud at the expense of the other notches by simply suspending the leaf so that the selected notch dipped into water. This suggests, of course, that ordinarily the conditions for bud formation are not equally favorable in all notches and, therefore, only a few shoots arise from a leaf instead of one in every notch. These few then tend to suppress the origin of buds from other notches. Does any such set of comparable conditions exist in a developing egg or blastoderm before the initial line or axis of the embryo arises and begins development to form a complete animal?

The periphery of the blastoderm in the eggs of the bird and mammal or the germ-ring in a teleost's eggs is probably in some sense comparable to the notched order of the budding leaf. At a certain place along the germ-ring in the fish's egg a peculiarly rapid cell multiplication begins and the embryonic shield with the axis of the embryo buds away from this place. There is already evidence for believing that more than the one place may be capable of embryonic axis formation, and much is added to such evidence by the experiments now to be presented. There are many potential points around the germ-ring at which an embryonic axis might arise. Here again, as in the plant, when one bud or embryonic axis has arisen, it tends to suppress the potential ability of other points to form an axis, and normally only one individual is developed from the egg.

We are entirely unable to state the reasons why a certain point along the germ-ring should form the bud and not another. One can only imagine that this point has some peculiar advantage of position which gives to it a higher power of oxidation and a temporarily more rapid rate of cell proliferation than is possessed by other points, just as the notch which is dipped below the water surface possesses a budding advantage over the other notches around the leaf. Can the advantage of position possessed by a particular point on the germ-ring be reduced so as to equalize the budding tendency of several points and thus allow them all to express their ability to form embryonic axes? Could such a condition be brought about double embryos, twins, triplets, etc., would be produced.

The use of the word bud or budding in connection with double embryo formations as employed by Patterson ('14) has been criticised by Assheton, who suggests fission as the better word for the process. Such a discussion seems devoid of value and I employ the word bud to mean what is indicated above.

*a. Arresting development by low temperature and the production of
double embryos and twins in Fundulus*

A number of years ago I occasionally found a double embryo
or a twin condition in Fundulus eggs that were arrested in their
development by being kept in solutions of $MgCl_2$ (Stockard, '09,
figs. 22, 56, and 57). Such specimens, however, were so extremely
rare that their occurrence was never associated with the experi-
mental procedure. Chidester ('14) also found a twin among
Fundulus eggs arrested in ether solutions, and reported one
other in an egg which had developed in a crowded condition.

The eggs of Fundulus heteroclitus are extremely hardy and
twins or double monsters are practically never found among
these eggs developing under ordinary conditions. During four-
teen spawning seasons many hundred control embryos have been
examined and I have not found among them a twin or double
specimen. While on the contrary trout eggs are known to be
rather sensitive, and must be developed under very carefully
regulated conditions. In the trout hatcheries double embryos
and twins are very often found and have at times been collected
and studied in large numbers (Windle, '95; Gemmill, '00, and
others).

Recently I have found strong evidence of a causal relation
between slowing development and the formation of twins in
trout, this will be discussed beyond. The evidence led me to
experiment with Fundulus eggs in order to determine whether
here also there was a direct connection between arresting devel-
opment or slowing its rate and the origin of double individuals
and twins. During the past three spawning seasons, a number of
experiments have been performed and the general results of these
may be reviewed.

Two methods of slowing the rate of development have been
employed; lowering the temperature and reducing the oxygen
supply. The latter method will be considered along with the
occurrence of duplicities in trout eggs.

It was soon learned that double embryos and twins could be
induced, but only by treating the eggs during a limited develop-

mental period. Either stopping development or greatly reducing its rate during cleavage stages and before the germ-ring has formed, that is, at periods preceding gastrulation, frequently serves to cause doubleness in the subsequent embryo formation. Specimens subjected to any degree or kind of treatment after the gastrular period never produced double or twin embryos.

Subjecting Fundulus eggs to low temperatures during early cleavages, the four-, eight-, or sixteen-cell stages, not only arrests the cleavage process, but on later resuming development many eggs fail to establish a normal rate and balance for some time and the early processes of gastrulation would seem to be disturbed. The majority of eggs after a stoppage of cleavage are completely unable to resume development and may live for a few days in an almost stationary condition and then die. Other arrested cleavage caps undergo a breaking-down or falling apart of the individual cells before the death of the eggs. A small minority of these hardy eggs after an arrest during cleavage stages succeed in finally readjusting their development to a sufficient extent to give rise to apparently normal free-swimming young fish. The individual variations in resistance and developmental ability shown among Fundulus eggs are remarkable in all experiments performed on them. Our present consideration is to be centered on that group which is sufficiently viable to continue development, but not so resistant as to be able to completely readjust its developmental processes following the early interruption.

Not only does the entire experimental lot become divided into the three above crude classes, but the members of our selected group which is not completely capable of normal readjustment by no means all develop in a similarly defective fashion. These discrepancies again are due to individual variations in the manner of resuming development.

Certain specimens after removal from low temperatures resume their cleavages with a fairly normal rhythm and form a typical embryonic shield, but later the larger diverticula from the interior parts of the central nervous system fail to arise in a usual manner, or other processes requiring a high degree of developmental energy are not sufficiently expressed and various de-

fects become evident. Other individuals resume their cleavage
processes, form a typical blastoderm and begin the formation
of a germ-ring, which indicates the commencement of gastrula-
tion, but just here the degree of energy necessary for normal de-
velopmental processes is insufficient and a single embryonic bud
is not formed with that normal rate of growth which sup-
presses the appearance of other embryonic buds. Therefore,
instead of the one point proliferating at a disproportionate rate
to form the embryonic shield, two such points are established with
more or less equal rates of proliferation, both of which may be some-
what less active than the single one should be. The formation
of two embryonic shields or the initiation of two points of rapid
gastrulation away from which will grow the axes of the embryos
is in fact the initial or primary step in double formations. The
phenomenon is exactly the same as when two buds arise from two
notches on the leaf border instead of one bud growing from a
single notch. Every notch is a potential bud-forming point, and
in the same way many potential invagination points exist on the
blastoderm, and when more than one such place begins to grow
we have double formations. In this sense it may be appreciated
that the intrinsic conditions which give rise to double monsters
or twins exist in all eggs and are not produced by the experiment.
The experimental modifications of the external conditions simply
serve to allow more than the one growing point to express itself.

The actual results of several rather typical experiments may
be given as better illustrating the occurrence of the double
individuals.

Experiment 903. A particularly fine lot of eggs was obtained from
a large female and fertilized by a single male on July 5, 1919, during
the height of the spawning season. Three groups of these eggs were
selected, one serving as a normal control and the two others, A_1 and A_2,
at three and one-half hours after fertilization when dividing into the
8-cell stage, were placed in temperatures of 6° and 8°C., respectively.

The outside temperature was unusually warm and the control eggs
developed at a vigorous rate. When 22 hours old the germ-rings were
from one-third to one-half over the yolk-spheres in all the specimens
the embryonic shields were well formed with the embryonic axes already
indicated in the midline. Every egg in the control lot was developing.

The two lots in the refrigerator at 22 hours old had as a rule undergone only one cleavage further than when placed in the cold. All were in a rather typical 16-cell stage. The low temperatures had not quite completely stopped development.

At 48 hours old, the control embryos were in a very advanced condition. They were large in size with fully formed optic cups and lenses, about 10 to 12 pairs of somites, the pericardium distended and the heart formed, although not yet pulsating. Chromatophores were present and though small had already differentiated into the red and black types. Five hours later the hearts were pulsating, but the blood-vessels were not fully connected and there was no circulation. One familiar with these embryos will realize that such a condition of development is rarely attained in less than 70 hours, thus this control group was developing with unusual rapidity.

The eggs composing the A_1 lot when 48 hours old at 6°C. were in about 32- or 64-cell stages. Many of the blastoderms were discs of irregular cell arrangement and some presented large uncleaved protoplasmic portions. The A_2 lot were in a closely similar condition.

The control specimens when 72 hours old had a vigorous blood circulation, with the vessels already mapped out by the migrating chromatophores. During the cooler, earlier part of the season a similar condition was not reached in less than four days of development.

The eggs in lot A_1, after being 69 hours at a temperature of 60°C., all showed irregular segmentation caps, the cells of which seemed to be in a large vesicle or bubble-like formation. The caps appeared to contain approximately 64 to 128 cells loosely arranged and in every case located within the bubble-like area, which seemed to prevent the normal flattening down of the cap upon the yolk-sphere.

There seems to be a clearly marked surface film between the yolk and the region containing the cleavage mass. It is as if the cleavage mass existed in a drop of more transparent highly refractive fluid. The drop is not in a segmentation cavity, but probably consists of accumulated fluid such as normally exists in the cavity, but here located between the cell mass and the yolk, possibly on account of some peculiar osmotic effect.

The specimens of group A_2 kept at 8°C. were at 72 hours old in a closely similar condition to those of A_1. Both groups were removed from the refrigerator and placed at room temperature after this 69-hour exposure to the low temperatures.

After being out of the refrigerator for two days, many eggs in the A_1 and A_2 lots had failed to resume development and had died.

When 8 days old, or 5 days after removal from the low temperature, many more, 41 of the remaining 99 eggs in lot A_1, were dead and many of those living were grossly deformed. In lot A_2 a few more were dead, many were not developing, a number were grossly deformed, yet some were apparently normal.

When 10 days old, the eggs were all very carefully examined to determine as nearly as possible the exact nature of the abnormalities

which had occurred. The control consisted of 114 eggs, each of which contained a normal well-formed fish. In the A₁ lot 4 more had died, and thus the total mortality in this group after removal from the cold was very high, a little over 70 per cent. In all 54 individuals had survived to develop embryos, and of these 16, or 30 per cent, showed gross abnormalities. Five of the 16 abnormal ones showed double conditions. One was a complete twin, two were double-headed and two had double anterior halves with single tails, Y embryos. Thus 9.3 per cent of all surviving embryos were specimens exhibiting some degree of doubleness, and 33 per cent of the deformities which occurred were duplicities. When we consider the very delicate degree of arrest and the particular developmental moment that must be affected on the basis of our explanation of double monsters, the above result is a remarkably significant one and is as good as any I have obtained by this method during the past three seasons.

In the A₂ group at 10 days old 2 others had died and 88 were now alive. Among the 88 survivors eleven individuals, or 12.5 per cent of all, were grossly deformed and many others were pale in color and far behind the average in their degree of development. Two of the 11 grossly deformed specimens were double, one showed a slight degree of anterior duplicity and the other was a twin with the two embryos 180° apart on the yolk. One of the twin components was large, well developed and normal in structure, the other was a short embryo with almost no body but with a well-formed head containing eyes and a pulsating heart and good blood circulation. In this group only 2.3 per cent of the surviving embryos were double specimens, but almost 20 per cent of those actually deformed were of this type.

When 25 days old, many of the normal specimens in both the A₁ and A₂ groups had hatched, although all of these were far behind the control, which had begun hatching when 12 days old.

The actual percentage of double individuals induced by this experiment is not really large, yet it is comparatively very significant. From a long experience with these eggs I would venture to believe that under normal developmental conditions there is only a small chance for finding one double specimen among a thousand. During the past three spawning seasons a great number, certainly many thousand, of Fundulus eggs have been arrested in their development by being placed in low temperatures after the germ-ring had begun to form. These specimens were all examined with such care in connection with the various problems being studied that no double specimen could have escaped record. Yet among all these late arrests not one double individual existed.

In comparison with such facts, the occurrence of 9.3 per cent in lot A_1 and even the 2.3 per cent of doubleness in group A_2 would scarcely warrant any other interpretation than that such conditions had in some way been induced by the experimental treatments. There can be little doubt that the embryonic axis is initially expressed during a very critical and comparatively brief developmental moment. When the axis is once expressed, common observation teaches us that in some way it prevents the occurrence of other axes or other embryos on the same blastoderm. Doubleness very probably, as will be more fully discussed below, results from the almost simultaneous occurrence of two embryonic shields instead of one, and this is further due I believe to the probability that neither of the axes possesses the advantages which normally suppresses the expression of other potential budding points.

To further illustrate the occurrence of doubleness in Fundulus following treatment with low temperature, we may briefly summarize one other experiment.

Experiment 890. These eggs were developed during the early cool part of the season and the control itself progressed rather slowly. The lot B_1 was placed in a temperature of 5°C. 3 hours after fertilization when in an early 2-cell stage.

Twenty-four hours later the control had developed high segmentation discs which had not yet flattened to cap down upon the yolk-sphere. The night had been unusually cool and these eggs were thus considerably retarded in their development. This amount of retardation is not, however, particularly injurious, as is shown by the later development of the eggs. It would seem that Fundulus eggs were sufficiently resistant not to be noticeably deformed by the retardations in development induced by the degrees of low temperature which might occur during their spawning season in this climate. Nevertheless, embryos developed during the early cool part of the season are not so large in size or vigorous in behavior at the time of hatching as are those being developed during the warmer days to follow.

The eggs of lot B_1 after 20 hours at 5°C. are in 2- and 4-cell stages, they are, therefore, almost completely stopped, having divided only once during this time.

When 2 days old, the control had the germ-ring only about one-fourth over the yolk sphere, with the embryonic shield beginning to form, a stage not more than one-half as advanced as is usual for this age. Group B_1 contained eggs in the first, second, and third cleavage stages with many very irregular arrangements of the cells. These

eggs were now returned to room temperature, and many of them very
soon began again to develop.

The control at 3 days old showed the embryos well formed,
although the germ-ring was not entirely over the yolk-sphere. The B_1
lot, after being out of the refrigerator for 24 hours, had high seg-
mentation discs which had not begun to flatten down upon the yolk-
sphere. There was no indication of the germ-ring or embryonic-shield
formation. After 24 hours more the germ-caps had flattened and
grown about one-half over the yolk-sphere, the embryonic shield was
well formed in most of the specimens and the line of the embryo was
visible in the shield. Thus within the first 48 hours after removal
from the low temperatures many of these eggs have attained about
the same condition as was shown by the present control specimens
when 50 hours old. Many of the eggs after refrigeration failed to
recover, and died during the first two days at room temperature.

When 6 days old, the control embryos were twitching and moving
their bodies and were in all respects normal in condition. The B_1
group contained small embryos without a blood circulation, many of
them were abnormal at the head end, and many were short. Thus after
developing for 4 days at room temperature they are far behind a usual
four-day embryo.

The B_1 group were carefully surveyed for deformities when 9 days
old. Four eggs had yolk-sacs containing blood-cells and chromato-
phores, but without formed embryos. Six eggs still had an early cell
mass at the upper pole which had not developed, although even at 9
days it was translucent and alive. There were 10 deformed embryos
without a circulation, and 4 deformed but with a circulation. The
majority, 45, of all living specimens seemed normal, with vigorous
circulations. Thus more than 34 per cent of the specimens which
survived the low temperature were grossly abnormal. Three of the 10
eggs which contained abnormal specimens with circulating blood
showed double embryos. One was two-headed, and two were double
throughout their anterior halves, each having two heads and two
bodies with a single caudal half.

The control embryos were with two exceptions all fine normal speci-
mens. Two of the 86 individuals were small and considerably behind
the others in their stage of development, although their structures
were normal and they later succeeded in hatching several days after
their fellows.

This experiment again shows a pronounced difference between
the modes of development in the normal control lot of eggs and
in a similar lot which had been inhibited by lowering their tem-
perature before the time of gastrulation. More than 4 per cent
of the eggs which survived the inhibition contained double
embryos, and one-eighth of all the gross abnormalities was of this

nature. Here again it would seem to be strongly indicated that a connection of primary importance existed between the retardation of development and the origin of the double specimens.

A number of similar experiments with low temperature arrests could be reviewed, but they would differ little in their general results from those above. We may, therefore, pass to an analysis of another type of experiment before undertaking a general consideration of the significance of the results.

b. *Arresting development by reducing the oxygen supply and the occurrence of double individuals and twins in the trout and Fundulus*

Cellular proliferation which is so important an element in development is a great energy-consuming process. No doubt the interruptions in cell proliferation which were described in the preceding section as due to low temperatures are actually caused by a lower rate of oxidation which takes place at such temperatures. In nature development is not only interrupted at times by indirectly lowering the rate of oxidation through temperature changes, but also by directly reducing the oxidation rate through a lack of free oxygen. In the present section we may review some of the consequences of lowering developmental rate by directly reducing the available oxygen supply.

The methods employed have been extremely crude, just such methods as nature might frequently use. With such methods the results are, of course, more variable than might be obtained from highly refined manipulations, yet the variations themselves are quite instructive. Experiments with Fundulus eggs may first be considered.

1. Results with Fundulus. The eggs of Fundulus are demersal and are supplied with long thread-like processes which normally serve to entangle them on the blades of sea-grass or other objects among which they are deposited by the female. This arrangement serves to keep the eggs near the surface, and to insure contact with a better oxygen supply than might be obtained should they lie in the sand or silt of the bottom. When these eggs are developed in the laboratory they are kept in small glass

dishes, ordinary 'finger-bowls,' containing about 60 cc. of water. The thread-like processes from the egg membranes become entangled and cause the eggs to cluster together in bunches of from a few to even as many as one hundred or more.

It is a well recognized fact that in such clusters the conditions for development of the individual eggs are not equal, and the egg group fails to present a uniform mode of development. The common practice is to separate the eggs in a dish so that they lie apart and are not clustered together. The permanent separation of the eggs requires care and attention, since they may again become bunched by the agitation of the water. When they are properly kept apart the entire lot in a dish will develop with remarkable uniformity.

No control group of Fundulus eggs should serve as a standard for development unless the individual eggs are kept completely free from contact with one another. In my experience, under such conditions only the most insignificant percentage of developmental abnormalities ever occur. I am convinced that the high percentage of abnormalities recorded by certain experimenters among their control sets are due to a failure to properly separate the eggs. The clustered condition also vitiates the results obtained from experimental groups of eggs.

Advantage was taken of this tendency to become entangled into clusters in order to study the developmental reactions of eggs with more or less access to a free oxygen supply. The eggs about the outside of such a cluster are in contact with fresh surrounding water and a sufficient amount of oxygen for normally rapid development. Those specimens lying deeper and deeper in the cluster are more and more removed from a freely changing water supply, and, therefore, experience various degrees of a stagnating environment. Such eggs not only lack a constant oxygen supply, but no doubt exist in an environment containing an excess of waste products, such as the CO_2 given off by their neighbors. The developmental perfection attained varies directly with the distance from the center of the egg cluster, the further removed from the center the more perfect the development.

In many of the experiments the available oxygen supply was further reduced by first boiling and driving the air out of the sea-water into which the eggs were to be placed. The central eggs of large clusters in this boiled water frequently had their development stopped in various stages, while other specimens progressed at an extremely slow rate. One group of such experiments will be briefly reviewed as illustrating the general results from all.

Experiment 915. A large number of eggs, from three females, was fertilized by a single male. After 3 hours they were almost all developing and presented the typical 4-cell stage. About 75 of these eggs were placed in ordinary sea-water and separated apart on the bottom of a dish to be developed as a control. The other eggs were divided into three lots. Two lots were placed in dishes containing sea-water that had been boiled, and the third lot was put in ordinary sea-water. The eggs in the three dishes were then moved gently around until they became clustered into large groups of about 100 or more.

After 2 days of development, the control contained well-formed embryos with the optic vesicles prominently shown and with 8 to 10 pairs of somites present. Many eggs on the outer parts of the clusters in the unboiled sea-water were equally as far along, while others near the center of the clusters were still in segmentation stages, and still others were in various degrees of arrested development. The two lots in boiled sea-water were in closely similar conditions.

When 8 days old, the entire experiment was carefully examined and the following conditions found. The control eggs all contained normal embryos except for the fact that 3 specimens were smaller than the others and somewhat delayed in development. These, however, later succeeded in hatching.

The clusters in unboiled sea-water contained many dead eggs. The more superficial eggs of the cluster contained in general normal embryos, though some were behind the control in their degree of development. Almost all of the more centrally placed eggs of the group were several days slower than the control in their developmental stages. These embryos were small and pale with poorly expanded chromatophores, and 15 of them, or 13 per cent of the small embryos, showed gross abnormalities. They possessed narrow undeveloped heads, defective eyes, deformed hearts with no circulation, and other common defects, while 2 of the larger better developed specimens were double-headed embryos. This dish contained a few more than 200 eggs, thus only about 1 per cent developed double conditions.

The first group that had been clustered in the boiled sea-water showed a somewhat better record than the preceding. Here also many of the eggs had died. There were again a number of normal embryos in the superficial regions of the cluster. The more centrally

located specimens were small and far behind the control in their rate
of development, but here only about 10 per cent of them were actu-
ally grossly deformed, and there were no double conditions at all.

The second group in boiled sea-water was more decidedly affected
than any. Many of the eggs died. Many superficial ones were
almost up to the control in their state of development. But the great
majority of specimens were small, pale, and poorly developed, being
several days behind the control. Almost 16 per cent of these small
specimens were considered to show gross defects. Twelve specimens
had no circulation of the blood; 10 had decidedly defective eyes, minute
in size and poorly developed or deeply buried in the head, and two
were cyclopean.

Four specimens that were near the surface of the clusters and very
well developed presented double conditions. One egg contained sepa-
rate twins, both embryos being fully developed. The 3 other eggs
showed different degrees of anterior duplicity. Therefore, more than
2 per cent of the entire number of specimens developing in the dish
were double; this is much the highest record that was obtained among
ten similar experiments.

The other experiments with low oxygen supply gave closely
comparable results to the three above, and need not be reviewed
in detail. Only a very small number of double specimens oc-
curred in any of them. In all cases, the double individuals were
among those of fairly normal development and were not extremely
small and highly defective specimens. This, in my opinion, is
a fact of considerable importance, and is to be explained some-
what as follows.

The origin of two embryonic axes or growing points on the
germ-ring of the fish probably results from a rather mild or
slight reduction in the normal developmental rate at the time of
gastrulation or embryonic-shield formation. It is probably more
important to obtain the reduction in rate at an exact and very
limited moment than to have a definite degree of reduction.
That is, the reduction in rate may be little or much, but it must
occur during a very limited time and not continue for long after
the doubling has once been accomplished. Should the arrest
continue, it is possible that one of the buds, even though it had
begun to develop, might be suppressed, and the more vigorous
or more favorably placed one might later continue as an appar-
ently single individual.

Certain delicate or sensitive eggs will probably respond more readily and give double conditions more frequently than hardier eggs. The eggs of Fundulus are very hardy, and it may be that a treatment when acting in a delicate manner affects favorably for our purpose only the more sensitive eggs, while the large majority are too resistant to respond. Should the conditions be more severe they would act too harshly to obtain a double response from any. These speculations will appear to have a stronger foundation after we have reviewed the very remarkable tendencies on the part of the delicate eggs of the trout to give double and twin embryos.

2. *Double embryos in trout eggs.* The eggs of the trout unquestionably possess a stronger innate tendency to form double and twin individuals than do those of Fundulus. *Twinning and double formations, like all other unusual developmental phenomena, are not simply and entirely due to the action of an ususual environment, but also depend upon the internal structure of the given egg and its peculiar manner of development.* An environmental stimulus which would frequently induce double formations in one type or species of eggs might be completely ineffective in its action on the eggs of another species. The burden of evidence for the cause of twin formation as well as the means of artificially inducing it indicate an accessory budding or double blastopore formation as the primary step, and it is obvious that the early morphology of certain eggs more readily lends itself to the establishment of accessory blastopore formations than does that of others.

Not only is this morphological difference to be expected, but from what we know of the physiology of budding, it is also logically probable that in certain eggs the bud for the embryonic axis will arise with higher powers for dominating the entire budding region than in others. Different degrees of dominance of the apical bud in different plants is a well-recognized fact. In some plants the terminal bud grows to form a long slender stalk, without producing axillary or lateral shoots, while the terminal shoot of other plants grows to a limited extent only before axillary buds and branches make their appearance. The

embryonic axis in the vertebrate egg may be compared with the terminal shoot of a plant. A second embryonic axis formation is roughly comparable to the occurrence of an ordinary lateral or axillary plant bud. A better comparison, however, is made between the animal egg and the budding leaf, such as Bryophyllum. In both of these, the egg and leaf, we may recognize an area of potential budding capacity, and we know that not only one, but several equal buds may arise simultaneously from either of these two stocks. There are doubtless certain kinds of budding leaves which are more prone to form multiple buds than others. From such leaves instead of one shoot arising in a certain notch, several notches are equally capable of budding and several shoots are formed. It would seem that certain animal eggs also normally possess a disposition to produce several buds. Such eggs develop more than one embryonic axis and give rise to several individuals instead of the usual single embryo from a single egg. This is probably the case in the Texas armadillo.

The eggs of Fundulus and those of the trout, very probably illustrate two different degrees of capacity to form several instead of one embryonic bud. This much of the twinning process is truly inherited, and variations in the tendency may occur not only among the eggs of different species, but probably also exist among the individual eggs of the same species. For example, certain mothers may produce eggs highly inclined to give rise to two embryonic buds or twins, and such an inclination may be transmitted or inherited by her daughters or even through her sons. Davenport ('20) has recently found in a study of human twins that the males of a family carry or transmit the twinning tendency in equally as evident a manner as do the females. Double and twin individuals are also of much higher frequency in certain human families than in the community as a whole. All this indicates that the eggs of certain individuals are more inclined to form twins than are those from others. In the human cases the present consideration refers, of course, only to so-called identical and not fraternal twins. The latter, truly speaking, are not actually twins.

There can be little doubt from the experiments recorded above and the results to be given below that the environmental conditions or external factors are of greater importance than the internal tendencies in twin formation. It is evident that eggs, although capable of producing more than one embryo, rarely ever do. The number of twin formations in a given lot of eggs may experimentally be increased so greatly in excess of the natural occurrence of such individuals, that we are forced to believe even in the cases before mentioned of the armadillo and the excessive occurrence of twins in certain families; as cited by Davenport, that the environment may in these instances also be the actually direct cause. A peculiar uterine reaction may be inherited in the armadillo and in certain human families which prevents a ready or rapid placentation and thus primarily brings about an initial slowing of development. There is much evidence of a slow placental formation and a peculiar uterine condition in the armadillo which will be considered more fully beyond.

This brief estimate of the internal and external developmental factors concerned in twinning has been given just here in order that the reader may appreciate more fully the very different reactions shown by Fundulus and the trout. He may form for himself some idea as to whether this is due to a difference in morphological pattern of the germ-rings or potential budding regions in the two species or to differences in the physiological reactions to the environment or finally to a combination of both.

Double and twin trout are classical objects, they often occur in the hatcheries in various parts of the world and have been frequently figured and described since the early studies of Lereboullet by Rauber, de Quatrefages, Klaussner, Gemmill, and others.

All of the double individuals and twins recorded have been surprisingly well developed and normally formed. From figures and descriptions, it would seem as though the trout egg possessed a rather normal tendency to form double embryos, and the causes necessary to give expression to this tendency were so slight as not to be further injurious to the development of the individual

embryos. In other words, some very small and simple chemical or physical irregularity in the developmental environment is sufficient to cause two embryos to grow from the germ-ring, but is not so injurious as to induce a deformed or abnormal development in the young fish. When either component of these double specimens is deformed, the cause of such deformities may be more reasonably attributed to conditions other than the surrounding environment (see beyond).

Several years ago I obtained a large number of young trout, many of which were twins and others presented different degrees of doubleness. Since then I have visited several trout hatcheries and have found in all that double specimens very frequently occur. The practical fish culturists in two of these hatcheries thought that such abnormal double specimens were caused by early development under too crowded conditions, or in sluggish water where the eggs did not obtain sufficient aeration. Such views are very probably correct, since all of my experimental studies with fish eggs has indicated that some retardation in rate or interruption of development was the simple cause of unusual structural responses in the embryo. Only recently, however, could a satisfactory explanation of double conditions be worked out on this basis, and the trout specimens gave the key to the situation. The foregoing experiments with Fundulus were then conducted to further substantiate the conclusions.

The artificial production of double trout embryos is no doubt rather difficult to bring about, since evidently only a slight slowing of the rate of cell proliferation at a particular moment is favorable.

Plates 1 and 2 illustrate a series of double trout which are selected from the large number of such specimens that have been obtained. The series shows the various degrees of double formation, beginning with a partially double-headed condition, and passing through the double anterior regions on single bodies, to double bodies with single tails, and on to the condition of complete doubleness but with the two components jointed more or less intimately together. The final specimen shows two com-

pletely developed twins attached to the common yolk-sac. It will be noted at once that each of the final twin individuals is equally as large and perfect in form as is the single specimen at the beginning of the series. This fact is of importance in showing that up to this stage of development and growth there is no question of available food, since the amount to be had in each egg is here demonstrated to be sufficient to form two full-size perfect young trout instead of the usual one.

In studying the graded series of duplicities illustrated by plates 1 and 2, the question immediately presents itself as to why the two components in the several specimens show the different degrees of separation? What conditions or arrangements determined that the specimens in the upper part of the series should be double headed, while those at the end of the series are completely double bodied? Gemmill ('12) in his monograph on the teratology of fishes, considered these propositions and gave an explanation for the varying degrees of doubleness which I believe my studies completely confirm. On the other hand, Gemmill failed to give any explanation of the initial or actual cause of doubleness.

In accordance with the view that has often been suggested, the germ-ring was recognized by Gemmill "as a stock, able to give rise vegetatively, so to speak, to more than one embryo." The embryonic axis or body begins to form in the embryonic shield which arises from certain places along the germ-ring. When two shields arise, the degree of duplicity of the resulting double fish "varies directly with the original distance between the two centers of embryo-formation." When the centers of embryo formation are close together, only 5° to 10° apart on the germ-ring, the embryonic axes very soon become united so that a double-headed specimen with a single body finally develops. It may be stated generally that when the original buds are less than 90° apart the specimens formed will exhibit various degrees of double anterior halves on single posterior parts. When the distance between the initial buds is greater than 90° and on up to 180°, the resulting specimens will show the double condition

not only involving the anterior half, but extending into the posterior part of the body. Finally, at 180° apart, the two embryonic shields give rise to two completely separate twin individuals.

The accompanying diagram may serve to illustrate the manner in which such processes operate. In figure 11 the diagram on the left shows two early embryonic shields arising about 20° apart. When the germ-ring has descended further over the yolk-sphere, the dotted line indicates how the two embryonic
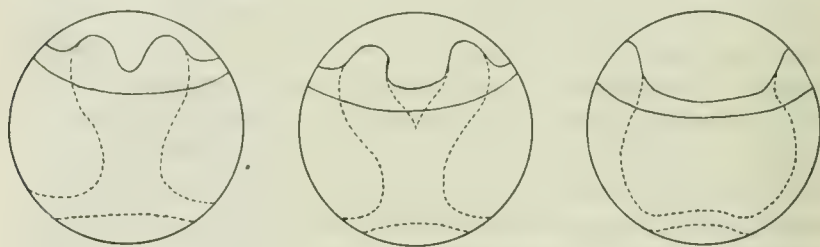
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Fig. 11 A series of diagrams illustrating the manner in which the degree of duplicity in embryos is determined by the original distance apart of the two embryonic shields on the single germ-ring. The solid lines indicate the early germ-rings with the two embryonic shields, and the broken lines show the resulting body outlines of the former embryos. The figure on the left has the embryonic shields less than 90° apart on the germ-ring and the dotted outline of the resulting embryo indicates it to be a double-headed specimen. In the central figure the embryonic shields are a little more than 90° apart, and the resulting duplicity extends throughout the upper half of the body. In the figure on the right the embryonic shields are 180° apart, or opposite one another, and two complete twin individuals result, as the dotted lines indicate.

axes become united or common in the body region, and such a condition would finally give rise to a single-bodied fish with two heads. The middle diagram in figure 11 illustrates similar steps in the history of a 'Y monster,' or individual with two heads and bodies and a single tail. The right diagram of figure 11 shows two embryonic shields arising 180° apart, or opposite one another on the yolk-sphere, each of these has an entire half of the germ-ring to develop from, and complete twins are produced.

In studying the early embryos of Fundulus these several steps have actually been observed. An observation of further importance in this connection has also been made, but unfortunately at present on very few specimens. In attempting to discover the earliest stages of doubleness from great numbers of eggs, I have selected all specimens seeming in any way to possess two early embryonic shields. On two occasions a fair number of such specimens were apparently found, one lot of seven such eggs and another of five. These seemingly double-embryo formations were isolated and observed during later stages, with the result that from among the seven specimens only two double-headed individuals arose, while the remaining five formed typically single embryos. The second group of five seemingly early double shields gave rise to five perfectly single specimens. There would appear to be only one interpretation for such a phenomenon: two initial buds may sometimes appear, but later one is completely suppressed by the other, or the two possibly fuse completely and only one normally single individual is developed. Therefore, it would seem that initial multiple buds are much more common than the resulting double specimens indicate, and that many secondary buds are suppressed or lost during early development. A comparison of the two components in older double monsters which is undertaken in a further section of this paper makes still more probable such deductions.

I wish to present these observations on the early double specimens with the chances of error fully in mind. In the first place, I succeeded in isolating a very few such probable early specimens, twelve from the many hundred eggs examined, and from these twelve only two actually showed double conditions during their later stages of development. The early embryonic shields were irregular and not strongly expressed. On the other hand, it seems to me significant that from the twelve specimens which were isolated two of them definitely developed double embryos, while it is recalled that among the great numbers of Fundulus eggs experimented on extremely few double specimens actually occurred. However, when one selects early specimens thinking them to be of a definite type and they later develop into indi-

viduals of another type, his confidence is considerably shaken in the validity of the selection. I have had similar experiences in attempting to isolate from large numbers of eggs those showing the earliest indication of the cyclopean defects. There are no doubt processes of regulation which may tend to correct and obliterate an early unusual arrangement, yet in spite of the recognized probabilities for mistake, I nevertheless feel that the foregoing indication of suppression of early buds has some real value, since two actually double specimens were certainly selected, as later development showed.

Kaestner ('98–'07) has figured very early double primitive streaks in the chick and Assheton ('08) double embronic shields in the sheep. Kopsch ('95) has described in Lacerta agilis, the European lizard, one blastoderm with two blastopores, and thus showed that a double gastrulation had taken place. From this observation he agreed with O. Hertwig that all twin formation as well as all anterior duplication arose from a double gastrula-infolding or proliferation. This position leaves, as Kaestner ('99) has stated, the question of doubleness or twins merely moved back to an earlier stage before the origin of the two blastopores, it remained to be answered why the double infolding takes place, and why it is so rare? In the present study it is felt that both questions are answered. A developmental arrest does away with the normal advantage of the usual growing point and permits a double gastrulation; the condition is rare for the same reason that the apical or dominant bud rarely fails to grow.

Returning to the consideration of the actual case in the trout, we may judge indirectly by the degree of separation of the two components in the several individuals as to the probable distance apart of the original embryonic shields or embryonic axes on the germ-rings. Gemmill ('01), has found a rather high proportion of complete twins among something more than seventy double trout specimens that he examined, while Windel ('95), had found only nine complete twins among 117 eggs containing double trout, or a proportion of one to thirteen. Among my double trout specimens there is one case of complete twins for every eight. From these observations it may be concluded that

when two embryonic shields arise from the germ-ring they occupy positions about 180° apart, or are opposite one another on the yolk-sphere, in nearly 10 per cent of the cases.

A further question bearing on the relative position of the embryonic shields on the germ-ring suggests itself. If the germ-ring is actually a potentially budding stock, why does not triplet and quadruplet formations appear almost as frequently as the double or twin condition? This question can at least be answered with a probable explanation, if not with a completely satisfactory one. In the first place, the extreme tendency of the eggs to form only a single rather than a double individual is important in this connection. There is certainly only a slight chance to form double specimens. The single bud is almost always capable of suppressing further expressions in the blastoderm. When this capacity is in any way lowered and a second bud arises, the stock is then still further dominated or preempted by the presence of the two, and the chance for still a third embryo formation is decidedly reduced. Yet among a hundred multiple cases one triplet may be found.

Gemmill found one case of three embryos in a trout egg as against over seventy doubles. This specimen had one almost perfect embryo and the other two were very abnormal and poorly developed. Among something less than 150 double fish embryos seen during the past few years I have observed only one triple specimen. This arose from a Fundulus egg that had been inhibited during early development by a weak solution of alcohol. One embryo, almost normal, was on the same blastoderm with a double-headed specimen.

I have never observed, nor found record of a fish's egg containing more than three embryos.

The conclusions seems warranted that one point of gastrulation, or embryo formation, has an extremely high tendency to prevent or suppress the existence of any other such point of excessive proliferation. When a second point is capable of expression the two almost without fail completely dominate the growth capacity of the entire germinal region and triplets are the rarest exception.

The relative conditions of the individual components in the double trout are of considerable importance and will be discussed in connection with their several particular bearings in the pages beyond.

c. An explanation of the frequent occurrence of twins and double chick embryos

It is extremely rare among birds for a double-headed or otherwise double individual to hatch from the egg; a few such irregular cases have been recorded. I have never, however, found record of complete twins hatching from the hen's egg. On the other hand, when the earlier stages of chick development are studied in the laboratory, one rarely fails, even in a limited experience, to meet with double and twin embryos. The prevalence of these early specimens has long furnished material for studies on twinning in the chick. Among many such investigations are those of Gerlach ('82), Burckhardt, Dareste, Klaussner, Erich Hoffman, Mitrophanow, five somewhat more recent studies by Kaestner ('98, '99, '01, '02, and '07), and most recently the description of several double chick embryos by Tannreuther ('19).

The almost abundant occurrence of double specimens among the limited numbers of eggs developed in the laboratory and the well-known high mortality among incubating eggs of the poultry farm, makes it highly probable that double and deformed embryos are not uncommon under natural conditions, but that they usually die during the early days of development.

From a survey of the literature on double monsters in the various vertebrate classes, it would be impossible to form anything like a correct estimate of the comparative frequency of such individuals in these several groups. It would be simply speculation to claim that doubleness was more frequent among the embryos of birds than among those of mammals. Yet the double condition in birds is just here of particular interest as probably being due to a somewhat definite and uniform cause arising out of their peculiar mode of development. The double bird embryos are very probably the result of a rather easily followed natural experiment.

It is a well-known fact, as mentioned in the early pages of this discussion, that the eggs of birds normally have a discontinuous mode of development. Fertilization takes place in the upper part of the oviduct and the egg begins its development in the high temperature of the maternal body and continues to develop as it travels down the uterine tube and becomes surrounded by its several accessory coats. Finally, at the time of laying, the blastoderm has passed the gastrula stage. The fall in temperature experienced on leaving the body of the mother causes development to stop in this early postgastrula condition, and the egg remains quiescent until the temperature is again raised to about that of the bird's body.

From the evidence given in preceding sections regarding the developmental time of inducing double embryo formations, it is seen that the bird's egg at laying has just passed the critical moment for causing double invagination or double blastopore formation. Since these double invaginations may be brought about by either interrupting development or slowing its rate before gastrulation, it would seem that the bird's egg had been piloted beyond this danger period within the body of the mother. How, then, is the frequency of double and twin chicks in the bird's egg to be accounted for?

In studies on the early stages of development in the bird's egg, it has been found by Patterson ('09) and others that the process of gastrulation takes place very close to the actual moments of laying. The time relationships between the moments of laying and finished gastrulation are, however, in general slightly variable, and the eggs of certain females, as I learn in conversation with Professor Patterson, differ decidedly from others in their tendency to be deposited at an unusually early stage. There would thus seem to be a strong probability that all eggs of the bird have not reached or passed the gastrulation process before the time of laying. This is a most important probability, and is believed to be true by some of those who have studied these early stages very extensively.

On the basis of my own experimental results, this probable variation in the moment of laying is entirely sufficient to account

for the double individuals and twins among the chick embryos. It also accounts most satisfactorily for the apparent frequency of such occurrences. The interruption of development following a fall in temperature at laying and before gastrulation has begun prevents the single gastrulation process from beginning at a rate sufficient to dominate the growth conditions of the entire blastoderm as it normally does. A second gastrular infolding or blastopore formation is established and thus two embryo formations are begun.

The usual interruption in the development of the bird has, with slight variations, been introduced at a most fortunately passive stage, just following gastrulation. This is a moment at which developing fish eggs may be stopped with impunity for considerable lengths of time and injurious results rarely ever follow. It is a moment following which no important embryonic structure need arise for a considerable length of time. After gastrulation only the linear growth to establish the embryonic axis immediately occurs. None of the highly energetic folding processes resulting from a localized excessive or unequally rapid proliferation take place until after a considerable interval of slow growth has passed. This interval of slow cellular proliferation following gastrulation is the fortunate occurrence that has preserved the birds as a class among present-day vertebrates. Had birds been so constructed that the egg was laid and allowed to discontinue its development before gastrulation had taken place it is conceivable that this condition could have eliminated them from the animal kingdom. There would have followed such a high proportion of deformed and defective specimens from eggs interrupted before gastrulation, that the individuals of a class having its eggs stopped at this time would very soon become so generally deformed as to be unable to maintain their existence. The important matter of a few hours' difference in egg-laying time lies between the successful class of birds and a hopelessly unfit monstrous condition.

Obviously, the evolution of the developmental environment has been of equally as great importance in the survival of a species, as has been its constant structural fitness. Nature's experi-

ment of temporarily lowering the surrounding temperature and stopping the developmental progress of the bird's egg has not proved fatal simply on account of the fortunate fact that the development is usually stopped during a very passive stage.

The slight individual variations in egg-laying time which cause certain eggs to be interrupted before gastrulation very probably furnish the material for the many descriptive studies of double avian embryos. On the other hand, it is a most significant fact to note that in spite of the many experimental studies on developing hen's eggs by Dareste, Fere, the writer, and others no double monster or twin conditions have been produced. This absence of double productions would naturally be expected, since the eggs were experimentally treated only after having been laid. They had thus passed gastrulation or the time after which double conditions cannot be induced.

Gerlach ('82) long ago thought that he had probably induced experimentally double anterior ends in chick embryos. His results were most uncertain, and have been interpreted as accidental by subsequent writers. He made injections over the blasderm so as to get fusions with the overlying shell. With such experiments he obtained double indications at the forward end of the embryos in two cases out of sixty eggs. Gerlach realized that conclusions could not be drawn from these meager results, but believed that if this method were perfected, it would yield more convincing results. Such experimental efforts to produce doubled conditions in hen's eggs are very probably futile, since the evidence at hand would indicate that there is only the rarest chance of the experimenter's striking an egg in the proper developmental condition to make possible the production of twin or double individuals. Should such specimens be obtained among the eggs employed in an experiment, there would always be the possibility that the natural interruption in development occurring in an egg laid at an unusually early stage was the cause of the doubleness, and not actually the experimental procedure.

d. An explanation of polyembryony in the armadillo

On examining the uterus in two pregnant specimens of a South American armadillo, von Jhering in 1885 discovered that each contained eight fetuses enclosed within a single chorion. He correctly concluded that all of the fetuses in each mother had been derived from a single egg by some process of division into separate embryonic rudiments. After this valuable discovery and interpretation, the study of the armadillo's development lapsed and nothing of importance was added for almost twenty-five years. Two series of investigations were then begun simultaneously, one on the South American species by Fernandez ('09) and the other on the Texas armadillo by Newman and Patterson ('09). The growth and expansion of these twin studies has brought our understanding of the phenomenon of polyembryony in the armadillo to a considerable state of maturity.

These authors readily agreed that in most species of armadillo the individual members of a litter, usually four in the Texas species and eight in the common South American form, are all derived from a single egg. It required considerable effort, however, to obtain the material that would furnish the morphological stages of the proccess by which this polyembryonic development was accomplished. We are finally indebted to Patterson ('13) for the very thorough and satisfactory manner in which he has collected and studied the early embryonic conditions; and particularly for having shown the first stages of the budding process through which the single blastocyst gives rise to four distinct embryonic areas, each exhibiting a typical primitive streak region.

In connection with the idea constantly advanced in the present study that twins and double vertebrate embryos arise from accessory growths or invagination points around the blastoderm, it now becomes important to ascertain exactly what degree of development has been attained by the armadillo blastocyst at the time the budding process begins. And since, according to our interpretation, these buds should arise at the time of gastrulation or blastopore formation, it becomes necessary to consider very briefly the germ-layers and gastrulation in mammals. The

decidedly precocious and highly modified method of forming the primary germ-layers in the mammalian blastocyst is not strictly comparable to gastrulation or the method of germ-layer formation found among the other vertebrates. On the other hand, the embryonic line or primitive streak of the mammalian egg is exactly comparable to the blastopore and embryonic process formation in the simpler forms.

The blastocyst of the armadillo has already, by a process of cell migration and delamination, separated off the primary entoderm from the ectoderm and further modified these layers before the budding which forms the embryonic primordia has begun. But it is in the primordia that the invagination of the entoderm forms the secondary entoderm of the gut and the embryonic mesoderm arises from a typical primitive-streak region much as in lower vertebrates. The precocious cell migration and splitting into layers in the mammal's egg is associated with the early implantation of the embryo upon the uterine wall of the mother, and the later primitive-streak formation may be interpreted as related to the actual gastrulation or blastopore formation away from which the line of the embryo always develops.

Whether the validity of the above briefly outlined interpretation of the germ-layer formation is admitted or not, we have in the armadillo a process of budding taking place from the blastoderm and associated with accessory or extra blastopore formation in much the same way as are the accessory embryos along the germ-ring in the egg of the bony fish. These buds also accord with Kopsch's ('95), description of a double gastrular condition with two blastopores in a blastoderm of Lacerta agilis, from which he concluded that twin formation as well as anterior duplication arises from a double gastrula—Einstülpungen. And, further, Assheton has described a similar condition in a blastodermic vesicle of the sheep. He, however, imagined the condition to have been due to a splitting during the morula stage.

The double primitive streaks in the hen's egg and other forms all lend themselves to strengthen the interpretation that double embryo formation first asserts itself by a double gastrulation or blastopore formation, which is initially a process of double

instead of single bud formation. Patterson's description of the origin of the quadruplet buds in the Texas armadillo furnishes the most striking case in the study of these conditions. And we may conclude that the budding or accessory embryo formation in the egg of the armadillo is exactly the same developmental process as that which gives rise to twins and double individuals in other vertebrate eggs.

However, the very important question yet remains to be answered: Why does this accessory bud formation occur so constantly in the Texas armadillo in contrast to the single embryo formation of mammalian eggs in general? Patterson ('13) failed entirely to answer this question, but he supplied some very significant data which Newman ('17) has appreciated as being intimately connected with the occurrence of polyembryony.

In connection with the collection of material Patterson ('13) discovered a 'period of quiescence' of the embryonic blastocyst. Regarding this he states: "The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collection in 1911." The following year be began collecting still two weeks earlier and again had a similar experience. "Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area)."

"It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15th) at which I have collected, possesses a free blastocyst. How long such blastocysts have been in the uterine cavity it is, of course impossible to determine; but I should judge not very long, because two vesicles taken from the fallopian tubes show a development almost as far advanced as that of some vesicles taken from the proximal parts of the horizontal grooves. Taking all the facts into consideration, I estimate the 'period of quiescence' to last

about three weeks; that is, from about the middle of October to the third or fourth of November.   .   .   .   Of the thirty-four free blastocysts obtained in 1911 and 1912, twenty-eight of them were secured within this period."

In a study of sections no mitotic divisions were found to occur in the blastocysts during the 'quiescent period.'

The only point of interest cited by Patterson in connection with this peculiar phenomenon of interruption in development, was the fact that in no other mammal except the deer, had such a condition been found.   Bischoff ('54) had long ago reported a 'period of quiescence' lasting for some weeks during a so-called morula stage of the deer embryo.

Newman ('17) has recognized the importance of Patterson's discovery of the 'period of quiescence' during the early development of the armadillo, and states in a discussion of twin formation that this 'period of quiescence' probably "holds the clue to the physiological explanation of polyembryony."   In this position Newman is, in my opinion, largely right, but this is as far as the data led him, and he finally remarks: "The problem is to locate the factors responsible for the slowing down of the developmental rhythm.   Whatever these factors may be, and we have no definite knowledge of them, the result of retardation is polyembryony."

Newman thus fails to appreciate the second point in Patterson's discovery, and that is, that the blastocysts always lie free in the uterus during the 'period of quiescence.'   This fact enables us to go one step further since the lack of attachment and, therefore, lack of oxygen supply are very probably "the factors responsible for the slowing down of the developmental rhythm."   The armadillo egg, like that of most mammals, undergoes its early development in the Fallopian tube and is, therefore, capable of reaching the blastocyst stage on its initial oxygen supply.   After this time however, it must become attached to the uterine wall for a further source of oxygen.   For some reason, in the armadillo the reaction between the blastocyst and the uterine wall is postponed and the blastocyst is incapable of further developmental progress until this reaction is established and the necessary supply

of oxygen becomes available. In exactly the same way the development of the blastoderm in the fish's egg is experimentally retarded or stopped by reducing the available oxygen and is again made to resume its development by supplying oxygen. In the case of the fish egg, the supply of ordinary nutriment is not involved, and reactions similar to those of the armadillo egg are only obtained as responses to changes in temperature or rate of oxidation.

I do not believe the retardation in the armadillo egg is of the nature of a starvation phenomenon, since we see nothing of the kind in other forms. Temperature changes are ruled out, since the temperature of the uterus is more or less constant. The absence of oxygen necessary for the energetic process of cell division is, therefore, in all probability the arresting cause, and the retardation results in polyembryony.

Thus Patterson has found the developmental interruption to exist, and he has also shown the blastocyst to be disconnected from the uterine wall and its necessary oxygen supply during this time. However, he has furnished no data bearing on the reason for the delay in uterine reaction and the consequent failure of immediate implantation of the blastocyst such as normally occurs in other mammals.

The consideration of the armadillo egg up to this point has taken account only of the external factors influencing its mode of development. It must now be remembered as a fact of serious importance that the production of quadruplets from the single egg of the Texas armadillo is an almost constant occurrence, while the experimental attempts to produce twins and double individuals in fish eggs and other forms have given at best only small percentages of such individuals among the large groups of eggs treated. It is also recalled that all eggs do not furnish equally favorable material for artificial twin production. The eggs of the trout seem unquestionably more disposed to give rise to twin formations than do the eggs of Fundulus. Thus some eggs would seem to have an hereditary or truly innate predisposition toward polyembryonic formations. There is much reason to believe that, aside from the external factors discussed, the

armadillo egg itself is highly disposed toward the formation of accessory embryonic buds.

There is the possibility, of course, that this natural experiment with the armadillo egg has become so exactly regulated as to influence the developmental processes precisely the same way each time, yet this is highly improbable for several reasons. The armadillo egg is not a case of simple twin growths from the blastoderm, but, as Patterson finds, there are primarily two buds, and then very promptly two secondary ones arise making the four, and after this the budding process ceases. In the South American species, however, it would appear as though a tertiary budding occurred giving the usual eight embryos; and in rare cases still another budding occurs from a few of the existing buds, giving a total of as many as twelve. It would certainly seem as though the blastoderm in these species passes through a stage of agametic reproduction or budding of a nature unknown among other higher vertebrates. But the possibility for such expression might only exist on account of the delay in implantation of the blastocyst and consequent shortage of the oxygen supply necessary for the rapid formation and growth of the single embryo.

It is important to keep in mind that there are species of the armadillo which produce only a single offspring from one egg. It is not known whether their embryos have a 'period of quiescence,' but if they have the period either occurs at a different developmental stage or the egg does not possess the inherent budding tendency of the other species.

It remains now to account for the fact that although the egg of the deer has a 'period of quiescence' during its development it does not give rise with any degree of frequency to twin individuals. In the first place, it is entirely uncertain from the scanty accounts as to what time in development the quiescent period occurs. Assuming that such a period does exist, it might occur at some indifferent stage when no peculiar result would be expected, for example, after gastrulation as it does in the bird with no subsequent effect. In the light of the experimental production of double individuals, it is readily understood that even though the egg of the deer is interrupted in its development at an early

stage, it might still be capable, on resuming development, of giving a normal single embryo. The egg of the deer may possess only a very slight tendency toward accessory embryo formations. A study of the experimental production of twin and double individuals among fish leads one to be surprised at the case of the armadillo and to expect the reaction found in the deer. The constant interruption occurring in the development of the birds and other animals at indifferent developmental moments with no subsequent ill effects renders commonplace the fact that the deer successfully withstands an interruption during its development without noticeable modifications in structural response.

In conclusion we may summarize the cases as follows. The development of the armadillo is interrupted on account of a failure to become promptly implanted on the uterus and a consequent exhaustion of available oxygen supply. The interruption occurs at a critical period just preceding the primitive-streak and embryonic-line formation. The internal qualities of this egg gives to it a decided tendency under conditions of arrest to form accessory embryonic buds. As a result of the interaction of these external and internal forces polyembryony is produced.

In the case of the deer only one probable fact is known, and that is that a 'period of quiescence' occurs. It is uncertain at what stage the arrest takes place, but it is probably due, as in the armadillo, to a delayed implantation of the blastocyst. Either on account of the stage of arrest or a lack of tendency to form accessory embryo buds, a typically single individual arises from this egg. The external factors may be the same as in the case of the armadillo, but they interact with different internal factors or different developmental moments to give a very different result.

c. 'Alternation of generations' and twins in vertebrates

Among plants and lower animals, particularly the coelenterates, there commonly exists a so-called alternation of generations. A given species at one time reproduces sexually by the union of gametes, egg and sperm cells, and the individuals derived from such gametes then give rise to a number of other individuals by a growth and fission or a budding process. Finally, sexually mature individuals again occur to reproduce another generation from germ-cells. In general this phenomenon is thought to be limited to these lower forms.

The suggestion has frequently been made but without sufficient emphasis that the blastoderm may be looked upon as a stock able to give rise asexually to more than one embryo. Since the natural process of budding to form four or more embryos in the armadillo is recognized, and accessory individuals may be produced experimentally from other vertebrate eggs, it becomes evident that even man and the highest animals may actually at times exhibit an alternation of the sexual and asexual processes of reproduction.

In a subsequent section of this paper the origin of various organs of the individual's body will be considered as arising initially through a budding process exactly comparable to the initial embryonic axis bud on the blastoderm. These buds may also be suppressed or inhibited in their expression in much the same way and by similar experimental methods as was described above in the case of the embryonic axis or initial embryo bud.

From a general biological standpoint the adult body of higher animals may be very correctly considered to be derived from a sexually produced embryonic axis the stock which gives rise by an asexual method of budding to the various special organs. The vertebrate body is thus composed of a group of different zooids, the organs. There are seeing, hearing, excretory zooids, and so on, comparable to the zooids of a siphonophore colony.

Alternation of generations is here considered a phenomenon, not limited as is generally taught to lower forms, but occurring throughout the animal kingdom.

6. STRUCTURAL DIFFERENCES BETWEEN THE TWO COMPONENTS IN CONNECTED TWINS AND DOUBLE INDIVIDUALS

As illustrated in plates 1 and 2, the components in connected twins and double individuals exhibit various degrees of separateness from partial double-headedness to completely double individuals. It has also been brought out in the previous section that the degree of doubleness shown by any such specimen depends upon the original distance apart of the two embryonic shields along the germ-ring of the fish's egg, as illustrated in the diagrams of figure 11. As Morrill ('19) has pointed out, the different extents of doubleness are in no way connected with different times of origin of the condition as was suggested by Newman ('17, p. 17-18), since every extent of doubleness is shown in this fish series and the time of origin from the developmental standpoint is the same in each case.

Irrespective of the degree of doubleness or the distance apart of the two components, there is a most significant competition, so to speak, between the components themselves, just as exists among several buds growing from a common stock. It is the results of this interaction or competition between the two components which we wish to consider in the present section, and their bearings, of very general importance, will be analyzed in the sections following.

a. Double individuals with identical or equal-size components

The two components in each of the specimens photographed in plates 1 and 2 are practically of equal size. The first plate illustrates the young trout from a dorsal view and the second plate shows the same individuals arranged in the same order from the ventral aspect. On comparing the two views of every specimen, it will be found that all heads are perfectly normal in appearance, each having two fully developed eyes, a perfectly formed mouth and branchial structures and a perfectly developed bilateral brain with its general contour clearly visible below the skin. On further comparing the two views in a given specimen, the body regions of the components are also found to be about equally

developed, except that in one or two of the cases one component is more decidedly twisted than the other. This twisted condition in some cases causes one component to appear considerably larger than the other. This, however, is only an appearance, and examination of the actual specimen shows the components to be very closely equal in size.

Correctly speaking, none of these components are structurally deformed. The application of the term 'double monster' to such individuals as these is actually a misnomer, since there is nothing whatever deformed or monstrous about their structures. The condition of being double is a perfectly normal result of the growth of two buds from a single stock. However, these individuals have arisen from unusual conditions acting on the developing egg during a particular interval and exhibit, therefore, unusual and modified developmental results. Similar conditions affecting other developmental periods are responsible for the production of all types of structural deformities and so-called monsters. The double series is, therefore, similar in so far as its causal origin goes to the ordinary monstrous forms, yet one could scarcely term two perfectly developed identical twins such as those shown by the last specimen of the series as monsters.

A study of the series here illustrated in addition to a large number of similar double specimens not only of fish, but of other animals as well as man, leads to the general conclusion that, *When the two components of a double individual are equal in size they are both normal in structure.* This means simply that such components are as strongly inclined to be normal as is a single individual and not that they are never deformed. All figures of double specimens in the literature further illustrate this point. One may deduce from these facts that if there was a competition of any kind between two such components, the advantages of each in the struggle have been equal. When the advantages are unequal, it will be found that a very different state of affairs results.
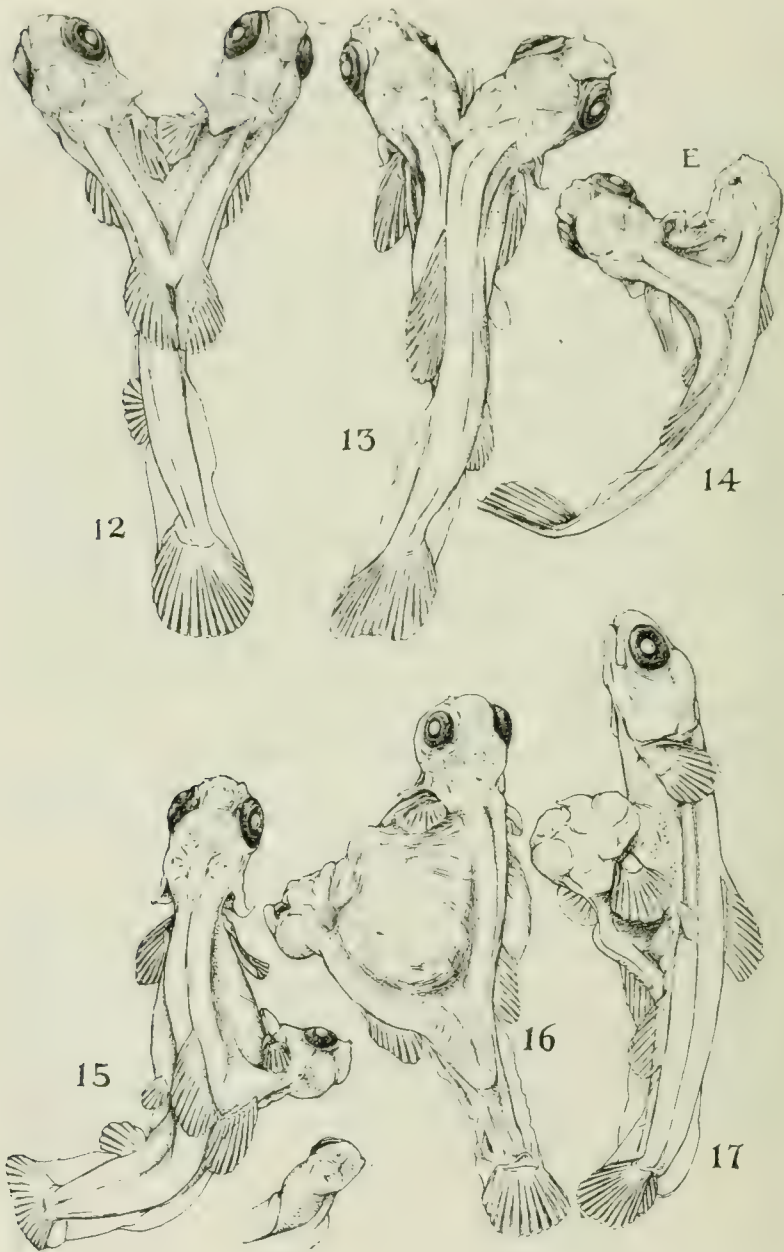
b. Double individuals with unequal components

In every extensive collection of double specimens we not only have those with components of similar size, but also a number of double individuals presenting two components of different size. The discrepancies in size between the two components may be arranged in a graded series beginning with only a slight size difference and finally ending with a very small mass attached to the larger component. Figures 12 to 17 illustrate such a series in cases of anterior duplicities, and figures 20 to 27 show various size differences between the components in completely double specimens.

Associated in all cases with these size differences are strikingly noticeable and important structural differences between the components.

Figs. 12 to 17   A series of double-headed trout specimens some time after hatching, and illustrating the fact that when the two components of a double individual are unequal in size the larger component is normal in structure and the smaller component is invariably defective.

Fig. 12   The two heads in this individual are equal in size and both are structurally normal.

Fig. 13   The left head is slightly smaller than the right, and the right eye of the smaller head is defective with a wide coloboma. The right head is entirely normal.

Fig. 14   The difference in size between the two heads is more marked than in figure 13 and the smaller head is also more decidedly deformed. Its right eye is entirely absent and the left eye is extremely defective, being only a small choroid body with a protruding crystalline lens. The mouth and gills are unopened with considerable structural distortion. The larger left head is in all respects perfectly normal.

Fig. 15   The left head is normal in size and perfect in structure, while the smaller right head is completely deformed with a twisted irregular shape and no definite outer indications of mouth and gills. The right eye is absent and the left eye is defective. A somewhat different view of the smaller head is shown immediately below the entire figure.

Fig. 16   A double specimen with the left head still smaller in size and more completely deformed. It has a cyclopean eye, and a narrow tubular brain, and the branchial parts are entirely distorted.

Fig. 17   Completes the series with a perfectly formed larger component, while the smaller left head is represented by an amorphous mass as seen from surface view. Should this specimen have attained adult size, it would probably have been a normal trout with a small nodule representing the lesser component projecting from its body wall.

1. Condition of the larger component. Whenever the components of a double individual are unequal in size, the larger component, with one exception in more than seventy such specimens that I have studied, is invariably normal in structure. A careful examination of a large number of illustrations of such specimens through the literature, without exception confirms the above fact. *It would seem to be a rule, that the larger component of a double individual is no more likely to be defective in form or structure than is a single individual of the same species developing under a similar environment.*

2. Condition of the smaller component. Whenever the components of a double individual are unequal in size the smaller component, in all cases examined, is always abnormal in form and structure. A survey of the figures in the literature also shows this to be constantly the case.

A study of the types of deformities and defects exhibited by these smaller components is most instructive, and is further extremely suggestive in an analysis of the causes underlying all abnormal development.

Examining first the cases of anterior duplicities, figure 12 shows two heads of equal size, both structurally normal. In figure 13 the left head is only slightly smaller than the right. The right head is normal, but the right eye in the left head is small and defective in form, with a ventral coloboma and a protruding crystalline lens. The size difference between the two heads is slight and the abnormalities shown by the smaller are not of an extreme type.

In figure 14 one head is decidedly larger than the other, the larger head, as usual, is normal, the smaller is very abnormal. There is only one minute deeply buried eye, E, and the structures of the mouth and branchial arches are peculiarly distorted. Figure 15 shows a still more marked size difference between the two components, and the smaller one here is decidedly twisted, with two poorly developed eyes almost in apposition on the ventral surface of the head. Mouth and gill formations are superficially suppressed, but there are certain contorted structures representing these parts. The brain lacks its usual bilaterality and has a

twisted tubular shape. The small figure immediately below the
defective head represents the opposite view of this head.

In figure 16 the smaller component presents a typical cyclopean
eye beneath the anterior tip of a narrow, almost solid brain.
Here again the mouth and gill structures are grossly deformed.

Finally, figure 17 shows only an amorphous mass representing
the smaller head on a perfectly normal larger component. This
head mass contained no ophthalmic structures at all, the brain
was entirely distorted and the mouth was completely absent,
with the gill structures greatly deformed. Behind this head mass
the pectoral fins were fairly developed and a short anterior body
portion representing the rest of the component is shown in the
figure.

Figures 18 and 19 give two views of the only case observed
among these individuals in which the larger component was
also deformed. In figure 18 the larger left head is seen, in dorsal
view, to have a left eye, but no right. The ventral view shown
in figure 19 illustrates the normal left eye and also shows the
normally well-formed mouth and gill arrangements in the superior
component. The right head, or smaller component, is shown
from both views to be much more decidedly deformed than the
left. It is completely anophthalmic and the brain, mouth, and
gill structures are clearly abnormal.

Since in all the other specimens the larger component is normal,
we may claim with justification that the larger component in
this specimen simply happens to be deformed as any single
individual might chance to be. But the smaller component is
more decidedly deformed than the larger, and the deformity in
this instance no doubt results from the same reasons which have
brought about similar deformities in all other smaller components
of the entire group of double specimens studied. It is only to be
expected that the larger component developing under somewhat
modified conditions, such as those necessary to induce the initial
doubleness, will occasionally be further affected and present
some structural deficiency. Such abnormalities are not uncom-
mon among those members of the experimental group which are
not induced to double formations, but continue to develop as single

individuals. In other words, monophthalmia, cyclopia, anophthalmia, deformed brains, and branchial structures occur among single specimens developing along with the double ones.

We may now consider the condition of the smaller component in double specimens in which the components are two complete individuals, or conjoined twins.
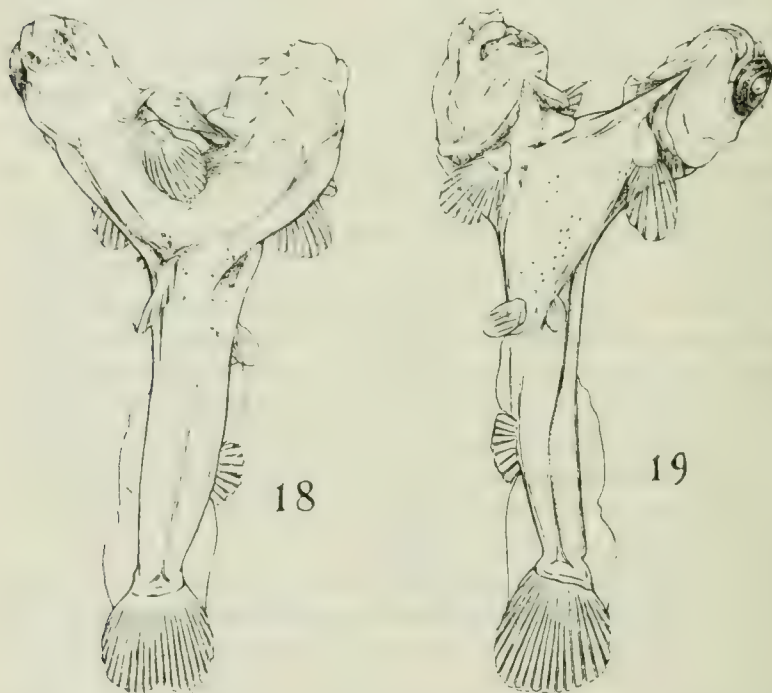
Figs. 18 and 19 Two views of a rare double specimen in which the components differ slightly in size yet both components are deformed. The left larger head has only one eye, the left; it is otherwise perfect, as the figures show. The right smaller head is completely eyeless and grossly deformed in the anterior portion. In this case the larger left head is by chance defective just as any single individual might be.

Figure 20 illustrates normal equal-sized identical twins attached to a common yolk-sac. The development of a teleost embryo on a large yolk-sphere and the structure of its yolk-sac prohibits a free separation of identical twins and they always remain joined as shown in this figure.

In figure 21 the lower component is somewhat smaller than the upper and there is a complete absence of one eye. A drawing of the opposite side of this head shown below figure 21 represents the other eye with an extreme coloboma, its entire ventral part being deficient. This component seems otherwise normal.

Two views of another double individual are illustrated by figures 22 and 23. The larger component is perfectly normal except for the fact that its tail is somewhat unusually bent. The smaller component is completely anophthalmic and its brain presents a very abnormal contour.

In figure 24 the smaller component is still more reduced in size as compared with the larger normal member. Here also the extent of deformity is still more marked than in the two foregoing specimens. There is one small deeply buried eye in a more or less shapeless head. The mouth and gills are distorted and poorly developed and the brain is deformed. The body is small and abnormally developed.

The specimen shown in figure 25 carries the condition a step further. A normal well-formed trout has attached to its ventral surface a greatly coiled and twisted twin. This small component shows a minute almost buried eye, E, and the head is in many ways, grossly deformed. But for the extreme coiling, the body would present almost as good an appearance as that of the smaller component in figure 24.

In figure 26 the small twin has a still more malformed head with no eye, but a more or less anteriorly protruding crystalline lens just beneath the skin. The body here is shorter than in the figure above and has only a single twist.

Finally, in figure 27, the last of the series, the large component is a splendidly developed young fish with little more than a nodular twin attached to the ventral portion of its yolk-sac. The little component has one small eye deficient ventrally, no external mouth or branchial formations, the brain is tubular and the entire head knob-like in shape. The middle body portions are suppressed and only a conical stump-like tail end is shown. The entire growth of the lesser component has been but a small fraction of that attained by the larger member. One might

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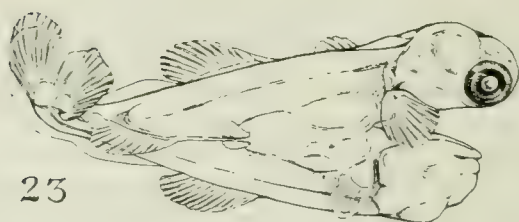
readily imagine that if this specimen had grown from its present length of 3 cm. up to a size of 30 cm., the small component would have been so outgrown by the larger as to appear a tiny almost unnoticeable nodule on the ventral surface of the large fish. The little component might possibly have become entirely included within the ventral body wall of the larger one. A twin inclusion would thus be formed.

3. *The small component and the frequency of double or twin individuals.* The frequency of double and twin individuals is probably much greater than realized. No doubt such specimens as the last one considered in the foregoing section might often attain the adult state without being suspected of their twin nature. It is also likely, in view of the fact that a graded series of reductions in the size of the smaller components in double specimens can be arranged down to the conditions here illustrated, that still more decided reductions exist. There probably are specimens with merely a trace of the smaller component, or it is possible that the small component might entirely disappear. Thus an individual appearing as a typically single specimen might in truth partake of the qualities and nature of the major component of a double individual.

In connection with such probabilities the condition of situs inversus viscerum is of interest. Morrill ('19) has found in an examination of certain of these double fish that a reverse arrangement of the viscera occurs in one of the components with a far greater frequency than has ever been known to occur among any group of single vertebrate individuals. The reverse arrange-

Figs. 20 to 23 A series of united twin trout, some time after hatching, further illustrating the principle that in double individuals with components of different size the larger one is normal structurally and the smaller is deformed.

Fig. 20 Twin trout, both of equal size and normal structure. Each twin is fully as large as a single specimen of the same age.

Fig. 21 The upper individual is the larger and is structurally normal, the lower specimen is slightly smaller with no eye on the right side and the left eye, shown in the small accompanying figure, is deformed with a decided coloboma.

Figs. 22 and 23 Two views of the same united pair. The upper larger individual is structurally normal, and the lower smaller twin is eyeless and somewhat further deformed, with a twisted caudal region which also causes a twist in the tail of the larger specimen.

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E

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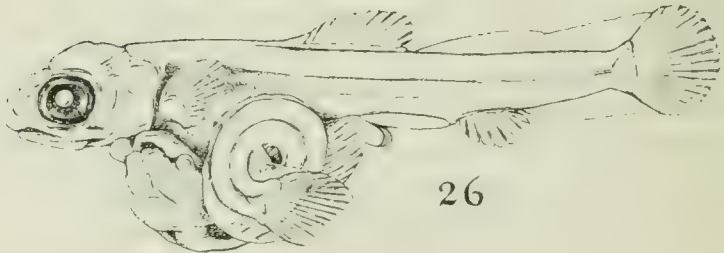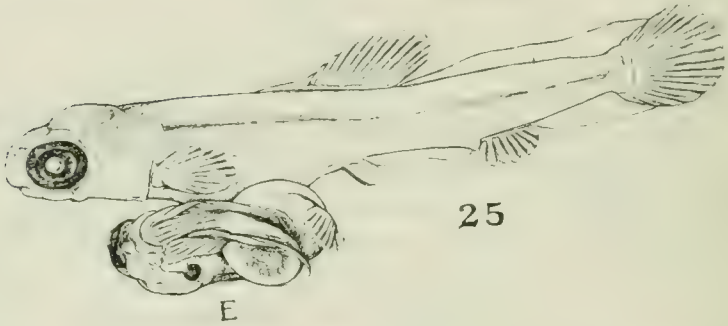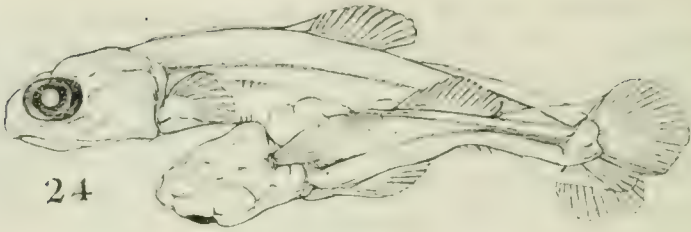
ment of the viscera in one component, though it by no means always occurs, would seem in some manner to be associated with the double condition. This reversed visceral arrangement also occurs very rarely among man and other mammals in single individuals. Its remarkable frequence among these double specimens would lead one to suspect very strongly that when a reversal of the visceral arrangement occurs, the apparently single individual is in reality a twin. All such specimens should be carefully examined for twin or embryonic inclusions as positive evidence of their double nature. Failure to find such inclusions would not, however, disprove the above suspicion, since the inclusions might be represented by structures so minute as to be readily overlooked.

4. The small component and certain theories of teratoma. Another much-debated problem may be somewhat illuminated by this study of double specimens. I refer to the various ideas of the possible origin of so-called teratomata or embryonal tumors. Such formations occur with greatest frequency in the lower abdominal or pelvic region. Certain pathologists have thought them to arise from a development of misplaced or arrested blastomeres, others have thought it possible that they might arise through some form of parthenogenetic development, and still others have looked upon them as a type of twin inclusion. The

Figs. 24 to 27   A continuation of the twin trout series shown in figures 20 to 23. In this group the smaller member is still more inhibited in size and more completely defective in structure. The larger component is perfect in all.

Fig. 24   The smaller twin is little more than half the size of the larger with an amorphous head containing one defective eye and the body is twisted.

Fig. 25   The smaller twin is here greatly twisted or coiled, its head is deformed, possessing a large defective left eye, and the right eye consists of a tiny choroid vesicle indicated by the dark spot, *E*.

Fig. 26   The lesser component is still smaller in size, short and twisted with a considerably suppressed eyeless head.

Fig. 27   The larger twin is a beautifully normal specimen, while attached to the opposite surface of the yolk-sac is a small individual represented by a badly deformed head with no mouth or gills and one defective eye. Almost the entire body of this component is absent and the tail is represented by a conical mass with no caudal fin. Should such a specimen attain adult size the smaller individual would be attached to the ventral abdominal wall of the larger as a nodular twin.

frequent occurrence of teratoma in the pelvic regions was in line with any of these explanations. Misplaced blastomeres might readily be in this portion of the body and twin inclusions or partially deformed twin bodies are frequently connected with the pelvic region.

The parthenogenetic theory which has received considerable support would necessitate the occurrence of all such formations in close proximity to the gonads and therefore would practically limit their occurrence to the pelvic region. It so happens, however, that teratomata occur with considerable frequency in the head and neck regions. This is most difficult to explain on the basis of a parthenogenetic origin. It might be possible, though not so probable (for the blastomere theory), that misplaced blastomeres arrested in the early egg might develop in such cephalic positions.

On the other hand, if teratoma arises from early twin inclusions, one would, on the basis of our series and a general survey of recorded double individuals, readily recognize that the head and neck regions should be places of frequent occurrence for these structures. The double specimens arrange themselves roughly into two groups, the anterior duplicities or double-headed lot, and the completely double specimens.

Should the smaller components of the first group become greatly inhibited and included within the larger components, we should have the inclusions in the head region. Should these then give rise to teratomata, such growths would not be expected to contain tissues found in the caudal regions of the body, such, for example, as nephric, gonadal, lower intestinal structures, etc. The rather frequent occurrence of teratoma in the head and neck would be what one would expect.

The inclusion of the smaller component of the completely double specimen would in most cases occur in the lower abdominal region. This would account for the great frequency of pelvic teratomata. Such teratomata in contrast to those of the neck region, may be found to contain tissues characteristic of any portion of the body, since these are inclusions of a possibly complete twin and are not limited to structures of either the

anterior or posterior regions. Nevertheless, from what we have
seen of the tendency on the part of the smaller component in
the completely double individuals to possess poorly developed
head ends, it would not be surprising to find that ophthalmic
tissue and other cephalic structures were frequently absent from
pelvic teratomata, though such structures might in certain cases
be particularly evident.

5. *Types of defects exhibited by the smaller component.* We may
now return to a brief consideration of the types of deformities
shown by the smaller components in the unequal pairs and
decide whether these defects are similar in kind to those which
occur in nature, as well as those experimentally induced, among
single individuals.

In the first place, it is noted at once that ophthalmic deformities
are particularly frequent. The illustrations show complete
anophthalmia, monophthalmia, and typical cyclopean conditions
as well as various degrees of imperfection in the individual eye,
such as coloboma and reduction in size of the retinal region.
Duplicities produced by any method such as the mechanical
constriction employed by Spemann, as well as those occurring
in nature, show in the smaller component the same ophthalmic
defects as are found among these double specimens induced by
development in low temperatures or with insufficient oxygen
supply.

The brain in the smaller components shows various abnormal
contours or may be simply tubular in shape without a normal
expression of bilateral diverticula or hemispheres.

The mouth is often deformed and frequently absent and the
operculum and branchial arches are distorted in shape. The
fins are often small and underdeveloped. The general body
shape may be variously modified, the caudal end being short
and stumpy or absent. The heart may be poorly developed
and pulsating feebly so that the blood fails to circulate and
becomes massed in various regions of the body.

It is unnecessary to do more than enumerate these defects
and examine the illustrations to convince anyone familiar with
the commonest developmental anomalies that the structural

modifications and defects of the lesser components are in every
sense identically the same as the defects which have been recorded
and illustrated as occurring in single individuals.

The types of defects most commonly found, such as those of
the eyes, are also the most frequently observed anomalies in
single individuals. Therefore, not only the kinds of defects,
but the frequencies of their occurrence are the same among these
lesser components as among single deformed specimens.

The fact that these malformed components are developing
in intimate union with larger normally formed components makes
it evident that the causal factors for the malformations are to
be sought in some difference that exists between the develop-
mental processes of the two. And, further, since the malforma-
tions of the one component are identical with those in single
specimens, the difference in conditions found between the larger
and smaller component may also furnish the clue to causes of
malformed structures in general. We shall attempt beyond,
in section 7, to give a logical explanation of abnormal structure
from this standpoint.

c. The components in double human specimens

In order to demonstrate that the conditions above described
as existing in double specimens of fish are in no way limited to
this class of vertebrates, I wish briefly to consider several very
interesting degrees of double development in human specimens
that have recently come into my laboratory.

All of these specimens have been examined by my colleague
Doctor Morrill for conditions of situs inversus viscerum, and the
left component in one case of anterior duplicity, as reported by
him ('19), shows a reversal in the position of the viscera just as
was found in certain of the components among the double fish.

The three specimens seen in plates 3 and 4 to form again a
graded series. The series begins with a double individual pre-
senting two heads and anterior portions on a single pelvis, seen
in the upper photograph, plate 3, and passes on to a completely
double specimen with the components strongly united through

their ventral surfaces, the lower figure, and finally ends with the entirely separate unquestionably identical twins illustrated in plate 4.

The case of separate twins differs in many ways from the other two and will be considered alone.

The first two specimens are in general alike. In each the two components are practically of equal size, and all of the four components present entirely normal structures. These specimens follow exactly the rule stated in reference to the double trout; that is, when the two components of a double individual are equal in size they are both normal in structure with almost the same frequency as a single individual would be.

In the first specimen, of plate 3, each head shows a perfectly formed face with all the sense organs fully developed. A dissection of this body shows the vertebral columns to be separate down to the sacrum. The pelvic skeleton is single with the normal single pair of lower extremities arising from it. The median arms of the two components have their soft parts fused or peculiarly arranged, a synbrachium, the details of which are being studied by Mr. H. B. Sutton. Each arm possesses a complete skeleton. Further details of the visceral structures are of no importance in the present connection. This specimen is in general comparable to the fourth case of anterior duplicity shown in the equal component series of trout (plates 1 and 2).

The second human specimen, in plate 3, is a case of full-term united negro twins. There are two completely formed components with a very wide ventral union into which the two livers and other viscera are drawn. The babies are females and each would weigh more than 5 pounds. A careful examination shows all organs and parts to be perfectly formed and of normally large size. This specimen is comparable to the last ones shown in the equal component series of trout (plates 1 and 2).

Here again we are warranted in attributing the different degrees of doubleness to the different distances apart of the two original embryonic lines or axis as they appeared on the blastoderms. In the first case the primitive streaks were not far apart and in the second case they were in positions almost 180°

apart on the blasto-disc.   Such an interpretation would certainly seem proper in the case of the fish and the bird.

The third case, that of the separate twins, plate 4, differs from the others in that both babies are deformed.   Yet the deformities and other peculiarities of this case make it unique in value.   It has been seriously questioned, on the basis of psychological and other studies (Thorndyke '05), whether actual cases of human
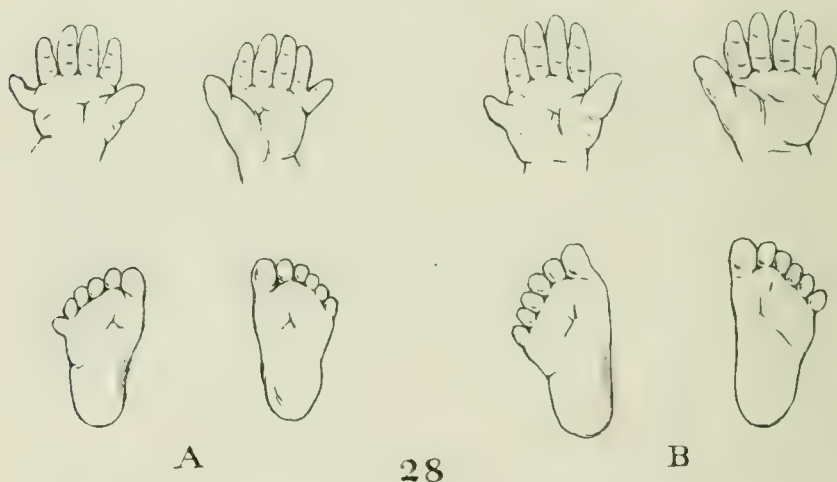
Fig. 28   Drawings of the ventral surfaces of the hands and feet of the identical human twins shown in plate 4.   A, from one individual, and, B, from the other.   The four hands are all polydactylous, having an accessory finger on the ulnar side, and the four feet are similarly polydactylous, all having an accessory toe on the fibular side.   The polydactylism is practically identical in the two individuals.

identical twins do exist.   The structural conditions of the two male twins in this case renders it practically certain that they arose from a single fertilized egg.   There are six fingers on each of the four hands, as shown in plate 4, and more distinctly in figure 28 A and B; there are also six toes on each of the four feet, as the illustrations show.   Such a polydactylous condition is known to be derived from a peculiar germinal complex and is not produced by the developmental environment.   The chance is one against thousands that two fertilized eggs carrying exactly

the same capacities for polydactylous development should occur at one time in this mother. The expression of polydactyly in a family in which it is hereditary, is most variable. Neither the father nor the mother of these twins were said to have polydactylous hands or feet, and it was claimed that they had another child with an ordinary number of fingers and toes. This, however, was a case of a 'seven-month' stillbirth in a New York hospital, and it proved impossible to obtain a family history of positive value.

These twins show further deformities that are almost identical in the two. All of the four kidneys are cystic and the left kidney in each individual shows the cystic condition in a more exaggerated form than does the right. The two heads have posteriorly protruding meningoceles, one being slightly larger than the other. The meningeal hernias are probably due to the action of some environmental influence that produced closely similar responses in these two individuals of identical germinal composition. In this case both members of the pair are malformed in addition to their unusual characters of genetic origin.

In every way these twins are structurally about the same with the exception that the one on the left is smaller than the one on the right to just the extent carefully shown in the two figures.

This is a most positive case of identical human twins and would certainly seem to leave no reason for question as to the occurrence of such individuals.

From these examples it is probable that the two equal components in double human individuals are in about the same relationship to one another as are the equal components of other double vertebrate specimens.

The double human specimens seen in many museums as well as those illustrated in the literature in which the two components differ considerably in size also follow the rule found for similar fish specimens. In double human specimens the larger component is usually normal in structure and the smaller component is always deformed. Extreme cases of this type are exhibited among the freaks in 'side shows.' In these living specimens the larger component has a well-formed human body with the

smaller component represented by a malformed partial body, attached to or protruding from it.

The cause of doubleness and twinning in these human specimens is in all probability the same as in the other cases discussed. The rate of development of the egg was probably arrested during an early stage, and perhaps on account of some interference or delay in implantation on the uterus. Very recently I have obtained a specimen through the kindness of Dr. Frank Erdwurm, of New York, which is of great value in an understanding of twin development in man.

The specimen secured by Doctor Erdwurm is shown by photograph in plate 5. A living female baby weighing 6½ pounds was enclosed within the upper membranes seen in the photograph. The cord from this baby is connected to the upper placenta near its lower border. After delivering the child, a second chorionic sac ruptured and discharged its fluid to the surprise of the observer. Later the two dead fetuses seen in the picture were delivered along with the placental mass. The fetuses proved to be identical twin girls enclosed within a common chorion and attached by their cords to a common placenta. The photograph clearly shows the single membranous sac in which they were enclosed and the positions of attachment of their cords to the placenta.

The size and other conditions of the fetuses indicate a stage of about six months' development. They had evidently been dead for a long time, probably about three months. The heads and bodies were somewhat macerated and shriveled and the blood-vessels had broken down in their placenta so that this no longer had any circulatory communication with the uterus. The two umbilical cords had become so wound around each other and knotted, as to completely cut off the connection of the fetal bodies with the placental circulation. The two fetuses were no doubt asphyxiated after six months of development.

This structural evidence is substantiated by the behavior of the mother. She had passed through the first six months of pregnancy in a normal fashion and then became greatly disturbed, so that it was feared that her pregnancy might be inter-

rupted entirely. The severe condition gradually subsided and she was able to carry the living child to full term. The reaction of the mother was doubtless due to the death of the two fetuses and the cut-off in the placental circulation. The uterus was able to adjust itself to the condition and the fetuses remained aseptically enclosed within their membranes.

This was the mother's second pregnancy; exactly twelve months before, lacking one day, she had given birth to a single normal child.

My interpretation of this triplet condition is as follows: The mother liberated from the ovary two eggs, both of which became fertilized and began development. One became implanted slightly before the other and developed into the single living girl. The second egg was not so favorably implanted as the first; this is indicated in the specimen by the lower placenta riding up over the larger one. The delay in implantation, due to the presence of the first egg, caused a slow rate of development at an early stage in the second and two embryonic buds arose instead of one, just as was described on the germ-ring of the fish. In this human specimen there is fortunately present the physical cause that might have produced the delay.

The woman gave birth to triplets, two of which were female identical twins derived from one egg and the other was a single sister individual derived from another egg.

Doctor Erdwurm furnishes a further very important record. This woman's mother had eleven pregnancies, nine of which resulted in living single births and two in abortions during the first half of pregnancy. One abortion, the tenth pregnancy, consisted of twins, and the eleventh pregnancy resulted in the abortion of triplets. The nature of these twins and triplets, unfortunately, is not known.

Evidently there is here a family tendency to ovulate more than one egg at a time. This may be due to simultaneous ovulations from both ovaries, from two follicles of one ovary, or from the rupture of a single follicle containing two or more ova.

7. THE DOUBLE INDIVIDUAL WITH UNEQUAL COMPONENTS AND AN UNDERSTANDING OF THE CAUSE OF ALL MONSTROUS DEVELOPMENT

The most valuable material that falls into the possession of the investigator attempting to analyze the causes of abnormal development is furnished by the united twins and anterior dup- licities where one component is fully developed and perfectly normal in structure, while the other component presents in a series of such forms various degrees or combinations of mal- formation and deformities. There are practically numberless attempts, from the time of Aristotle until now, at theoretical explanations for the cause of monstrous development, but none · of these, as far as I know, have recognized as crucial the con- dition presented by the combination of a larger normal twin developing in actual union with variously deformed lesser indi- viduals. Certainly, all theories that conflict with this condition of fact may be discarded as being inadequate in general. And, as mentioned before, the explanation of abnormal development probably lies in the differences between the factors operating on the development of the two connected individuals.

In the first place, one could scarcely state in the presence of these specimens that the abnormalities of the lesser compo- nent are of germinal origin. Yet similar deformities in single individuals have been interpreted in Wilder's ('09) theory of 'Cosmobia' as always being of such origin. The larger and smaller members of the double complex have both arisen from a single fertilized egg. There is no trace of either direct or col- lateral evidence to indicate that the hereditary factors are not equally distributed in the cells of both components. The germinal origin of one component could in no way be different from that of the other, since the entire specimen was a single individual up to about the stage of gastrulation. Further, when the two components are of equal size their identical genetic composition and character is evident. Obviously, then, defects similar to those enumerated as occurring in the smaller component are not in general of germinal origin.

It is further evident that 'identical twins' and the components in double individuals need not necessarily exactly resemble each other as is commonly thought. The two members of the pair may be structurally very different; in extreme cases one may be normal and the other actually deformed.

What influences could act on the smaller component that do not also act on the larger? Evidently there can be nothing in the external environment that would not come in equal contact with both components, since they are intimately united and enclosed within the same egg membrane. There can be no case of injurious substances inducing a 'blastolysis' in the one component and not acting on the other, or causing an early 'cellular disorganization' (Kellicott, '17), which would not affect both components. There is also no question of an insufficient supply of nutriment of the ordinary type, since it is shown by many specimens that two normal embryos equally as large as the usual single one may develop on the yolk-sac of the fish's egg, compare the first and last specimens shown by photograph in plates 1 and 2.

There must, however, be some sort of competition between the two components other than a competition for the appropriation of ordinary yolk material. Much evidence suggests that an interaction exists between the components similar to, if not identical with, the interaction between two plant buds growing from a common stock. When the growing tip is cut from the shoots of certain plants, e.g., the ordinary privet, Lagustrum, as a rule the axillary buds of the two leaves immediately below the cut give rise to growing shoots. In many cases two shoots grow at equal rates and are about equal in size, in other cases one of the shoots evidently possesses some advantage and grows much faster and becomes larger than its companion. Finally, in a few cases a single vigorous shoot arises from one of the resting buds and the opposite bud is entirely unable to grow. There is here involved a factor in addition to available food material, just as Loeb has found in the Bryophyllum leaf, and whatever this factor may be, through it the growing parts exert an inhibiting influence on one another. In the first case cited for the plant, the growing impulse was balanced between the two upper axillary

buds and they grew equally, in the second and third cases one bud occupied a position of advantage over the other; this advantage may have been due to a slightly more favorable exposure to sunlight, heat, or moisture, or to a better flow of sap material on its side of the stem. Its more rapid rate of growth in some way imposes an inhibiting influence on the expression of the other bud, causing it to be smaller, sometimes ill-formed or suppressed entirely. If the larger bud be pinched away in any of the cases, the smaller immediately improves its condition and grows large, provided it has not been held back too long (figure 29 A, B and C).

The advantages of certain positions on a stem over others is strikingly shown by privet branches growing in dense shade. These branches are slender shoots with long intervals between the pairs of leaves until finally they reach the sun. After a certain length of the stem has grown into the sunlight, the axillary buds of a particular pair of opposite leaves grow into shoots. Later, when still further in the sun, the two axillary buds immediately below those that grow first, now grow to form the second pair of shoots. Still later the axillary buds from the leaf pair immediately above the first shoots send out the third pair. I have observed this exact order of growth in nine cases of shaded stems. The first shoots to appear have an advantage of position over the second and third on account of a proper exposure to sunlight and at the same time occupying a certain distance away from the growing tip. The second buds then come into sufficient sunlight and grow out despite the inhibiting influence of the first pair, and finally the third pair of buds now grow on account of having become more mature and further removed from the growing tip.

The two embryos developing from a single blastoderm compete in a comparable way, and the results of the competition are also similar to the case of the plant buds. In the present state of our knowledge, it is impossible to say what the primary cause is that gives one of the growing parts an advantage over the other. We may merely express this in a non-committal way as an 'advantage of position.' It would in no sense relieve our ignorance of the situation to state the likely probability that one of the growing points has a higher rate of metabolism or a more rapid oxidation

than the other.   No doubt this is a fact, and should it be demonstrated, we still have the question: why is the rate of metabolism or oxidation higher?   Why does this difference in rate of oxidation exist in some instances and not in others?   What is there in these apparently similar points around the germ-ring that brings

29

Fig. 29   Outlines of branches from the common privet in which the terminal portions had been cut away, as indicated at the upper ends of the stems.   In the first and usual case, A, following removal of the tip each of the upper axillary buds have given rise to equal-size shoots.   In B the shoot from the right axillary bud is large and strong, while the shoot from the left bud is slow-growing and small.   In C the right shoot is normally expressed, but the left upper bud has failed to grow entirely, yet if the right shoot were pinched away the left bud then readily grows out.   In A the advantage of position in the two upper axillary buds was equal, but in B the right bud had an advantage in growth position over the left bud, and in C this difference in growth advantage was still greater.   In both of the latter cases the growing shoot exerted an inhibiting influence over the opposite bud.

30

about this higher affinity for oxygen at one point than at another? Certainly, we do not at present know!

This unknown factor acting between the growing buds or embryos when out of equilibrium, inhibits to an unusual degree the rate of oxidation and through this probably the rate of cell-multiplication, and certainly the rate of development in one of the components. All the defects observed in the inferior component are simply due to a slowing of its developmental rate or are strictly what I have always termed developmental arrests. This problematical factor, then, simply tends to lower the rate of development in the one component and thereby does what the experimenter is able to do in various ways with any developing single individual. I ('06, '07, '09, '10 a b, '13, etc.) have experimentally produced in single embryos all of the deformities seen in the smaller components by arresting the developmental rate of eggs with a large number of different chemical and physical treatments. Newman ('17) has observed exactly the same types of monsters among slowly developing hybrids. As Newman very correctly points out, one obtains similar monsters by any method, either treating the eggs with injurious chemicals, strange physical conditions, or by heterogenic hybridization. Each of these methods simply lowers the rate of metabolism and the rate of development. Newman, in agreement with my position, recognizes all of the monsters as being primarily due to a lowering of developmental rate and, therefore, generally speaking, they are actually developmental arrests. From an extensive study of monstrous individuals, Dareste ('91) long ago believed that all developmental abnormalities were in general arrests, yet he lacked proof for such a position.

The present study, however, enables me to state the case in far bolder and more definite terms than it has been possible to do before. In the first place, *every type of developmental monster*

Fig. 30 The terminal portion of a long privet stem which had grown upward in a shaded position. On reaching direct sunlight the axillary buds three leaves from the bottom of the figure grew into lateral shoots. Very soon after this the axillary buds of the pair of leaves immediately below the first shoots give rise to the second pair of lateral shoots, and finally the buds of the leaf pair immediately above the first shoots grow into a third pair of lateral branches. In nine cases this budding sequence was invariably followed.

known in the literature may be produced by one and the same experimental treatment. For example, simply by lowering the surrounding temperature or by treating with a weak ether solution all monsters may be produced. Secondly, *the same structural abnormality may be induced in the embryos of various species by a great number of different experimental treatments.* Thirdly, *in all cases the initial effect of the experimental treatment is a lowering of the developmental rate, and the resulting deformity is always secondarily due to this slow rate of development.* Fourthly, *the type of monster or deformity is determined by the developmental period during which the slowing in rate is experienced.* An early slowing will induce the growth of accessory embryonic axes or duplicities, while a similar reduction in rate at later periods may produce anophthalmia or cyclopia, simple tubular brains, malformed otic structures, deformities of the mouth or branchial arrangement, etc., depending upon the time at which the rate of development was slow. Slowing by a number of different ways if done at the same developmental time will give closely similar defects.

We may finally state a common law of both normal and abnormal development as follows: *Structural quality may be affected by many things, but always depends directly upon the rate of development of the part or of the individual.* I have many reasons for believing that this law equally applies during postnatal growth and change in higher animals, as well as during their prenatal development.

In a study of a number of different embryos it will be observed that a particular structural modification is more common in one species of egg than in another. With trout eggs for example, duplicities more readily occur than in the egg of Fundulus. While, on the other hand, cyclopia and certain eye defects are more common among deformed specimens of Fundulus than among those of the trout. It would seem as though particular moments and localities were more susceptible to modifications in one egg as a result of slow development than in another. Further, certain eggs are in general much more sensitive than others and, as is well known, more frequently deformed. Their developmental rate is less strongly regulated than is the case in the more resist-

ant types. These facts are due to the different hereditary backgrounds on which the modifying conditions act.

Finally, if one admits the above generalizations to be true, studies and descriptions of individual monsters and deformities lose much of their interest and so-called value. It is evident from the present standpoint that a single deformed specimen, whether human or lower vertebrate, must be considered as resulting from an arrest or slowing of its developmental rate during a particular period. Observing the nature of the deformity or the parts involved enables one to estimate the developmental period during which the arrest was effective. Such individual monsters in no way supply evidence to determine what the initiating cause of the deformities may have been, since we know that the same type of deformity may be experimentally caused by many different treatments. We can estimate simply that the exciting cause acted to lower the rate of development during a definite interval.

The great number of descriptions in the literature of isolated monsters have added very little to our understanding of the causes of abnormal development. The writer believes after a prolonged study of this subject that the only benefit to be derived from examinations of such isolated specimens is possibly to obtain aid in studying the normal sequence of development. This of course is valuable and only to this extent are such descriptions worthy of record. Detailed descriptions of monsters occupy the same level of scientific value as do records of ordinary structural anomalies observed in the dissecting room.

Having stated the above conclusions derived from an extensive study of abnormalities in single individuals as well as the double specimens under present considerations, it is not deemed necessary to enter into a general discussion of the various views regarding abnormal development with which morphological literature abounds. Many of these positions have been discussed in my previous papers. I shall here only attempt to consider briefly the last contribution by Mall ('17) who devoted so much study and masterly consideration to this subject. The investigations by Mall are far the most valuable that have been made on human material.

In his last study on the frequency of localized anomalies in
human embryos and stillborn infants, the data from a thousand
specimens are recorded.

Mall's method of arranging this material may be considered
in brief in order to attempt fitting it into our above conclusions.
The material was primarily divided into normal and pathological
specimens. Some of the 'normal' may possess localized anomal-
ies, such as cyclopia, etc., and a study of the pathological group
Mall believes justifies this inclusion of localized anomalies among
the normal embryos.

The pathological group was then subdivided into seven classes
of specimens: first, those consisting of only degenerate chorionic
villi; second, of only the chorion with the extra embryonic coelom;
third, of the chorion and amnion; fourth, of the embryonic mem-
branes containing a nodular embryo; fifth, of cylindrical embry-
os; sixth, stunted embryos, and, seventh, dried and deformed or
soft and macerated specimens. The series thus begins with the
most degenerate conditions and passes on to those specimens
which maintained their integrity fairly well, though evidently
malformed and dead for some time before being aborted. Un-
questionably, all members of such a series have suffered develop-
mental arrests of the severest types. In some cases the arrest
has come at an early stage and been followed by a disorganiza-
tion or cytolysis and subsequent absorption of the embryonic
material. In guinea-pigs one frequently finds similar stages of
embryonic degeneration in utero, and here also the placenta and
membranes are the last parts to disappear. In almost all of these
cases portions of the pregnancy must have remained in the uterus
for some time after the embryo died before being discharged.

If these pathological specimens are primarily due to develop-
mental arrests, what, if any, evidence is there that conditions
may have existed which could probably have induced such
arrests? Or, is there evidence that human embryos are affected
very readily by strange conditions? Very valuable data bearing
on both of these questions are supplied. In the one thousand
specimens considered, about 33 per cent of the ova and embryos
from the uterine lot were pathological, while as many as 66 per

cent of the ectopic specimens were of this nature. The double frequency of pathological specimens in ectopic pregnancies shows at once the influence of unfavorable environment. These facts are of primary importance and Mall discusses them in a most instructive way. He states, to account for human monsters: "It would have been quite simple to conclude that the poisons produced by an inflamed uterus should be viewed as the sole cause, but when it is recalled that pathological ova occur far more commonly in tubal than in uterine pregnancy, such a theory becomes untenable." It is then stated further: "For this reason (meaning the records from ectopics) I have sought the primary factor in a condition buried in the non-committal term, 'faulty implantation.' " The faulty implantation acted to injure the development, in Mall's opinion, on account of supplying insufficient nutriment. I should be inclined to accept the faulty implantation as the primary factor, but the injurious effects of such an arrangement are due to an insufficient oxygen rather than food supply. This difference in interpretation is only of academic value. Malnutrition effects developing individuals in a general way causing a condition of undersize, while insufficient oxygen decidedly slows the rate or may completely interrupt development and thereby induces various structural deformities.

Mall in this paper is inclined to drop the cruder term 'nutrition' and admits that, "Probably it would be more nearly correct to state that change in environment has affected the metabolism of the egg." This would be entirely in accord with the interpretation of arrest as being due to lowered oxygen supply.

Again, Mall reaches significant conclusions when considered in connection with the foregoing general principles of abnormal development. For, on page 72, he states: "Accordingly, when an embryo through changed environment is profoundly affected, the development of one part of the body may be arrested, while the remaining portion may continue to grow and develop in an irregular manner. In very young embryos, tissues or even entire organs may become disintegrated, as can easily be recognized by the cytolysis and histolysis present, and the resultant disorganized tissue cannot continue to produce the normal form of an

embryo. If this process (evidently meaning disintegration) is sharply localized, for instance, in a portion of the spinal cord or in the brain, spina bifida or anencephaly results. To produce a striking result, as in cyclopia, a small portion of the brain must be affected at the critical time."

The one position with which we are entirely unable to agree is that the arrested development must so constantly be "associated with the destruction of tissue." This tissue destruction is not at all essential to the production of such defects as spina bifida or anencephaly. It may be demonstrated in many experimental cases that the tissues fail entirely to arise or differentiate without there being any indication whatever of a previous destruction.

As stated in the beginning, Mall included localized anomalies among his normal specimens, yet such anomalies occurred about twice as frequently among the pathological individuals as among the normal. This is closely in accord with what has been found for the abnormal fish embryos.

After this review of monstrous development in general, and an analysis of its causes from the conditions found in the smaller components of double individuals, we may consider in the following section the interaction, if such can be observed, among the early organs in the single individual. It may be possible that these organs are related to one another in their development in somewhat the same manner as are the components of double specimens. A further test, therefore, of the correctness of my interpretations for abnormal development may be had in an analysis of the relationships among the developing organs in the single individual.

8. THE DOUBLE INDIVIDUAL WITH UNEQUAL COMPONENTS AND AN ANALYSIS OF THE DEVELOPMENT OF ORGANS IN THE SINGLE INDIVIDUAL

By way of introduction, we may further consider certain conditions in developing plants on account of their apparent simplicity and also their very striking suggestiveness in connection with an analysis of the origin and growth of organs in the vertebrate embryo. The imaginary elements involved in comparisons of

plant conditions with animal development I very fully recognize. There is, however, evidence of certain actual similarities which, along with deductions from my experiments on embryos, may serve to elucidate the problem of organ formation to a considerable extent. Particularly suggestive is an examination of—

a. The growth influence of the apical or primary bud over the secondary and potential buds in plants

It is commonly observed that when a number of beans or other seeds are planted in a row under similar conditions of soil and moisture, the initial bud from each seed sprouts upward and grows to a definite extent and then temporarily stops. On examining the row of young shoots, each with two horizontally spread terminal leaves, it is generally found that all are very nearly of the same height. Should a certain part of the row occur in a more favorable environment than another the sprouts in this part may grow higher than in the others, or should certain seeds have been defective or their environment in the row unfavorable, the sprouts in such cases are lower and smaller than the average. These low small plants seem as a general rule unable to overcome their inferior condition during later growth and either die or form very poor specimens. The small sprouts would appear to have suffered an arrest during their early development in consequence of which they generally fail to be normally large fruiting plants.

The original shoot is entirely formed from the food contained within the cotyledons and the water of absorption. After attaining a definite length, it stops or slows its progress until the roots become sufficiently established to obtain further food and moisture from the soil. On becoming properly rooted the apical bud then grows upward from a point between the two original leaves and from this the development of the plant proceeds. We thus have an interruption, after the formation of the original sprout, similar to that found in the development of many vertebrates and from a somewhat similar cause. Here the plant could not continue to grow until certain substances were supplied by

the roots, through the assimilation of which, cell multiplication was made possible. In the birds and in the experiments with fish eggs, the initial development is interrupted by a sudden lowering of temperature and through this the chemical processes necessary for cell multiplication are slowed or stopped and development ceases. Although the stuff is available, the conditions prevent its use.

The case of the mammal is more closely analagous to that of the plant. Here the fertilized ovum within the Fallopian tube begins to develop and continues until it exhausts its initial supply of oxygen, though there may possibly be here also an exhaustion of nutriment as in the plan. Following this, the development of the embryo is either stopped for a considerable time, as in the extreme cases of the deer and armadillo, or it is temporarily interrupted or slowed until the membranes have become established or embedded in the uterus of the mother and a further source of oxygen and nutriment is thus acquired. The placentation of the mammalian ovum and the rooting of the plant in the earth as a mother, are comparable processes. Any lack of perfection in the process is either fatal or lowers the supply of necessary stuffs and thus causes an abnormally slow rate of development and growth with a resultant imperfection in structural formation.

After the original linear sprout of the plant has rooted, and a certain extent of linear growth has taken place from the apical bud, growth in length gradually slows as if the apical bud had passed beyond the point at which it could dominate the growth activities throughout the length of the plant. When this time is reached, the axillary buds at the base of the leaves are able to express their growth capacities and the plant develops its lateral branches. Though all the branches of a plant have a more or less similar function, yet each may be looked upon as an organ, and their origin and subsequent competitive growths are in many respects similar to the origin and growth of organs in the vertebrate embryo.

Such a statement of the situation in plant development is rendered further justifiable by a very common experiment. If, instead of allowing the apical bud to gradually exhaust its suprem-

acy by continuous growth, it be injured or pinched away at an early stage, the lateral buds very quickly grow out, showing their liberation from some controlling influence possessed by the apical bud. In other words, *each growing bud (also true of the embryonic organs) exerts a depressing influence on the growth of all other buds in the individual plant. As a shoot gradually ceases to grow its depressing influence also gradually ceases.*

b. The initial linear growths, subsequent lateral buds, and the interactions among the organs of the vertebrate embryo

When the first trace of the embryonic body begins to express itself in the blastodermic matrix it appears as a linear growth, the head process extending forward from the blastopore or primitive streak. This very soon becomes surrounded by, or associated with the linear outline of the arising neural folds, the beginning central nervous system.

The neural folds indicating the early nervous system are originally of more or less straight outline and their first growth is largely a growth in length. When in a given species the neural groove has attained a certain length, it then begins a series of lateral outgrowths, or branches. The first and largest of these are the two optic outpushings and after them follow in a general way, a series of bilateral outgrowths designated the three primary brain ventricles.

The initial linear origin and growth of the nervous system is very probably due to an equal rate of cellular proliferation along the entire extent, with perhaps a somewhat more rapid rate at the tip. The lateral outgrowths arise on account of an excessively high rate of proliferation occurring in a given region during a certain time. For some unknown reason the rate of metabolism, or actually the rate of oxidation becomes disproportionately high in a particular group of cells, and these begin to multiply rapidly as compared with the multiplication rate of neighboring regions, and thus a folding or outgrowth occurs to produce, for example, the optic vesicles. Since other portions of the brain seem not to be proliferating so rapidly at the same moment, it may be that

the growing optic vesicles exert a depressing influence over the growth of other parts. There is indirect experimental evidence for such a statement.

The initial moment of high cell multiplication for a particular organ outgrowth is a most critical instant in the development of this organ. Thus, if the general developmental rate of an embryo be reduced by exposure to low temperature or cutting off the oxygen supply at the time when the rapid proliferation of the optic anlage should occur, the disproportionate growth of this region is prevented, and the result of such an experiment will be either the complete suppression of eye development, anophthalmia, cyclopean eyes, monophthalamia, or some other degree of defective eyes. This result ensues in spite of the fact that after the critical moment for eye origin has passed the embryos may have been again developing at the usual rate in a normal environment. The eye has only one favorable period for its origin, its moment of supremacy so to speak, and when it is unable to express itself at this time, the opportunity is largely, if not entirely, lost. This is probably due to other organ anlagen having arrived at their controlling moments, the optic inhibition being no longer sufficient or capable of suppressing them, but they, on the contrary, now suppress the optic bud.

The arrest in development necessary for suppression of the optic vesicle must be induced in the early embryo, before the embryonic shield stage in the teleost, or before the optic anlage is at all visible in the neural plate. This I ('09, '13) have shown by a number of different experiments, and now also find to be true in case of treatments with low temperature and scant oxygen supply.

I ('09) have reported a number of experimental cases of fish embryos in which the eye was absent or was cyclopean, while the general brain structures were as usual bilateral and normal. Such specimens are viable and swim actively about. It is evident in these cases that the arrest was limited in its effect to the optic outgrowths and was no longer effective when the primary brain ventricles were forming.

Specimens have again been recorded from my experiments, and these also may be induced in a great number of ways, showing either two poorly formed eyes, cyclopia, or anophthalmia, accompanied by a narrow tubular brain. Here the arrest or slowing in developmental rate has affected the optic outgrowths only slightly in some cases, in other cases severely, but in all cases it has persisted or continued to act for a longer period and has thereby also suppressed the outgrowths which normally form the series of primary bilateral brain ventricles, hence the final narrow tubular brain. Depending, then, upon the rate of development at a given moment, we may obtain: first, as is normally the case, optic vesicles on a brain with three bilateral primary ventricles; second, no optic vesicles, yet a brain with the bilateral primary ventricles; in the third case, we may or may not obtain optic vesicles on a brain with no growth of the bilateral ventricles —a simple tubular brain (fig. 31).

We may describe the development of the central nervous system in the vertebrate embryo very simply and schematically as follows. At first a more or less straight linear growth takes place until a given length for the given species is attained, then the linear growth possibly becomes slower in rate and lateral branches or outgrowths begin to appear, first the optic vesicles and then the first, second, and third primary brain ventricles. A competitive element is involved in the origin and growth of the lateral outpushings so that should one of these fail to express itself during the usual time for such expression, it is later unable to grow out normally or may not grow out at all (fig. 31).

We know from experimental demonstration (Lewis, '04; Spermann, the writer, Leplat, '19, and others) that the optic vesicles are derived from a definitely located group of cells in the neural plate of the embryo. When they do not arise from this group of cells no other cells are capable of forming optic vesicles and they do not appear at all.

In addition to our knowledge of this definitely located optic anlage in the embryonic brain, I have now to contribute a fact of equal importance in the development of the eye which may be stated thus. When the optic vesicle does not grow out from the

brain at a definite developmental moment, it is subsequently unable to grow out and develop normally or it may be unable to grow out at all. I have definitely inhibited development during this period in a large number of experiments and have either suppressed or modified the development of the eye. It may be concluded that, *such an organ as the eye is not only derived from a*
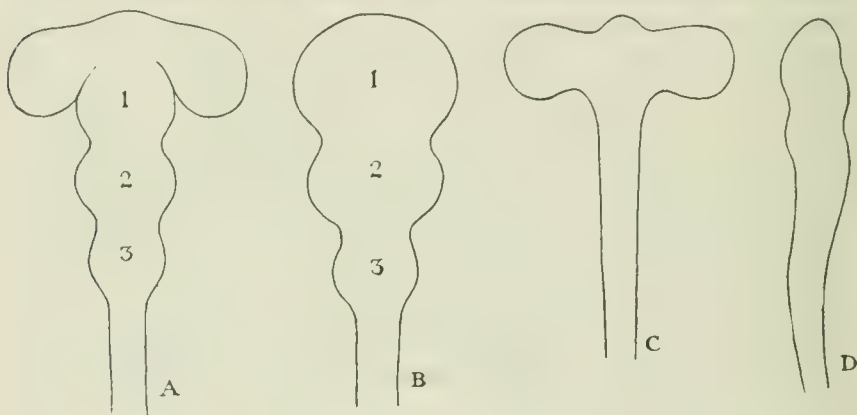
31

Fig. 31 A series of diagrams indicating modifications in the lateral outgrowths or budding processes of the anterior region of the central nervous system. A, outlines the normal case with the optic-outgrowths shown above and followed by the first, second, and third primary ventricular outgrowths of the brain. B, shows the outline of a brain in which the optic outpushings were suppressed, but the three brain ventricles succeeded in their lateral expansion. C, indicates the opposite case in which the optic outpushings were expressed, but the three brain ventricles were suppressed. This is a narrow tubular brain with eyes developed from it. D, outlines the condition of complete suppression of all lateral outgrowths, there being neither eyes nor bulging brain ventricles. A simple tubular brain.

definitely located primordia, but must also be derived during a limited moment of development.

This time-limited opportunity for origin is probably due to a growth competition between organs. The eye, not attaining a maximum growth rate at its proper moment, may permit an excessive growth to commence in a neighboring part and such a growth may then further prevent the initial growth of the eye.

There is also a possible chemical interpretation of the limited moment. The great activity and high oxidation rate of a given group of cells might result from the formation of certain specific compounds of a highly labile nature within these cells. Should the available oxygen be insufficient or the temperature be too low at the moment of origin of such molecules, they would be unable to produce the usual cellular activity, and on account of their labile nature would soon become changed. The opportunity for unusual growth activity of the specific kind on the part of the given cellular group would be lost. No doubt some such peculiar chemical process must be taking place during the different stages of cellular growth and differentiation in a complex vertebrate embryo. When such labile compounds do break down we may also imagine that a more generalized chemical condition of the cell is produced. And such cells may subsequently take part in the formation of the more general tissues and may not necessarily be lost on account of not having succeeded in giving rise to the specific tissue intended. Certainly, one does not find necrotic and disintegrating cells in all brains of anophthalmic embryos.

Ralph Lillie ('17) has described structures simulating organic growths arising from electrolytic local action in metals. He also shows the formation of filaments from one metal to be inhibited by contact with another metal. ''The inhibitory influence of zinc upon the formation of ferricyanide filaments from iron may be shown as follows: a straight piece of thin bright iron wire some centimeters long, one end of which is wound with a small strip of zinc, is placed in a 2 per cent K_2FeCy_6 solution in dilute egg-white. Filaments put forth rapidly from the zinc, especially near the iron, but the iron itself remains perfectly bright and bare, and may show no development of filaments for hours. If then the wire be cut in two by scissors, the part remaining in connection with the zinc remains unchanged, while the isolated part quickly develops the characteristic blue-green filamentous growth of ferrous ferricyanide. Evidently this growth had previously been repressed by the influence of the zinc   .   .   .   .''
Or when the zinc becomes completely covered by a growth of zinc ferricyanide the growth of ferrous ferricyanide will begin.

Such reactions resemble in general the inhibiting effects of one growing bud or organ over the growth of other buds in the plant or organs in the embryo.

The consideration up to this point has been limited to the developing nervous system and its organs. Does a similar relation of linear and lateral growths and evidence of a similar competition among organ buds exist in other systems of the embryo? And, further, is there any evidence of a wider competition between the different systems of the embryo?

The development of the foregut from which is derived a large portion of the alimentary tract in the vertebrate embryo is closely similar in many ways to that outlined above for the nervous system. The initial anteriorly directed conical evagination of the entoderm first undergoes a linear development or growth, simply becoming longer. When a certain length has been attained by this early tubular foregut, here again lateral outgrowths begin to appear, and a series of them is formed in order from the anterior end backward in much the same way as the early neural tube gives off the optic vesicles followed by the three primary brain ventricles. The first and largest of the early foregut outgrowths is the pair of mandibular pouches, in association with which the mandibular arches arise to form the lower jaw. This pair of outgrowths is soon followed by the hyoid pair and this by the series of branchial pouches associated in later development with the several gill arches. An outline scheme of these growths is simply represented by the three accompanying diagrams in figure 32.

The further development of the alimentary canal also shows in a very definite way a continuation of this process of lateral outgrowths or buds to give rise to other organs. The lungs in higher animals bud away from the floor of the entodermal canal immediately behind the branchial pouches. And again in the branchial region the thyroid and other glands arise by a definite budding process from the epithelial wall.

The development of the stomach itself is due to an excessive proliferation or diffuse budding in a limited region, giving finally the local sacculation in the otherwise narrow tube. Fol-

lowing closely behind the stomach, the canal buds off its most striking secondary growth.   This begins as an evagination following rapid cell multiplication, the excessive growth becomes too great to be longer retained by the wall and the liver pushes out, always maintaining the original connection through the bile-duct, its old stalk.   This large liver bud generally contains some cells
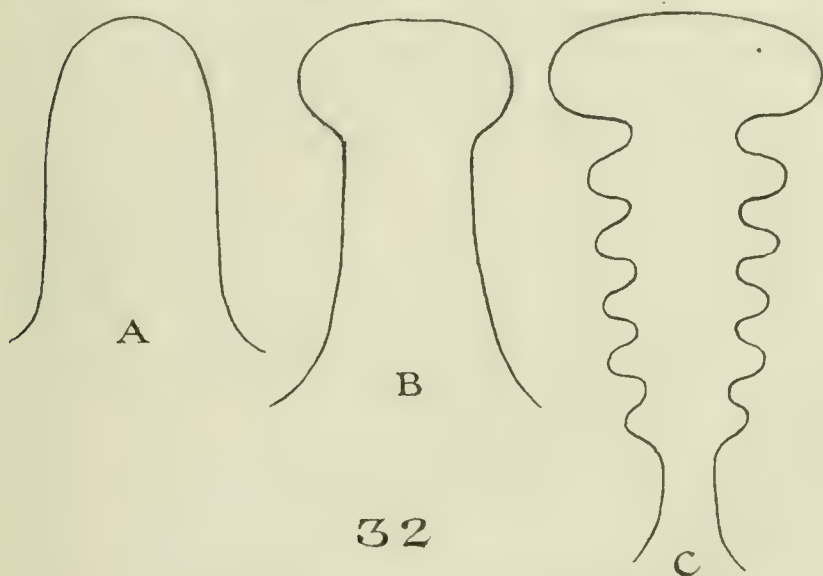
Fig. 32   A series of outlines indicating the primary linear, or cephalad, growth of the foregut, and the subsequent lateral branches or outgrowths from it.   A, outlines the simple forward growths of entoderm to form the foregut.   B, lateral outgrowths have begun from the forward end to form the mandibular pouch.   C, a series of lateral branches, following the mandibular, now grow out to form the hyoid and branchial pouches.

not exactly of its own kind, and these later begin to increase and again bud away from the wall of the bile-duct as the ventral pancreas.   Other cells of a similar kind are left in the wall of the tube, and these now grow out as the dorsal pancreas.   This is the behavior of the pancreas in higher forms, while in lower animals it may arise from more than two separate buds or may fail entirely to grow away from the tube, and remain as scattered masses of

cells in the gut wall of this locality. The pancreas in different vertebrate groups illustrates the phylogenetic steps in the development of a budding outgrowth from the wall of a linear canal.

The entire alimentary tract in the lowest vertebrates was no doubt originally a simple tube and the lateral outgrowths or buds are the highly specialized organs that have become so excessively developed as to necessitate their separation from the tube. Thus in phylogeny as well as ontogeny of the vertebrate gut it would look as though the primary growth was linear and its complexity has been added by lateral buds and offshoots.

The above being the general state of affairs in the development of the foregut, we come now to the point of experimental importance in the dynamics of these organ-forming processes. And that is, that each of the organs derived from the entodermic wall is in its critical or sensitive stage at the moment of its outgrowth, or at the time of the excessive cell proliferation in its region of the wall. Should any condition be introduced which would lower the general developmental rate, that organ will be most affected which happens at the time of the arrest to be in or nearest its critical moment. Thus an arrest during very early development will inhibit the growth of the mandibular pouch and through its malformation distort the formation of the mandibular arch, causing deformed and strangely developed mouths (see figures of the deformed fish). Since the hyoid and other branchial pouches arise so nearly at the same time as the mandibular pouch, they, with later accompanying structures, are likewise almost invariably deformed along with deformities of the mandibular structures. Such deformities as these may, however, exist in individuals with perfectly normal stomachs, livers, etc. In these cases, a normal or fair rate of development had again been established before the critical moment in the origin of the latter organs had arrived.

It would thus seem possible that an experimenter might inhibit at will the rate of development during particular intervals and thereby succeed in suppressing and deforming the mouth and branchial structures and leave the more caudally situated organs uninjured. Or, reverse the experiment and obtain normal mouth and gill structures in an embryo with suppressed and underde-

veloped liver and pancreas. I have repeatedly succeeded in performing the first experiment with Fundulus embryos by early arrests. The second experiment is more difficult and is not yet completely perfected, though among a large number of cases certain specimens arise in the experiment with underdeveloped livers, but normal mouth and branchial regions.

The experiments with the alimentary organs are more difficult than those on the eyes and brain, since the former are more difficult to observe and are not all so decidedly expressed in the young embryo. The study of the liver and pancreas must also be largely limited to examination of microscopic sections, while the mouth and branchial arrangements and the eyes and brain are very readily examined in total specimens after some experience.

The experiments on the nervous and alimentary systems as they now stand make very probable the correctness of the following proposition. The organs arise as a series of buds which bear a relationship to one another very similar to that existing among the buds of a growing plant. A given bud is dominant or has an 'advantage of position' for a limited time during which its rate of oxidation and cell proliferation is higher than that of other potential buds in the system. It grows at this moment and continues to dominate the situation until it has exhausted the advantage, when its proliferation rate decreases and another region attains the advantage and begins to bud to form another organ. If the entire embryo be depressed or has its developmental rate reduced at a moment when a certain bud is proliferating at its height, this bud is more decidedly reduced in its rate than any other portion of the embryo. On resuming a more rapid rate the other slow-going parts are able again to attain their ordinary rates, but the bud in question is unable to regain its extraordinarily high rate and therefore loses its exceptional advantage. This bud may be subsequently unable to express itself, since other parts now arrive at the stage of advantage.

The problem is then to locate the given critical moments for the several developing organs. By depressing development during a period which would cover a definite moment one might be able to suppress any given organ at will. With sufficiently re-

tined technique we could get not only embryos otherwise normal, but without eyes, without normal brain hemispheres, without normal mouth and branchial structures, and without ears, as can now be done, but also simply without a liver, without a pancreas, etc.

In many of the arrested embryos it would seem highly probable that the total number of red blood-cells in the yolk-sac and body was greatly reduced. The red cells may be considered as a diffuse organ, and this organ seems at times reduced in size as a result of arrest. It cannot be positively stated that the entire embryo in such cases is not proportionately reduced. Thus the probability of having specifically arrested the early blood formation is still questionable.

The development of one other organ, however, may be frequently interfered with by arresting the rate of development of Fundulus embryos for a time immediately following the early embryonic-shield formation. Specimens so arrested by low temperatures, treatment with alcohol solutions (Stockard, '10), or by reduced oxygen supply, often show various abnormal conditions in the development of the otic vesicle. The vesicle on one side may be absent and the other normal or poorly formed. Or the semicircular canals may not arise and only the ampullae or small cysts may represent the entire ear.

In such cases, as I pointed out and illustrated in 1910, the cartilaginous capsule representing the hard parts of the inner ear forms immediately around and exactly fits the defective membranous arrangement. The cartilage development would seem to be regulated by the membranous portion of the ear. The details of these experiments may be more fully presented when a larger number of these critical moments in organ origin are more exactly located by a further refinement of the experimental method.

It is most difficult to apply a treatment that is not permanently injurious in such a way as to have the rate of development very low at a given brief interval of time and again restored to the normal rate shortly following. The crudeness of the experiment necessitates bringing on the arrest some time before the particu-

lar moment desired and it later continues until further critical stages are interfered with. In this way, as a rule, we obtain specimens with several regions or organs deformed and rarely secure a specimen simply defective in respect to the state of a single organ or part.

In plants the conditions are far more simple, and it is possible to suppress or bring out a given bud at will. In spite of the fact that we may not understand exactly how it is accomplished, it is definitely the growth of one bud in a plant that prevents the growth of another particular one. Similarly in the embryo probably the growth of a given organ holds back the initial growth of another organ until the first organ has exhausted its power of suppression.

The two components of a double individual interact on one another in a way which would strongly support the foregoing interpretations. When the components arise in positions of equal advantage on the germ ring their interaction is balanced and both develop normally and are equal in size. When one component possesses an advantage over the other, its growth tends constantly to suppress the growth and development of its fellow, and the inferior component is, therefore, deformed and arrested in its development.

When a growing shoot of a plant, such as the common privet, has finally exhausted itself, the terminal bud goes into a dormant or resting stage and stands only a little above the axillary buds of the two uppermost leaves. After a certain interval of rest the shoot may again begin to grow, and then one of several possibilities may occur. In the first place, the terminal bud generally possesses an advantage or occupies a more advantageous growth position. It again shoots up continuing the line of the original shoot. Its advantage is so complete that the uppermost axillary buds are unable to express their growth potential and remain dormant (fig. 1, plate 5). In the second place, the terminal bud may again shoot up, but its growth is not so pronounced and it fails to completely suppress the two uppermost axillary buds. One of these being in some way more favorably located than the other, also begins to grow a shoot in a direction at an angle to

that of the shoot from the terminal bud. The other top axillary bud, however, was not so fortunate as its fellow and was not capable of overcoming the inhibiting influence of the terminal bud and so remained dormant, as is shown in figure 2, plate 5.

Finally, the third possibility very rarely occurs and all three of the uppermost buds are able to grow. In this case both of the axillary buds had a potential or tendency of growth sufficiently strong to successfully compete with the inhibiting influence of the terminal bud (fig. 3, plate 5).

We may imagine that the growth of the terminal bud in the second and third cases was not normally vigorous. For some reason the advantages of the two or three potential buds were equalized and we find twin and triplet shoots growing out. Similarly, we may imagine the potential buds around the germ-ring of the fish's egg to vary in their degrees of supremacy, ordinarily only one grows and suppresses the growth tendency of all other potential points. But if the developmental rate be slow, the one bud fails to suppress all other points on the blastoderm, and twin or triplet buds may become capable of expressing themselves.

In conclusion, these experiments and observations make it seem highly probable that influences similar to those acting between a growing plant bud and its resting buds, or between the stronger component in a double vertebrate embryo and the smaller component, are also acting between a rapidly growing organ bud and other potential organ buds in the embryonic individual. Such a conception has the decided advantage of being of practical scientific value. Since on this basis the experimenter has a logical working scheme for a study of the abnormal and through this, the normal development of a given organ. Such a method in an analysis of the development of the eye, for example, has been most valuable.

Summarizing the present status, I have succeeded in locating more or less definitely the critical moment of origin in the following individual developmental processes:

1. The growth of the primary embryonic axis: If this be slowed by arresting early cleavage stages or pregastrulation stages, the

usual single axis does not arise with sufficient influence to suppress the origin of other axes, and twins or double individuals result. Twins and double monsters are, therefore, types of developmental arrests.

2. The suppression of the eyes or modification of their structure: When an arrest is induced later than the above, but before the origin of the embryonic shield in the Fundulus embryo, all known modifications in the structure of the eyes may result in otherwise normal individuals or in further deformed specimens.

3. Suppression of the primary brain ventricles inducing subsequently deformed or tubular brains: Arrests induced before and just about the time of first appearance of the embryonic shield result in malformation of the embryonic brain ventricles. These do not express their usual bilateral outgrowths and are frequently of a simple tubular outline.

The periods of arrest necessary to induce the eye and the brain modifications are so close together or so nearly the same, that one generally finds combinations and mixtures of the defects among the same experimental group of embryos. Arrests at the earlier moment give a majority of eye conditions, many without brain involvements, while arrests at the slightly later stage give a majority of brain modifications, a few with fairly well-formed eyes. The individual variations in developmental moments among the embryos of a group also tend to contaminate the results and give mixtures of the two classes of deformities.

4. Modified or contorted mouth and branchial systems: Arrests during the embryonic shield stage, and earlier, frequently cause deformities of the mouth and branchial regions of the Fundulus embryo. In a few cases these deformities have existed in individuals otherwise normal. They, therefore, must possess a critical moment occurring at a time more or less distinct from that of the other organs. The close association of mouth abnormalities with those of the eyes is probably not due to identical critical moments of origin in the two cases, but more likely to the fact that when a slow rate of development exists during the eye moment it is rarely completely overcome and the normal rate reestablished before the critical moment for the bilateral outgrowths of the mandibular pouch is reached.

There is also probably some overlapping between the critical moments of origin for the ectodermic organs, for example, those of the central nervous system and the entodermic organs of the alimentary canal. There is, further, the possibility that the interaction or inhibiting influence existing among the buds of one system may not extend to the organ buds of a different system. I believe, however, that this is not the case, and it is more probable that the growth of each organ affects to a degree the growth of all other parts in the entire embryo. The degrees of effect exerted by one growing organ over the others may differ, for example, the growth of the optic vesicles very probably affects the primary brain ventricle growth more strongly than it does the growth of the branchial organs, etc.

5. Modifications in size and structural outline of the inner ear: Arrests during a closely similar stage to that in case 4 sometimes show their effectiveness on a different bud. In such cases the ear as well as the mouth and gills becomes affected. The otic vesicle may be completely suppressed or develop into a simple tiny cyst with no outgrowth for the semicircular canals.

6. Faulty development of the liver and pancreas: Later arrests, after the embryonic line is distinctly seen, in the Fundulus embryo may cause an abnormally small outgrowth representing the liver. Such conditions make it appear as though the primary liver bud had been inhibited in its outgrowth from the intestinal wall or the later rate of multiplication of liver cells had been reduced. The pancreas evidently arises and has its critical moment at a somewhat later instant than does the liver. Yet the two moments are so close together that it would require a very delicate difference in time of arrest to affect the one and not the other.

The findings in these six attacks on the problem make evident this very important fact: That each organ arises at a definite moment during embryonic development and not during widely different moments just as truly as that an individual organ arises from a very definite embryonic area or anlagen and from no other.

The organ defects found in nature further confirm the results of experiments on the Fundulus embryos. It is well known from

the studies by Mall ('17) and many others that localized anom-
alies are quite frequent in both normal and pathological human
specimens. The localized anomaly may involve only the eye,
only the bilaterality of the brain, only the ear, only the mouth
structures, only the kidneys (I have dissected two fetuses at
term neither of which possessed any evidence of a kidney, but
one of which was otherwise structurally normal), only the geni-
talia, etc. It is evident that such anomalies could not occur
unless there was a certain moment of specific and peculiar sus-
ceptibility on the part of each organ during which any unfavor-
able condition would act on it in a selective way. Of course, the
specific action of certain substances on certain embryonic buds
would give a possible explanation, but there is so much strong
positive evidence in the present study as well as from other sources
against this once attractive possibility that it can well be dis-
carded. A strong point of evidence is the fact that a typical
defect of one organ can be induced by a great number of treat-
ments, and different defects of many different organs can all be
induced by one and the same treatment. In every case the result
depends upon the developmental time during which the treatment
acts and not upon chemical or physical properties of the sub-
stance used. As repeatedly shown, the primary effect in all in-
stances is simply a slowing in the rate of development.

c. Developmental rate and postnatal changes

The relations between the rate of growth and particular de-
velopmental moments found in the embryo probably continue to
be of importance during postnatal development as well. The
numerous studies on inanition in rats and other mammals bear
on the same general problem as that considered in the present
report. The one important effect that inanition might have on
the subsequent history of a developing individual would be the
malformation and arrest in development of certain tissues or
parts. After birth a number of important organs and tissues
still have a considerable degree of growth and differentiation to
accomplish, and very probably the same rules apply to this activ-

ity during the postnatal period as are found to apply in the embryo. The important question at once arises as to whether there are periods during which starvation might produce no subsequent ill effects, alternating with more or less critical periods during which a similar treatment might be followed by considerably more serious results. For example, if a given treatment be administered to one group of animals from the second to the fifth week after birth, and to a similar group from the fourth to the seventh week, the results might be the same or they might be very different. The results would depend very largely upon whether significant tissue changes susceptible to variations in developmental rates had occurred during the time intervening between the two experiments.

The interaction or competition among the growing and developing organs found in the embryo certainly continues during postnatal development. The suppression of development in certain organs and tissues by the activity of another organ is splendidly illustrated by the glands of internal secretion. The further development of certain secondary sexual characters, such as hair and plumage, after subdued activity of the gonads is a case in point.

The difference in importance between two developmental moments in the postnatal individual is, however, of far less significance than in the early embryo, just as arrests during early cleavage stages are of more far-reaching consequence than similar arrests after gastrulation has occurred. Differences in developmental rate during postnatal periods incline to affect the finer features or the type of the individual rather than cause actual malformation or pathological deficiencies in the tissues. Such effects are readily observed in many of the arrested, status, and infantile human types. A fuller conception of the significance of developmental rate and rhythm in the determination of human types and appearances will be given in a separate communication.

9. CONTINUITY OF THE SERIES FROM MONSTRA IN DEFECTU
THROUGH THE SINGLE NORMAL INDIVIDUALS TO MONSTRA
IN EXCESSU AND FINALLY IDENTICAL TWINS

It has long been recognized that certain types of monsters exhibit their characteristic defect to varying degrees. The cyclopean series, for example, may present individuals not only with a single median eye, but with a bilaterally wide eye, hour-glass eyes, and finally closely approximated separate eyes. The series of diplopagi likewise exhibit all degrees of doubleness, as illustrated in plates 1 to 4.

In studying monsters belonging to these groups, Wilder ('08) went a step further and called attention to the fact that the so-called series of defective monsters passed by degrees up to the normal individual and continued from there through the excessive series on to identical twins. He was impressed by the 'orderly development' of the members in such a series and termed these individuals 'Cosmobia.' The treatment of the series as variations about the normal as a standard was a most important advance in an analysis of their structural conditions. Wilder further emphasized the important fact that monstra in defectu and monstra in excessu are both due to the same kind of cause and should be considered together in any general treatment of the subject, especially concerning cause.

However, after enunciating this clear arrangement of the problem, Wilder was entirely misled in his interpretation of the cause of these individual anomalies. The fact of their 'orderly' and symmetrical structure, and the further evident fact that normally formed identical twins represent the termination of the diplopage series, led him to consider all such forms as due to a definite germinal variation. It seemed to him more probable that orderly deviations from the normal would arise in the germ-plasm than that they should occur as a result of some modification during individual development. The burden of evidence, however, is unfortunately against such a proposition, and weighs decidedly more at the present moment than when Wilder published his account.

From what we know of germinal variations and mutations, they
do not *necessarily* give rise to individuals that gradually grade
away from the usual type.   There may be wide structural breaks
between the parent stock and the mutant.   On the other hand,
we now know that unusual environmental conditions tend to
modify the normal course of structural development to varying
degrees and give rise to the exact series of defects on which Wild-
er's conceptions were based.   The present contribution clearly
demonstrates the underlying factors and the very probable cause
of this orderly series of beings deviating from the normal indi-
vidual as monstra in defectu and monstra in excessu.

The idea is entirely correct that double monsters and twins are
due to the same cause as cyclopia.   And both may be experi-
mentally produced by an identical physical change in the envi-
ronment, lowering the temperature.   Both conditions also result
from a slowing of developmental rate, but one differs from the
other because of the difference in the developmental periods dur-
ing which the slowing in rate was effective.

10. THE NECESSITY OF A CONTROLLED OR REGULATED ENVIRON-MENT IN WHICH TO DEVELOP HIGHLY COMPLEX INDIVIDUALS

From the foregoing considerations it has become evident that
normal development of the vertebrate embryo depends acutely
upon the stability of certain factors in the environment.   Changes
in the conditions of moisture, temperature, or oxygen supply are
the most frequent causes of embryonic death as well as monstrous
development.   Any degree of actual dryness is fatal to the ver-
tebrate embryo, and sudden lowering of the surrounding tempera-
ture and reductions of the oxygen supply interrupt development
with the significant consequences discussed above.   A normal
amount of ordinary food materials is not, however, so acutely
necessary for perfect structural expression.   The rate of develop-
ment under malnutrition is slow, but the depression does not
come on suddenly nor is it often sufficiently complete to cause
serious structural anomalies.

Vertebrate animals are faced with the problem of the necessity
of a regulated environment in which to develop their eggs into

the free living individual. The lower vertebrates are almost entirely aquatic and their eggs undergo only a short embryonic development before reaching the swimming larval stage. The birds and mammals, however, at the moment of birth or hatching have, as a rule, attained a complexity of structure greater than that of the adult stage in fishes and lower forms. The period of their prenatal development is extremely long, offering far greater opportunity in time for changes in the environment and, therefore, necessitating some means of control on the part of the parent generation.

The marine and fresh-water fishes live in a more or less homogeneous medium which rarely undergoes sudden or marked changes during the spawning seasons. Their eggs are deposited in the water in instinctively chosen places during definite times when the conditions of oxygen and temperature are generally favorable for the given species. This developmental environment may in unusual cases fail in one or all respects. The water may become so stagnant as not to supply oxygen, or it may suddenly become either too hot or too cold for the welfare of the developing eggs, or in a dry season it may become evaporated or carried off, allowing the eggs to dry. The instinct of the fish helps to guard against such accidents, and the eggs are deposited at a season when the temperature changes are least likely to be harmful, and localities are chosen where the water is properly supplied with oxygen and is sufficient in amount to escape rapid drying.

The higher land-living vertebrates have no such surroundings in which to develop their eggs. In becoming terrestrial, these animals must have evolved not only appendages for locomotion on land, but also some means of controlling or providing an environment in which their long embryonic development could take place.

The eggs of reptiles and birds, as is well known, are provided with comparatively enormous amounts of food-yolk surrounded by layers of other food and enclosed in protective membranes and shell. These arrangements not only supply food, but insure a moist environment essential to all development and permit free

access of oxygen from the surrounding air. The one element essential for development of these eggs, not yet provided, is a constant high temperature. The reptiles are largely confined to warm regions and deposit their eggs during the hottest periods of the year in sand or other heated places, and in this way the proper temperature is usually provided. The birds, however, with the extremely high temperature of their own bodies, supply in a more definite way the proper amount of heat for the incubation of their embryos. Lack of moisture and oxygen very rarely causes the death or abnormal development of the eggs of reptiles and birds. But failure to maintain a uniform temperature and unfavorable degrees of heat and cold are the chief causes for embryonic mortality and deformity in these animals.

The mammals have advanced a step further in perfecting a controlled developmental environment. The internal development of the embryo not only insures a properly moist condition, but the high temperature of the maternal body is sufficiently uniform never to cause interruption of the normal progress of development. The supply of oxygen is derived from the blood of the mother through the placental circulation, and this is the one element in the mammalian developmental environment which most frequently becomes deranged. Faulty placentation cuts down the supply of oxygen to the mammalian embryo and lowers its rate of development, producing as a result prenatal death and all varieties of malformation. Yet we may well believe that the long and highly complex development of the mammalian embryo could not take place unless it was protected by a fairly well regulated environment. Abnormal development in the embryos of birds may very rarely result in nature from poor ventilation on account of a coated egg shell, but more frequently it results from failure to maintain a uniform temperature. While in mammals the temperature changes are eliminated by the internal mode of development, the one great danger to normal development still not completely controlled is the chance of a low oxygen supply brought about by a delayed or poor implantation of the placenta. The great majority of monsters in mammals are very probably due to an insufficient oxygen supply during development, and this results as a rule from faulty placentation.

The ready manner in which the structures of the developing individual are modified by changes in temperature and oxygen supply makes it evident that the existence of the species often depends upon some means of regulating the developmental environment. We may readily believe that species have been lost during evolution not only on account of failure of their adult structures to fit them for existence, but equally often as a result of failure to obtain an environment in which their embryonic development was possible.

No developmental environment in nature is constantly perfect, and this fact is the underlying cause of the frequently occurring malformations and monstrous productions.

11. GROWTH COMPETITION BETWEEN THE TWO COMPONENTS IN DOUBLE INDIVIDUALS AND THE TIME OF OCCURRENCE OF TERATOMA IN MAN

It has been clearly seen that in cases where one component of a double individual is larger because of a more favorable location, the smaller has been inhibited in its growth and development by the presence of the larger. In plants this inhibiting influence is readily demonstrated, since on pinching away a growing shoot the suppressed buds immediately spring into growth. There is much evidence to indicate that a similar interaction exists between two developing organs in a single individual. The alternating moments of rapid growth among the several organs of the embryo is a case in point.

With the preceding discussions of these propositions in view, if it be now admitted that teratoma in man often originates as a twin inclusion, we may expect an antagonistic growth reaction to exist between the teratoma and the host. In other words, while the host individual is rapidly growing, the teratoma will be suppressed and when the rate of growth of the host individual becomes slow, the teratoma will tend to grow more rapidly. If such an opinion be correct, there should be a marked correlation between the postnatal growth curve and the time of enlargement or recognition of teratomata. When the individual is growing very rapidly during the first year and a half of infancy, few tera-

tomal enlargements would be expected; following this period there is a decided fall in growth rate and the teratomata of early childhood may occur. The alternating periods of fast and slow growth should then continue to correspond with periods of few and many recorded teratomata. Dr. H. E. Himwich has undertaken a careful survey of the teratomata as recorded in the literature in order to ascertain whether any apparent relationship does exist between the time of occurrence of a teratoma and the periods of fast and slow growth rate in man. The results of his investigation are soon to be published.

12. CANCEROUS GROWTHS AND THE GENERAL CESSATION OF ALL NORMAL GROWTH IN THE OLD INDIVIDUAL

In an interpretation of the cause of cancer the fact that the condition is so much more frequent in the adult and old individual than in the young is to be recognized as of deep significance. The fact that there is an interaction and especially a growth-inhibiting effect exerted among proliferating tissues in the individual is a second point of great importance.

In the young rapidly growing and developing person almost all organs and tissues are increasing in amount through multiplication of their cellular constituents. The liver, for example, grows in actual mass until it reaches the adult size. This size, although decidedly variable in a group of individuals, has rather definite limits. The normal human liver is never indefinite or unlimited in its growth. Almost all other organs are similarly of limited size. Thus growth in general tends to cease as the body approaches its adult proportions. Finally, in the old individual, the only remaining cell proliferation becomes almost entirely confined to the germinative layer of the skin, the lining epithelium of the alimentary tract, the testes in the male, and the production of red blood-corpuscles. Even these proliferation processes become feeble with increase in age and new cells are not abundantly supplied. This is the normal course of events.

The size and proportion of parts are largely determined by heredity, but may be seriously interfered with by irregularities in the environment.

A slowing of the developmental rate at particular times may largely suppress the growth of certain organs, rendering them abnormally small in size and insufficient in their function. The normal proportion of things becomes distorted. Again it may rarely happen that one organ takes on an excessive growth and attains a size entirely out of normal proportion. There is thus a frequent lack of proper balance and adjustment among the several organs of the developing body.

The properly regulated balance among the organs is to a great extent due to the inhibiting and controlling effects of one growing region or part over other parts. This is readily demonstrated by the modifications which result in size and proportion of certain parts of the body following the experimental removal of other parts. All parts may be thought of as having more or less to do with the ultimate growth results of the whole.

On becoming adult, a state of apparent balance is maintained. Growth is considerably reduced and largely confined to the repair of natural loss and the maintenance of this state of adult balance. Under such conditions there still remains considerable regenerative powers following injuries of various kinds. Yet these regenerative processes are not so perfectly accomplished or so well controlled in the adult animal body as they were in the larval or immature condition. This fact may in some way be associated with the absence in the adult of general growth and the well-expressed regulatory processes which are necessary in the developing individual.

The regenerative growth following injuries to the adult animal may become morbid in degree and without regulation, thus giving rise to malignant conditions. Such a growth might rarely occur in the immature body, but in this case one would expect to find the growth proportions among the tissues in general to be abnormal and distorted. Thus, juvenile cancer conditions are rare and are probably associated with other deformities.

Cancer in the adult would be expected to occur more frequently in certain families, since the growth balance and proportions are hereditary characters, and on the state of these, the cancerous growth largely depends. Families or persons derived from

similar cytological complexes show more nearly similar growth
and tissue reactions than do random groups of individuals derived
from non-related parentage.

In the old individual with but little normal growth still in exist-
ence, there can be, on the basis of my interpretation, but slight
inhibition to any regenerative process that might be set up.
Such animals naturally on account of their old condition usually
regenerate very slowly, but following continued trauma, active
regenerative growths are frequently begun, and not being under
the inhibiting control of any other active growth processes, this
regeneration attains an excessive, distorted, and malignant con-
dition.

All very old animals no doubt experience a considerable amount
of trauma, and if they lived long enough almost all of them might
possess some cancerous growths.   The truth of this statement is
well illustrated by comparing the frequency of reported cancer in
rats and mice with similar growths in guinea-pigs, all constantly
used laboratory animals.   Rats are very old after three years of
life, and actually at two years old may properly be compared,
according to Donaldson ('15), with a man at sixty.   Mice attain
old age even earlier, and at two years are very old.   This being
the case, it frequently happens that the rats and mice used in
laboratories have actually become old individuals, having been
kept by the breeders and the laboratory for as long as two years.
Cancerous growths are common in these animals.

The guinea-pig under favorable conditions does not become
old until it has lived for about five years, and we have frequently
kept these animals for more than seven years; at this age, how-
ever, they are extremely old.   Thus, as a rule, the guinea-pigs
used in laboratories are really young individuals, generally less
than three or four years old.   Consequently, cancerous growths
are said to be uncommon among these animals.   However, among
the old individuals in our stock a considerable percentage of can-
cerous ones have occurred.   So it might be inferred that if as
great a number of really old guinea-pigs were observed as of old
rats and mice, cancer might be found to be almost as common
among guinea-pigs as among rats and mice.   And finally it may

be supposed that every mammal would develop some form of cancerous growth should it chance to live until extreme old age. The increased length of life in man may be associated with the increased frequency of cancer.

13. GENERAL SUMMARY

In considering the results of the present study it is necessary to recognize the fact that a given animal species passes through its embryonic stages at a specific rate of development, probably dependent upon the rate of oxidation in the protoplasm of the species. This developmental rate varies within certain normal limits; should variations in rate extend beyond these limits, the developmental result frequently becomes modified and distorted.

The rate of development is not uniform throughout the entire process, but periods of rapid progress alternate with moments of slow rate or almost quiescence. In spite of these changes in rate, development in most forms does not actually stop after it has once started, but progresses in a continuous manner until the fully formed animal is produced.

There are certain animals in which the continuous mode of development has become modified. In these forms development begins and attains a definite stage and then stops completely, to remain at a standstill for days or even weeks, until a change in the environment again permits the resumption of the developmental processes and the completion of the fully formed animal. Such a discontinuous mode of development is universal among the birds and is known to occur in several mammals.

With these points in mind, the results of the present study may be summarized as follows:

1. The continuous mode of development may be experimentally changed into the discontinuous by two very simple methods, temporarily lowering the surrounding temperature and thereby reducing the rate of oxidation and by directly cutting off the supply of oxygen.

The effects on subsequent development of interruptions caused by these methods depends upon the stage during which the interruption is introduced. There are stages of apparent indifference to a stop in development. Shortly after gastrulation is completed,

the development of the fish's egg may be stopped for a considerable length of time with impunity, no ill-effects resulting. This is the developmental moment at which the bird's egg is normally stopped on account of the fall in temperature experienced after passing out of the mother's body.

There are other stages during which a temporary interruption of the developmental processes will be followed by most disastrous effects. These critical stages are usually moments during which marked inequalities in rate of cellular proliferation are taking place in different portions of the blastoderm or embryo. The period preceding the process of gastrulation is just such a critical moment.

2. There are considerable differences in effect between greatly reducing the rate of development and actually stopping the process temporarily. The development of certain eggs may be slowed down to one-tenth or one-twentieth of the usual rate and be maintained in such a slow condition for days without the majority of specimens losing their power of regaining the normal rate and giving rise to structurally perfect individuals. If at similar stages the development of the same eggs be completely stopped instead of slowed down, they are in many cases unable later to resume the process and die, in other cases they may resume development in a most abnormal fashion, or finally a few may be capable of resuming the apparently normal process.

This difference in results between a severe reduction in developmental rate and an actual temporary stop is to be explained as follows: Slowing does not completely eliminate the normal inequalities in rate of developmental change existing among the several parts. Those parts that were in states of rapid development are depressed in the same proportion as other parts that were developing more slowly and inequalities in rate still exist in the slow-going embryo. When such specimens are allowed to resume a faster development the several portions of the embryo are able again to maintain normal differences in developmental rate and a proper balance is assured.

A complete stop in development reduces the rate of all parts to zero and eliminates normal inequalities. On resuming develop-

ment from such a state, parts that should progress at a disproportionately fast rate are unable to attain such supremacy and all portions of the embryo start at about the same rate. The usual developmental balance and inequalities in rate among the parts are lost and thus the typical form of the individual which actually depends upon these inequalities in rate of growth becomes modified.

3. The types of deformities following a stop in development as well as those occasionally resulting from a slowing of the rate are similar to the defects produced by all experimental methods. Practically any deformity recorded in the literature other than those resulting from germinal variations or mutations may be induced by lowering the temperature and thus modifying the developmental rate.

4. By an interruption of development during late cleavage stages a considerable percentage of twins and double individuals may be produced. When the eggs of the sea-minnow, Fundulus heteroclitus, are subjected to temperatures of 5° or 6°C. during cleavage stages, development is almost stopped. On returning such eggs to a summer temperature, after several days' sojourn in the refrigerator, there will follow a high mortality, but many specimens will resume development producing a significant percentage of twins and a number of variously deformed conditions along with a good proportion of normally formed young fish.

Arresting or stopping development of the same eggs during the same developmental stages by diminishing the available supply of oxygen will be followed by closely similar results.

The eggs of the trout are naturally much more inclined to develop into double individuals than are those of Fundulus. When the oxygen supply during early development is not abundant, a great many twin and double trout specimens are frequently found to occur.

All of these double conditions result from arrests during very early stages of development, invariably before the process of blastopore formation has in any way begun. No duplicities or twins have been found to occur among the great numbers of fish eggs which have been arrested during postgastrular stages of development.

5. The bird's egg is usually laid, according to investigations on this subject, after the process of gastrulation has commenced. Yet double chick embryos are not uncommon among the developmental stages observed in the laboratory, although in nature such specimens almost never exist at the time of hatching.

The cause for the double chick embryos is the same, I believe, as that indicated above in the case of the double fish. Although the great majority of hen's eggs are laid and their development stopped by the fall in temperature after gastrulation has begun, still it is recognized by those who have investigated the subject that there is considerable variation in the developmental stages of the eggs at the time of laying, and a minority of eggs are laid before gastrulation has begun. When an egg in this stage is stopped by the fall in temperature following laying, it would be expected from the experience with the fish that just such eggs would frequently give rise to two points of gastrulation and two embryonic fundaments instead of one. The interruption in the process of development at this critical time and the resumption of development at an equally slow rate in all regions of the blastoderm, permits more than one potential embryo-forming region to express itself. The interruption at this particular moment is the very probable cause of twin and double specimens.

6. Polyembryony in the armadillo is in all probability explainable on a similar basis to the cases above. Development begins as in most other mammals in the fallopian tubes and continues until the egg passes down into the uterus as an early blastocyst. Development then stops in the armadillo for a period of several weeks with the blastocyst lying free in the uterus, as Patterson ('13) has reported. The stop here is not due to a temperature change, since none has occurred, but is very probably on account of an exhaustion of the original oxygen supply derived from the ovarian blood. The uterus fails to react immediately to the presence of the blastocyst, implantation is delayed, and no means of obtaining oxygen necessary for continuing development is possible until the egg becomes implanted. After the delayed implantation has taken place, development is slowly resumed in a way which gives rise to multiple embryo formations or budding, as

has been fully considered above. The 'quiescent period' in the armadillo egg is probably the result of lack of oxygen and thus the cause of polyembryony.

Twinning or polyembryony may be considered a typical method of asexual reproduction, and its occurrence in mammals and other vertebrates makes the phenomenon of so-called 'alternation of generations' universal among animals.

7. The degree of duplicity in double individuals depends upon the original distance apart of the embryonic buds on the blastoderm.

The relative sizes of the two components in double specimens vary widely. In many double individuals the two components are practically equal, while in others one component is of normal size and the other component in a series of specimens varies from slightly below normal size down to a very small mass. This size difference between components is in no way associated with the degree of duplicity.

8. In double individuals in which the two components are equal in size they are both normal in structure. When the two components of a double specimen are unequal in size, the larger component is almost always normal in structure, and the smaller component is always deformed. The degree of deformity in the smaller component varies directly with the extent of difference in size between the two components.

9. As the large component reaches adult size the lesser component may have become so relatively small as to be represented by a nodular mass on the body of the larger, or it may be lost to sight entirely as a twin inclusion. Such conditions make it evident that doubleness and twinning are actually more frequent than records would indicate.

10. The types of defects and the degree of deformity exhibited by the smaller component are exactly similar in kind and degree to the deformities found among single individuals. This fact renders the double individual with unequal components a most valuable key to an understanding of the cause of all monstrous development. The two components are from identical germinal origin and are developing in organic connection in exactly the

same environment, yet one is structurally perfect while the other smaller member presents all types of deformities. The difference between the two is in their developmental rate, the larger having a normal rate and the smaller progressing more slowly and in an arrested fashion. The depressed state of the one component is the result of an inhibiting influence exerted by the other.

11. The deformities of the small component in the double individuals and the similar defects induced by stopping the development of single individuals make it evident that all developmental monstrosities are the results of simple arrest. During my experiments with Fundulus eggs it has been possible to induce a single type of defect with a great variety of different experimental treatments. The reverse is also true; all varieties of defects may be induced by subjecting the embryos to one and the same experimental treatment.

The primary action of all the treatments is to inhibit the rate of development, and the type of deformity that results depends simply upon the developmental moment at which the interruption occurs. All monsters are the result of the same cause, and the type of monster depends upon the time at which the cause was in operation.

Several developmental moments have been located at which rather definite defects of particular organs may be induced. These are the moments during which the organs are in their most rapidly proliferating condition. Arresting the rate at such a moment gives decidedly injurious results. When an organ is developing at a slow rate the arrest fails to affect it.

12. The development and growth of organs in the single individual are interrelated in a way similar to the interrelations between the components of a double specimen. When one organ or one component has a higher rate than another, it develops at this rate for a limited time and tends to inhibit development on the part of other organs. This is readily demonstrated by the inhibiting effect of the growing shoot over all the potential buds of a plant. When the growing tip is pinched away, the inhibited buds immediately express their capacity to grow. There is much evidence to indicate that a similar interaction exists among the developing parts of an animal embryo.

13. The initial growth giving origin to an embryonic system, such as the brain and spinal cord, is linear in type, until a definite length is attained when linear growth subsides. This is followed by a series of lateral outgrowths in consecutive fashion. These lateral outgrowths from the central nervous system may be experimentally suppressed by slowing development at definite times, and when all are absent a simple tubular brain is the end result. The same plan of development holds for the foregut and its lateral outgrowths to form the mandibular pouch, etc., and the development of this system may also be modified in a manner similar to that mentioned for the brain.

14. Monstra in defectu and monstra in excessu, which have frequently been treated as such distinctly different classes of conditions, are as a matter of fact closely similar. Both classes of anomalies are due to a common cause and may actually both exist in the same specimen. For example, an arrest of development before gastrulation may cause a blastoderm to form two embryonic processes which later develop into a double-headed individual—a typical monstrum in excessu. At a very early stage one of these embryonic processes may become inhibited and later form a cyclopean eye instead of the usual two lateral eyes; this head is then a typical case of monstrum in defectu. The fact that the normal individual stands between these two arbitrary classes of monsters has no other significance than that the monsters themselves are simply modifications of the normal condition resulting from an unusual reduction in the rate of development during certain critical periods.

15. The great importance of developmental rate in influencing the type and quality of structure is not confined solely to embryonic development, but postnatal development, and structures are similarly influenced by the rate at which the processes are accomplished. This phase of the subject is to be presented in a subsequent communication.

16. In view of experimental results, it becomes evident that normal development of the vertebrate embryo depends acutely upon the stability of certain factors in the environment. Changes in the conditions of moisture, temperature, and oxygen supply

are the most frequent causes of embryonic death as well as monstrous development. The existence of the species may frequently depend upon some means of regulating the developmental environment. Species may be lost during evolution not only on account of failure of their adult structures to fit them for existence, but equally as a result of failure to obtain an environment in which their embryonic development is possible. The highly complex forms, such as birds and mammals, with a long embryonic period have partially succeeded in controlling their developmental environment. But in no case is the regulation constantly perfect and this fact is the underlying cause of frequent malformations and monstrous productions.

17. The double fish specimens with unequal components and the growth reactions between these components are important in connection with certain teratomal conditions in man. If teratoma in man frequently originates as a twin inclusion, we may expect an antagonistic growth reaction to exist between the teratoma and the host. While the host individual is rapidly growing the teratoma will be suppressed and when the host slows its growth the teratoma should tend to grow more rapidly. There should thus be a correlation between the postnatal growth curve and the time of enlargement or recognition of teratomata. Dr. H. E. Himwich has undertaken a survey of this subject which will soon be published.

18. The interaction between the growing organs of a developing individual has been discussed in its relation to regeneration and cancerous growths of old age. In the old individual with but little normal growth still present there can be but slight inhibition to any regenerative process that may be set up following a continued trauma.

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PLATE 1

EXPLANATION OF FIGURES

A series of young trout that started development with a slightly insufficient supply of oxygen. The series begins with an ordinary single individual and passes through increasing degrees of anterior duplicity, shown in the two upper rows. It then continues with specimens showing step after step of completely formed double bodies and tails and finally ends with perfectly formed identical twins, in which both members of the pair are equally as large and perfect in structure as is the first single individual.

The photographs were all made at one magnification and show as nearly as possible the dorsal aspect of each specimen. On careful examination it will be found that in every specimen the two components are practically identical in size, and when the anterior halves are considered all heads are found to be normal in structure.
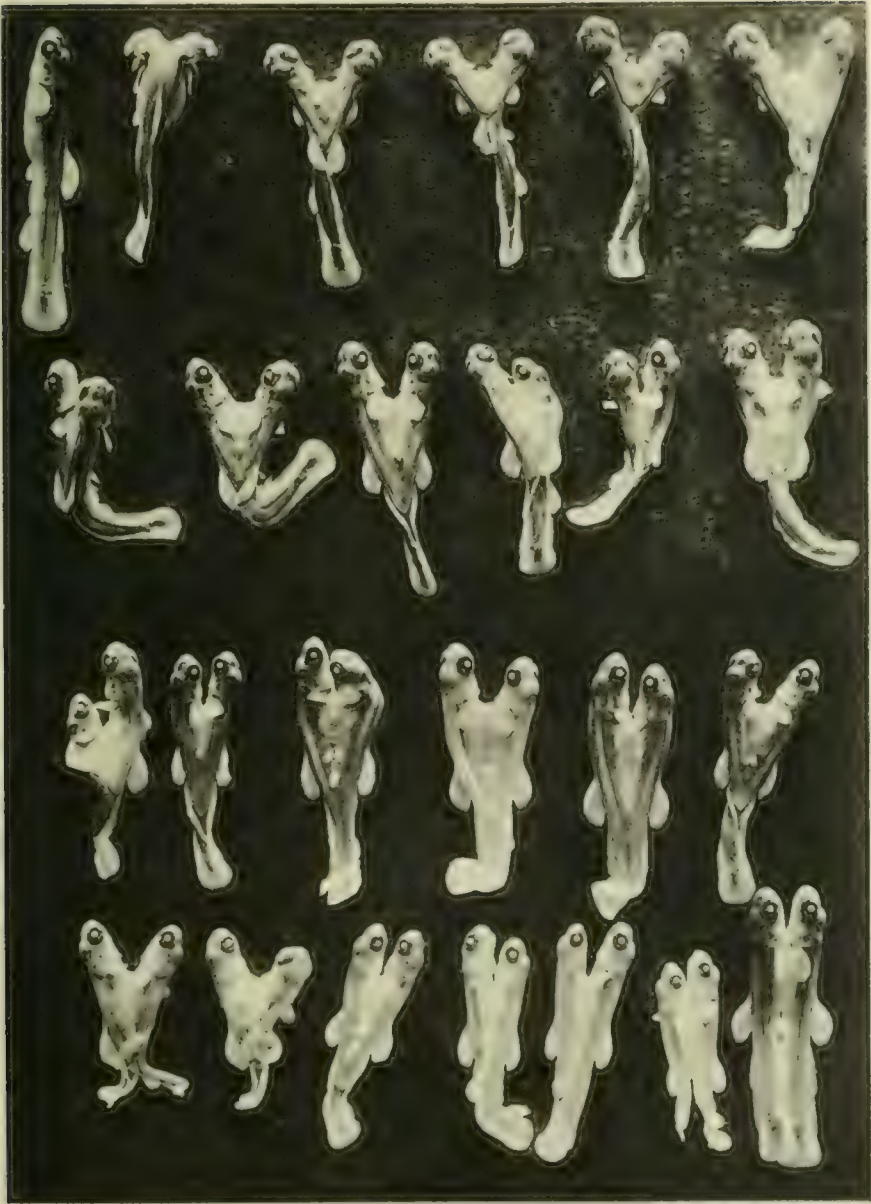
The same series of trout specimens and photographed in exactly the same order as illustrated in plate 1. The individuals are here shown from as nearly as possible the ventral aspects. Selecting any given specimen and comparing its dorsal and ventral surfaces, as shown in plates 1 and 2, it is clearly seen that in all cases the two components are equal in size and both are structurally normal. These are not 'double monsters,' but perfect individuals. The condition of doubleness is unusual, but not deformed or monstrous. The identical twins could not be considered monsters, and they only differ in degree of doubleness from the other members of the series.

EXPLANATION OF FIGURES

Two degrees of duplicity in human individuals. The upper photograph illustrates a doubled condition extending superficially only to below the shoulders, but internally the doubleness extends to the sacrum in the skeleton and to the lower ileum in the intestine. The lower photograph shows two complete babies extensively united by their ventral walls.

In both of these specimens the components are of equal size and their structures are normal throughout. In all specimens of human duplicities examined or found recorded in which the components were of equal size they were both structurally normal.

PLATE 4

EXPLANATION OF FIGURES

Drawings of male identical twins. These specimens were 'stillborn' after seven months' gestation. They were inclosed in a common chorion, and in addition have identical characters of germinal origin which make it practically certain that they were derived from a single egg. There are six fingers on all of the four hands and six toes on the four feet. Polydactylism is so variable in expression that it is scarcely possible that two brother individuals would exhibit it to exactly the same degree unless they were of identical origin. All of the kidneys are cystic, but the left in each is the larger and has the more exaggerated cystic condition. Both babies have meningoceles protruding from the back of their heads. The right individual is slightly the larger, but the hereditary characters as well as the developmental arrests are identical in the two.
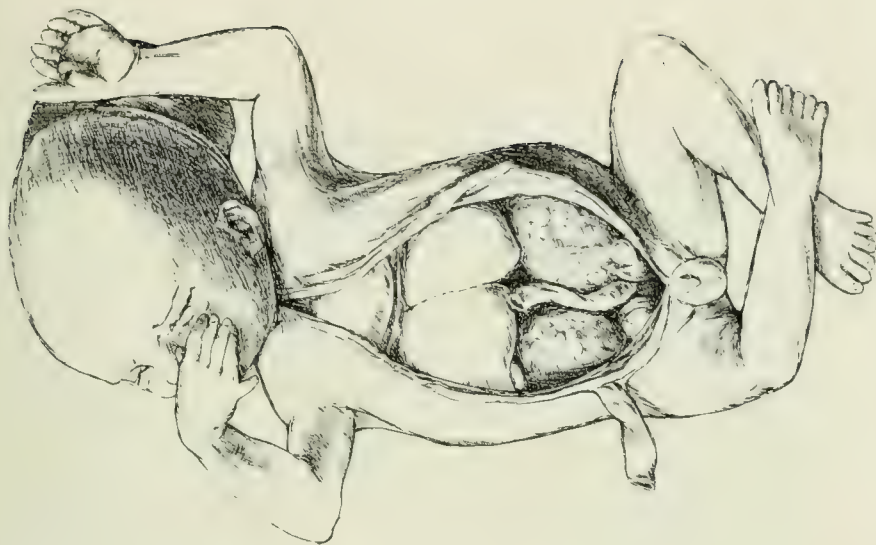
PLATE 1

PLATE 5

Photograph of a specimen delivered by Dr. Frank Erdwurm of New York. A living female child was attached to the cord leading away from the upper placenta. This baby was enclosed within its own chorion, the upper membranes shown in the picture. The two fetuses of about six months' development shown below were enclosed in a common chorion and their cords are attached to a common placenta. These are identical twin girls. About three months before birth their cords became so twisted that the placental circulation was cut off and they died. The mother was disturbed for a time until the uterine situation became adjusted and this placenta shunted off. The fetuses remained enclosed within their membranous sac and at birth were considerably shriveled and somewhat macerated.

Probably the implantation of the single individual in some way delayed implantation of the other placenta and caused the arrest which resulted in the twinning of the second egg.

PLATE 6

EXPLANATION OF FIGURES

Outline drawings of terminal branches from the common privet.

Fig. 1   A branch in which the terminal bud is in a resting state.   The common condition following a limited period of growth.

Fig. 2   A new shoot grows from the apical bud, all of the axillary buds at the base of the leaves remaining in a resting state.   This is the usual manner of resuming growth.

Fig. 3   An unusual case in which two shoots grow out after the resting period, the usual one from the apical bud and the second one from the upper right axillary bud.   The potential impulse to grow on the part of the axillary bud was as great as that of the apical bud and twins developed from the two potential growth points.

Fig. 4   The very rare case in which not only the apical bud grows into a shoot, but shoots also grow from both upper axillary buds.   Here all of the upper potential growth points produce individual shoots, and 'triplet' branches arise.
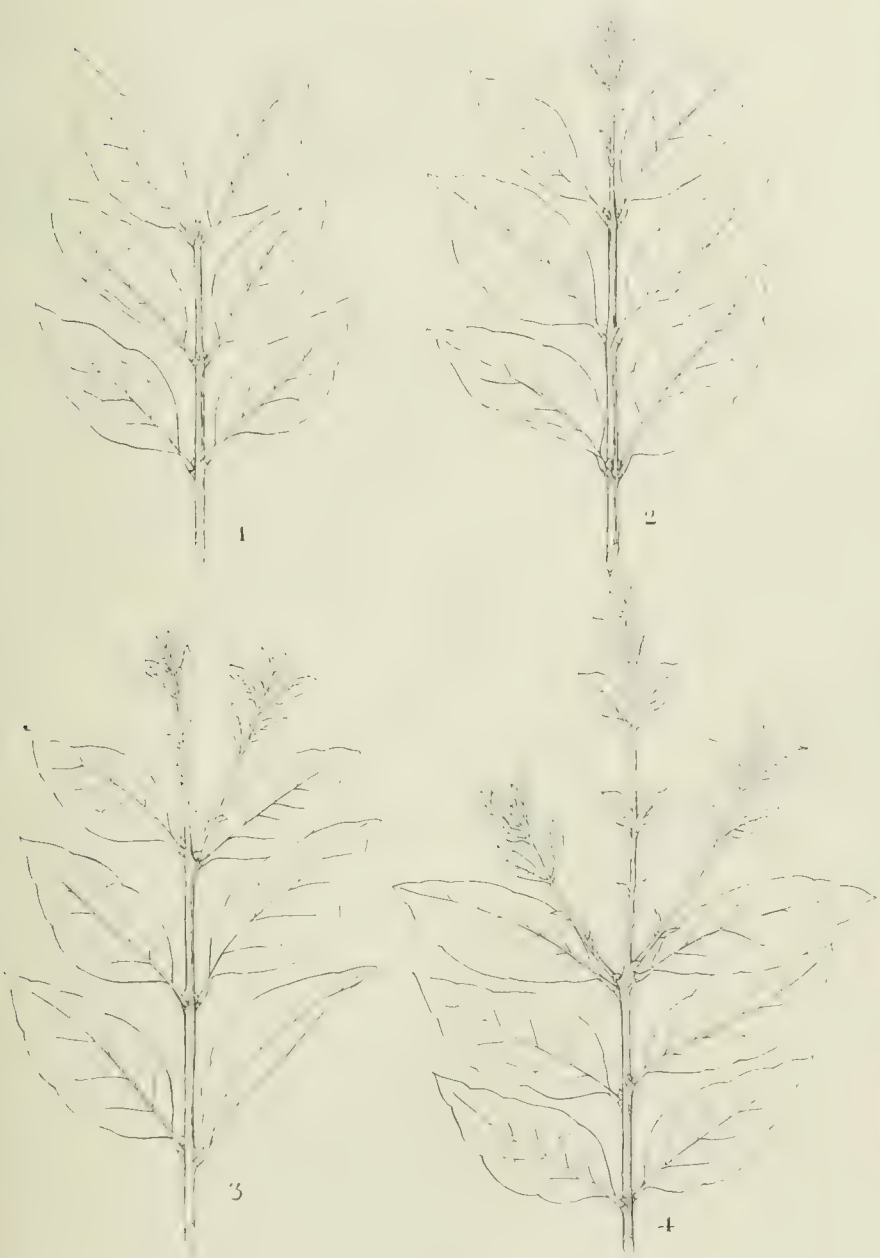
A PROBABLE EXPLANATION OF POLYEMBRYONY IN THE ARMADILLO

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By arresting the development of the fish's egg during early stages double individuals and twins are frequently induced. The interruption or arrest makes it possible for more than one potential growth point along the germ-ring to give rise to an embryonic shield. In other words, accessory invaginations or blastopore formations occur as the initial structural step in doubleness. The interruption in the development of the fish embryo must be introduced during the cleavage stages and before gastrulation in order to produce such phenomena. Among hundreds of eggs arrested during later developmental stages no double monsters or twins ever occurred. A complete account of these experiments is soon to be published but for our present purpose two facts are important: First, accessory embryo formations result from arrests in the developmental process; and second, the arrest must occur before gastrulation has taken place.

In the light of these experiments it has seemed possible to interpret somewhat more clearly than has formerly been done the remarkable phenomenon of multiple embryo formation in the armadillo.

On examining the uterus in two pregnant specimens of a South American armadillo von Jhering, in 1885, discovered that each contained eight fetuses enclosed within a single chorion. He correctly concluded that all of the fetuses in each mother had been derived from a single egg by some process of division into separate embryonic rudiments. After this valuable discovery and interpretation, the study of the armadillo's development lapsed and nothing of importance was added for almost twenty-five years. Two series of investigations were then begun simultaneously one on the Texas armadillo by Newman and Patterson,[1] and the other on the South American species by Fer-

[1] H. H. Newman and J. T. Patterson, *Jour. Morph.*, Vol. 21, p. 359, 1910.

nandez.[2] The growth and expansion of these twin studies has
brought our understanding of the phenomena of polyembryony
in the armadillo to a considerable state of maturity.

These authors readily agreed that in most species of armadillo
the individual members of a litter, usually four in the Texas
species and eight in the common South American form, are all
derived from a single egg. It required considerable effort, how-
ever, to obtain the material that would furnish the morphologi-
cal stages of the process by which the polyembryonic development
was accomplished. We are finally indebted to Patterson,[3] for
the very thorough and satisfactory manner in which he has col-
lected and studied the early embryonic conditions; and particu-
larly for having shown the first stages of the budding process
through which the single blastocyst gives rise to four distinct
embryonic areas, each exhibiting a typical primitive streak
region.

In connection with the fish experiment it now becomes im-
portant to ascertain exactly what degree of development has
been attained by the armadillo blastocyst at the time the bud-
ding process begins. And since, according to my interpretation,
these buds should arise at the time of gastrulation or blastopore
formation, it becomes necessary to consider very briefly the
germ-layers and gastrulation in mammals. The decidedly pre-
cocious and highly modified method of forming the primary
germ-layers in the mammalian blastocyst is not strictly com-
parable to gastrulation or the method of germ-layer formation
found among the other vertebrates. On the other hand, the em-
bryonic line or primitive streak of the mammalian egg is ex-
actly comparable to the blastopore and head process formation
in the simpler forms.

The blastocyst of the armadillo has already, by a process of
cell migration and delamination, separated off the primary ento-
derm from the ectoderm and further modified these layers be-
fore the budding which forms the embryonic primordia has
begun. The primordia are first formed by a thickening of the
ectodermal layer of the blastocyst. The primary entoderm then
invaginates into the primordia to form the secondary entoderm
of the gut. The precocious cell migration and splitting into
layers in the mammal's egg is associated with the early implanta-

2 M. Fernandez, *Morph. Jahrb.*, Bd. 39, p. 302, 1909.

3 J. T. Patterson, *Jour. Morph.*, Vol. 24, p. 559, 1913.

tion of the embryo upon the uterine-wall of the mother, and the later primitive streak formation may be interpreted as related to the actual gastrulation or blastopore formation away from which the line of the embryo always develops.

Whether the validity of the above briefly outlined interpretation of the germ-layer formation is admitted or not, we have in the armadillo a process of budding taking place from the blastoderm and associated with accessory or extra blastopore formation in much the same way as are the accessory embryos along the germ-ring in the egg of the bony-fish. These buds also accord with Kopsch's description of a double gastrular condition with two blastopores in a blastoderm of Lacerta agilis, from which he concluded that twin formation as well as anterior duplication arises from a double Einstülpungen. And further, Assheton has described a similar condition in a blastodermic vesicle of the sheep. He, however, imagined the condition to have been due to a splitting during the morula stage.

The double primitive streaks in the hen's egg and other forms all lend themselves to strengthen the interpretation that double embryo formation first asserts itself by a double gastrulation or blastopore formation, which is initially a process of double instead of single bud formation. Patterson's description of the origin of the quadruplet buds in the Texas armadillo furnishes the most striking case in the study of these conditions. And we may conclude that the budding or accessory embryo formation in the egg of the armadillo is exactly the same developmental process as that which gives rise to twins and double individuals in other vertebrate eggs.

However, the very important question yet remains to be answered. Why does this accessory bud formation occur so constantly in the Texas armadillo in contrast to the single embryo formation of mammalian eggs in general? Patterson failed to answer this question, but he supplied some very significant data which Newman,[4] has appreciated as being intimately connected with the occurrence of polyembryony.

In connection with the collection of material Patterson[3] discovered a "period of quiescence" of the embryonic blastocyst. Regarding this he states:

The fact was first made apparent in 1911, when, after I had started collecting two weeks earlier than in the preceding year, I failed to

[4] H. H. Newman, "The Biology of Twins," Univ. of Chicago Press, 1917.

obtain the cleavage stages, although judging from the condition of development in the vesicles collected in previous years, one would naturally expect to find these early stages during the period of my first collection in 1911.

The following year he began collecting still two weeks earlier and again had a similar experience.

Practically all of these vesicles lie free within the uterine cavity, either in the horizontal groove or in the region of the attachment zone (placental area).

It is evident from these data that the embryonic vesicle remains for some time lying free within the uterine cavity. Just how long this period lasts, I am unable to state; for practically every old female taken at the earliest date (October 15) at which I have collected, possesses a free blastocyst. . . . Taking all the facts into consideration, I estimate the "period of quiescence" to last about three weeks; that is, from about the middle of October to the third or fourth of November.

In a study of sections no mitotic divisions were found to occur in the blastocysts during the "quiescent period."

The only point of interest cited by Patterson in connection with this peculiar phenomenon of interruption in development, was the fact that in no other mammal, except the deer, had such a condition been found. Bischoff had long ago, 1854, reported a "period of quiescence" lasting for some weeks during a so-called morula stage of the deer embryo.

Newman[4] has recognized the importance of Patterson's discovery of a "quiescent period" during the early development of the armadillo, and states in a discussion of twin formation that this "period of quiescence" probably, "holds the clue to the physiological explanation of polyembryony." In this position Newman is, in my opinion, largely right, but this is as far as the data led him, and he finally remarks:

The problem is to locate the factors responsible for the slowing down of the developmental rhythm. Whatever these factors may be, and we have no definite knowledge of them, the result of retardation is polyembryony.

Newman thus fails to appreciate the second point in Patterson's discovery, and that is that the blastocysts always lie free in the uterus during the "period of quiescence." This fact enables us to go one step further since the lack of attachment and, therefore, lack of oxygen supply are very probably "the factors responsible for the slowing down of the developmental rhythm."

The armadillo egg like that of most mammals undergoes its early development in the fallopian tube and is, therefore, capable of reaching the blastocyst stage on its initial oxygen supply. After this time, however, it must become attached to the uterine wall for a further source of oxygen. For some reason in the armadillo the reaction between the blastocyst and the uterine wall is postponed, and the blastocyst is incapable of further developmental progress until this reaction is established and the necessary supply of oxygen becomes available. In exactly the same way the development of the blastoderm in the fish's egg is experimentally retarded or stopped by reducing the available oxygen supply and is again made to resume its development by supplying oxygen. In the case of the fish egg, the supply of ordinary nutriment is certainly not involved, and reactions similar to those of the armadillo egg are only obtained as responses to changes in temperature and rate of oxidation.

In the armadillo egg I also do not believe the retardation is of the nature of a starvation phenomenon, since we see nothing of the kind in other forms. Temperature changes are ruled out, since the temperature of the uterus is more or less constant. The absence of oxygen necessary for the energetic process of cell division, is, therefore, in all probability the arresting cause, and the retardation results in polyembryony.

Thus Patterson has found the developmental interruption to exist, and he has also shown the blastocyst to be disconnected from the uterine wall and its necessary oxygen supply during this time. However, he has furnished no data bearing on the reason for the delay in uterine reaction and the consequent failure of immediate implantation of the blastocyst such as normally occurs in other mammals. However, from what is known of the dependence of uterine reactions on conditions in the ovary (Leo Loeb,[5] Stockard and Papanicolaou[6] and others) it may very probably be that some peculiarity in corpora lutea formation is primarily responsible for the entire series of reactions leading to polyembryony in the armadillo.

The consideration of the armadillo egg up to this point has taken account only of the external factors influencing its mode of development. It must now be remembered as a fact of serious

[5] Leo Loeb, *Jour. Morph.*, Vol. 22, 1911.

[6] C. R. Stockard and G. N. Papanicolaou, *Am. Jour. of Anat.*, Vol. 22, 1917.

importance that the production of quadruplets from the single egg of the Texas armadillo is an almost constant occurrence, while the experimental attempts to produce twins and double individuals in fish eggs and other forms have given at best only small percentages of such individuals among the large groups of eggs treated. It is also a fact that all eggs do not furnish equally favorable material for artificial twin production. The eggs of the trout seem unquestionably more disposed to give rise to twin formations than do the eggs of Fundulus. Thus some eggs would seem to have a hereditary or truly innate pre-disposition towards polyembryonic formations. There is much reason to believe that aside from the external factors discussed, the armadillo egg itself is highly disposed toward the formation of accessory embryonic buds.

There is the possibility, of course, that this natural experiment with the armadillo egg has become so exactly regulated as to influence the developmental processes precisely the same way each time, yet this is highly improbable. The armadillo egg is not a case of simple twin growths from the blastoderm, but as Patterson finds, there are primarily two buds, and then very promptly two secondary ones arise making the four and after this the budding process ceases. In the South American species, however, it would appear as though a tertiary budding occurred giving the usual eight embryos; and in rare cases still another budding occurs from a few of the existing buds giving a total of as many as twelve. It would certainly seem as though the blastoderm in these species passes through a stage of agametic reproduction or budding of a nature unknown among other higher vertebrates. But the possibility for such expression might only exist on account of the delay in implantation of the blasto-cyst and consequent shortage of the oxygen supply necessary for the rapid formation and growth of the single embryo.

It is important to keep in mind that there are species of the armadillo which produce only a single offspring from one egg. It is not known whether their embryos have a ''period of quies-cence'' but if they have, the period either occurs at a different developmental stage or the eggs do not possess the inherent budding tendency of the other species.

We have further to acknowledge the fact that although the egg of the deer has a ''period of quiescence'' during its development it does not give rise with any degree of frequency to twin indi-

viduals. In the first place it is entirely uncertain from the
scanty accounts as to what time in development the quiescent
period occurs. Assuming that such a period does exist, it might
occur at some indifferent stage when no peculiar result would be
expected, for example after gastrulation, as it does in the bird
with no subsequent effect. In the light of the experimental
production of double individuals it is readily understood that
even though the egg of the deer is interrupted in its development
at an early stage, it might still be capable, on resuming develop-
ment, of giving a normal single embryo. A study of the experi-
mental production of twin and double individuals among fish
leads one to be surprised at the case of the armadillo, and to
expect the reaction found in the deer. The constant interrup-
tion occurring in the development of the birds and other animals
at indifferent developmental moments with no subsequent ill
effects, renders commonplace the fact that the deer successfully
withstands an interruption during its development without
noticeable modifications in structural response. A full considera-
tion of the different results following interruptions at critical
and indifferent developmental moments will be published in a
forthcoming number of the *American Journal of Anatomy*.

In conclusion we may summarize the cases as follows: The
development of the armadillo is interrupted on account of a
failure to become promptly implanted on the uterus and a con-
sequent exhaustion of the available oxygen supply. The inter-
ruption occurs at a critical period just preceding the primitive
streak and embryonic line formation. This egg appears to have
a decided tendency under conditions of arrest to form accessory
embryonic buds. As a result of the interaction of these external
and internal forces polyembryony is produced.

In the case of the deer only one probable fact is known, and
that is that a "period of quiescence" occurs. It is uncertain at
what stage the arrest takes place but it is probably due as in the
armadillo to a delayed implantation of the blastocyst. Either
on account of the stage of arrest, or a lack of tendency to form
accessory embryo-buds a typically single individual arises from
this egg. The external factors may be the same as in the case
of the armadillo, but they interact with different internal factors
or different developmental moments to give a very different
result.

77 (1537)

Effect of underfeeding on ovulation and the œstrous rhythm in guinea-pigs.

By George N. Papanicolaou and Charles R. Stockard.

[*From Cornell University Medical College, New York City.*]

Under well-regulated food conditions the œstrous cycle in the guinea-pig is almost uniformly 16 to 17 days in duration.

Underfeeding with a diet of 20 grams of carrots per day produces a prolongation of the diœstrum and, at the same time, a congestion in the ovary and uterus and a degeneration of developing graafian follicles.

The extent of prolongation of the diœstrum depends upon the stage at which an animal is underfed.

Underfeeding during the first 5 to 7 days of the diœstrum has only a slight effect, postponing the next œstrus for one or two days, while underfeeding during the later part of the diœstrum gives much more marked results.

When an animal is underfed for 5 days, from the 12th to the 17th day after an ovulation and œstrus, the next ovulation and œstrus is delayed for about 7 days, being expressed at the 23d to 25th day instead of at the 17th.

Should an animal be underfed for 7 days, from the 10th day to the 17th day after œstrus, the next ovulation and œstrus is postponed for 10 to 11 days, arriving at the 27th to 28th day, instead of the 17th day.

This variation in the effect of the underfeeding when applied at different periods of the diœstrum is associated with the fact that the conditions of the ovary differ at the different times.

Shortly after an ovulation the ovary contains almost entirely small primary follicles. These follicles are not so unfavorably affected by food conditions as are the large graafian follicles, which begin their growth and development during later stages of the diœstrum.

A large follicle at the height of its development seems to require much better nutrition than a small primary follicle, and the lack of proper food arrests its progress very readily. Thus a late underfeeding has a more injurious effect than an early one, and the postponement of the next œstrus is correlated with a postponement of the development of new ripe follicles in the ovary. The entire œstrus activity depends chiefly upon the conditions prevailing in the ovary.

The fact that following a late and long underfeeding the next ovulation is delayed about 11 days after the underfeeding has been stopped is in accord with the results of operation experiments which Papanicolaou has performed on the corpora lutea in guinea-pigs.

These experiments show that after removal of all young corpora lutea following an ovulation, the next ovulation arrives in about 11 days instead of 16 to 17 days as would be expected. This acceleration of 5 to 6 days is due to the absence of the corpora lutea, which if present evidently inhibit the maturation, or prolong the time necessary for the development, of ripe follicles in the ovary.

These experiments all demonstrate the sensitiveness of the follicles within the ovary to environmental conditions and when considered in more detail than is here possible, they throw light on many peculiar reproductive phenomena observed in nature. The extreme variations in the œstrous cycles recently recorded for the rat by Long and Evans (Proc. Am. Ass'n of Anatomists, Anatomical Record, April 1920) may be in part, at least, due to the variations in the diet taken by the individuals. When rats are fed a mixed diet no doubt certain individuals receive a ration quite different from that eaten by certain other members of the colony.

23 (1483)

Some studies on the surface layer in the living egg cell.

By ROBERT CHAMBERS.

[*From Cornell University Medical College.*]

The results recorded here were obtained through the use of Barber's mechanical pipette holder somewhat modified for microdissection purposes.

The cells experimented upon were the egg cells of the starfish and of the sea urchin. The eggs, which are somewhat over 1/10 of a millimeter in diameter, were placed in a drop of sea water hanging from the roof of a moist chamber. The microscopically fine tips of the glass dissecting needles projected into the moist chamber and up into the hanging drop. By manipulation of the screws of the mechanical pipette holder the cells in the hanging drop could be dissected with considerable accuracy and an estimate ascertained of their physical consistency. Detailed accounts of Barber's apparatus and its application to microdissection have already been published.[1]

The egg cells studied consist of a decidedly fluid interior surrounded by a more solid surface layer of appreciable thickness. This surface layer is most solid on its external surface. Internally its consistency seems to merge insensibly into that of the fluid interior. The inner surface of this layer adheres to the touch. This is demonstrated by introducing a microdissection needle into an egg and pushing the needle through until its tip comes into contact with the inner boundary of the surface layer on the side of the egg opposite the puncture. On withdrawing the needle the layer adheres to the needle tip and strands are drawn into the interior of the egg.

If the surface layer be torn while the egg is kept under compres-

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[1] Barber, *Philippine Journal of Sc.*, Vol. X, Sec. B, Tropical Medicine, 1914; Chambers, *Biol. Bull.*, Vol. 34, 1918.

sion the fluid interior will bulge out through the tear. The cytoplasm, on coming into contact with the surrounding water, tends to establish a definite surface film which prevents the cytoplasm from mixing with the water. If the internal pressure be not too great this film persists and, in time, strengthens into a definite ectoplasmic layer. The bulge then slowly retracts until the original contour of the egg is reëstablished. If the neck of the protruding mass of cytoplasm be small it may pinch off a spherule of cytoplasm which to all appearances is normal. If the internal pressure be too great a succession of films may form as, one after the other, they succumb while the escaping cytoplasm disperses and disintegrates in the surrounding water and the film which finally holds out may enclose only a fraction of the original cell but what it encloses will be normal protoplasm.[1]

Churning of the contents of a mature unfertilized sea-urchin egg causes the ectoplasmic layer to revert to the fluid condition of the interior. The surface film of such an egg is very thin and very easily tears upon which the entire egg disintegrates. On standing, however, the surface film steadily strengthens until the normal condition is reëstablished.

That the distribution of substances throughout the egg cell is not uniform can be demonstrated by the following experiment on the starfish egg: If the surface of a mature unfertilized egg be torn while the egg is kept under compression almost all of the internal cytoplasm may be made to flow out to form a spherule of cytoplasm which pinches off from the rest of the egg. What is left behind is a collapsed remnant consisting mainly of protoplasm which originally enveloped the egg. This remnant consisting largely of the more solid ectoplasm tends only slowly to round up. The extruded mass, which is very fluid, immediately assumes a shape approximating that of a sphere. This may be termed an endoplasmic sphere. The remnant containing the original ectoplasmic substance of the egg is readily fertilizable and undergoes segmentation. The endoplasmic sphere is unfertilizable. If, on the other hand, the endoplasmic sphere remains for some time connected by means of a bridge of protoplasm with the remnant containing the original ectoplasmic substance it is fertilizable.

[1] Chambers, *Amer. Journ. Physiol.*, Vol. 43, 1917.

The ability of the endoplasmic sphere to approximate normal conditions of segmentation is a function of the length of time that it remains in organic continuity with the original ectoplasmic mass. Possibly there exists a substance necessary for development which normally accumulates in the surface layer of an egg. This substance is diffusible and will distribute itself over new protoplasmic surfaces. If a bridge of protoplasm connects the ectoplasmic remnant with the endoplasmic sphere this substance will diffuse into the sphere thereby rendering it fertilizable.

The nature of the surface film produced by cutting an egg cell differs in an unfertilized egg from one which has been fertilized. Before fertilization the needle may be pushed vertically into the side of the egg and moved through the egg from one side to the other without cutting the egg in two. The cytoplasm closes behind the needle thus obliterating the furrow. Shortly after fertilization, however, such a procedure cuts the egg cleanly in two. The sides of the furrow produced by the needle do not fuse although contiguous. The character of the surface film which forms over a cut is thus changed upon fertilization. This change prepares the egg for the ensuing segmentation process by causing the formation of a type of surface film which prevents contiguous blastomeres from fusing with one another.

30 (**1612**)

Dissection and injection studies on the Amœba.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

The species used was *Amœba proteus*. By means of a micropipette liquids of various kinds were injected and the effect noted.

Oils form spherical droplets which are carried about in the cytoplasmic currents. A large drop is usually expelled. Immediately on being extruded the drop tends to flow over the surface of the *Amœba* thus partially engulfing it.

Distilled or spring water diffuses through the granular endosarc diluting it. The dilution is followed by a contraction of the endosarc and the massing of a hyaline fluid between the endosarc and the external pellicle of the *Amœba*. This dilates the area usually termed the ectosarc. The fluid soon accumulates on one side of the *Amœba* in the form of a blister which is ultimately pinched off.

A number of acid indicators were injected. The color reactions showed that the protoplasm of the *Amœba* is more acid than its environment. Upon death the colors change to those characteristic of the surrounding medium.

The difference in behavior of living protoplasm to "basic" and to "acid" dyes is striking. The "basic" dyes used were all chlorides of colored basic radicles and the "acid" dyes, potassium or sodium salts of colored acid radicles. In every case the "basic" dyes had a coagulating and the "acid" dyes, a liquefying effect on the protoplasm.

In the case of the "acid" dyes, when the effect is local, the healthy non-colored portion of the endosarc shrinks away from the colored liquefied area. This liquid accumulates under the pellicle in the form of a blister and is ultimately pinched off.

If the "basic" dye be relatively nontoxic its injection results in a coagulated area which is localized as a colored lump of inert material. This lump is carried about in the protoplasmic currents. The color gradually diffuses out of the lump and stains many of the cytoplasmic inclusions in the *Amœba*.

Dissection indicates that the granular endosarc is capable of easily reverting from a fluid to a solid state and vice versa. Peripheral to the endosarc is a hyaline liquid zone, the ectosarc, which is bounded externally by a very thin, extensible, pellicle. The extosarc can be enlarged by a hyaline liquid extruded from the endosarc.

In the formation of a pseudopod a localized area of the pellicle softens. The accumulation of liquid in the ectosarc immediately under this area produces a bulge. The more jellied endosarc at the base of the bulge liquefies and a liquid suspension of granules streams into the bulge and up to its tip where it spreads out and flows back peripherally in the manner of a fountain flow. The granules heap up around the base of the bulge where, by means of a jellying process, a semisolid wall is built about a central liquid channel. Retraction of a pseudopod is accompanied by a reversal of the jellied to a liquid state.

An undisturbed *Amœba* usually forms numerous pseudopodia. Upon continued agitation a broadly lobate pseudopod is formed. The jellying process of the backward flowing endosarc is diminished. The base of the pseudopod, consequently, broadens more and more until all of the endosarc reverts to a liquid state and the entire body of the *Amœba* becomes transformed into what one may term a single pseudopodium within which vortical currents occur analogous to those of a chloroform drop creeping along a bed of shellac under water.

The motile activities of an *Amœba* depend upon a delicate balance between the liquefying and solidifying tendencies of its protoplasm. The most recently solidified regions are the ones that most readily liquefy. In this way a gradient exists with a definite antero-posterior axis. The posterior end consists of a heaped up mass of jellied material which is more resistant than other parts to the liquefying process necessary for the formation of pseudopodia. In an actively moving *Amœba* the amount of

such material is very small and pseudopodia may form on either side thus tending to mask its presence. Exceptionally the posterior end may be made to liquefy but usually the inert posterior end compels an *Amœba*, in order to retrace its path, to turn about.

98 (1558)

Disturbances in the development of mammalian embryos caused by radium emanation.

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As has been shown by various observers, the exposure of living tissues to the influence of radium rays leads to a severe injury and ultimate destruction of these tissues. In our work an attempt was made to study this destructive influence on mammalian embryos in utero, in the hope that a partial or complete destruction of one or more tissues might lead to definite abnormalities or malformations in these fetuses.

Bagg had lately used a method of applying radium, which was described in the *Journal of Cancer Research*, Vol. V, 1920. Radium emanation, carried in a very small amount of saline solution, was injected in measured quantities into adult rats, either subcutaneously or intravenously. This solution contained all the properties of the radium metal itself, and, no doubt, the resulting physiological changes were due mainly to the activity of α-rays. Such an injection produced peculiar destructive changes in the inner organs of the animals.

The same method was used in our experiments. After long experimentation we found that a dose of 5 mc. (= milli-curies, a standard unit in radium experimentation) was about the optimal dose. Such an amount was injected into female rats, pregnant and non-pregnant, with the purpose of either injuring the ovarian or uterine tissues, or, in case of pregnancy, the embryonic tissues. While the results were not those which we expected, viz., the production of various types of monstrosities, yet a definite influence of radium on the fetal and placental tissues was noticeable.

Radium-treated rats were killed at different periods of pregnancy, so as to procure a series of fetuses of various ages.

The most destructive results of radium emanation, injected subcutaneously, were seen in a number of pregnant females, in which the embryos were killed in the uterus and, instead of being aborted, remained attached to the uterine wall and were gradually absorbed (group I). Whether the embryos were killed primarily, or their death was due to the destructive influence of the radium on the maternal, placental tissues, cannot, of course, be determined. Probably the first assumption is correct, since other findings (group II) showed, that the toxic agent *does* pass the placenta and affects the embryos directly.

A number of such partially absorbed embryos were found, the age of which, naturally, could not be determined. Judging from the sizes of their respective placentæ, however, development must have proceeded to some extent before the radium was applied. The remnants of the embryos were small, nodular bodies attached to the placentæ (figures were shown) and had lost all resemblance to properly developed fetuses.

In one case a small, ovoid shaped sac was found, attached by a thin stalk to the uterine wall (figure shown). This apparently represented the remnants of a former embryo and placenta, although neither one could be recognized any longer. In the sac extravasated blood and cell detritus were found. A great many large cells of an epithelioid nature probably belonged to the former embryonic syncytium. The wall of this cyst was formed by fibrous connective tissue. ·

In a number of other cases (group II), the fetuses were not killed by the radium emanation, but peculiar macroscopic lesions appeared in their skin vessels.

When the fetuses were removed from the uteri, peculiar hemorrhagic areas were noticeable, in some cases just along the dorsal midline, in other cases, spreading over the entire body with the exception of the ventral surface. These extravasations took place in the vessels of the subcutaneous connective tissue and along the meningeal sinuses. In all cases, one or more hemorrhage. appeared in the midline, mainly in the head and thoracic regions It seems that the vessels in this dorsal median zone are especially

liable to injury. In one instance, there was a large area of hemor-
rhage extending over the thoracic and lumbar region. Its outline
was just symmetrical to the dorsal midline (figure shown). In
other cases, a great number of such hemorrhagic areas, some
extremely small, were found over the lateral aspects of the head
and body. Probably these affected fetuses would have died, if
left longer in the uterus, and would have undergone absorption.
In many animals which we killed in the early parts of the experi-
ments we failed to find any fetuses, although we definitely believed
that these animals had been pregnant before. We probably waited
too long after treatment, so that the embryos were completely
absorbed, when the animals were opened.

Not all of the fetuses of one litter are affected in the same degree.
In one case, for instance, we found among 7 fetuses 3 showing
hemorrhagic lesions, 2 beginning to macerate and 2 in the process
of absorption. This difference in resistence may be due either
to the higher or lower vitality of the embryos themselves or to
the amount of radium which passes the placenta. In another
case the fetuses, although injured, were carried to full term and
among 6 young of one litter we found two normal and four showing
hemorrhagic spots on head, face and along the dorsal midline.

In one very remarkable instance the female had been treated
22 days previous to conception and yet the fetuses, approximately
16 days old, showed areas of extravasation (one of considerable
size shown in figure). These lesions were much more widely
distributed than in previous cases, extending over both lateral
and dorsal surfaces (figure shown). These results cannot be ex-
plained at present. It would seem as if the treatment of the
mother previous to conception had lessened the faculty of the
later embryos to form proper endothelial walls. The wide distri-
bution of the lesions would seem to substantiate such a view.
This is in accordance with findings in adult animals treated with
radium in which the extravasations in the organs are due not only
to increased blood pressure, as would seem at first, but to the
actual breaking down of the endothelial tubes. In other words,
the effect of radium on endothelium might be selective.

When the radium was injected intravenously (group III)
instead of subcutaneously, the same lesions resulted along the

vascular channels. Females of about 19 days pregnancy were injected intravenously and the young, born dead 24 hours later, showed the hemorrhagic lesions along the dorsal midline (figures shown). In one case we found a striking difference in the size of the placentæ of different fetuses. One fetus, for instance, had a markedly enlarged placenta completely filled with blood, so that it had the appearance of a large hemorrhagic sac. This fetus did *not* show any hemorrhagic lesions, while their placentæ were of normal size and moderately filled with blood. It would seem as if in the first case the placenta functioned as an effective "shock-absorber," while in the other cases the radium emanation passed through the placentæ to the fetuses.

Lately Bagg exposed pregnant females, near full term, directly to the action of γ-rays (group IV). This radiation of the fetuses in utero, through the abdominal walls produced hemorrhagic lesions of the same nature as described above. However, the lesions did not appear until about 10 days after exposure. The young were born 2 days after treatment and appeared normal. After about a week they began to fail considerably, hemorrhagic areas appeared along the mid-dorsal line, especially in the head region and death followed. The hemorrhages in these animals were mainly along the meningeal sinuses (figures shown), in some cases frontal and occipital hemorrhages were just beginning, in others they extended considerably over the cerebral hemispheres. Additional lesions on the dorsal side of the thorax were found.

The interval of 10 days after treatment strictly corresponds to the time at which a primary skin erythema develops in radium treated patients. Again it seems as if the endothelial walls had been injured at the time of exposure and gradually gave way to the blood pressure.

In the course of the experiments, we also found numerous hemorrhagic areas in the uteri and especially in the ovaries (figures shown). Congestion of the uterine vessels always was pronounced.

While in experiments on adult animals reported by Bagg before, the injection of radium emanation led to considerable injuries in the internal organs, in our experiments the weaker

doses did not produce any macroscopically visible effects on the *maternal* tissues. However, the *embryonic* differentiating tissues were easily affected. This fact might be of some biological significance, when one remembers that radium rays have a decided effect on fast growing tumor and cancer tissues.

A Review of the Chromosome Numbers in the Metazoa

Part II

ETHEL BROWNE HARVEY

II. TABULATION OF CHROMOSOME NUMBERS

PART I. SUPPLEMENT (1915-1918)

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	OBSERVER	REFERENCE
A. INVERTEBRATA						
II. ARTHROPODA						
b. **CRUSTACEA**						
2. Malacostraca						
b. Decapoda						
1. Brachyura (Part I, p. 14)						
Cancer magister..........	100 + spg	60♂	60♂	Chromatoid body in half of spermatids	Fasten, '18	Biol. Bull., 34, p. 277
c. **INSECTA**						
2. Coleoptera						
d. Chrysomelidae (Part I, p. 16; see foot-note 1)						
Diabrotica vittata..........	21♂ emb / 22♀ emb	11♀		11♀ pron	Hoy, '18	Biol. Bull., 35, p 166
f. Coccinellidae (Part I, p. 17)						
Epilachna borealis........	18 cl			XX in ♀, XY in ♂	Hoy, '18	Biol. Bull., 35, p. 166
j². Lucanidae						
Passalus cornutus..........	26 spg / 26♀ som	13♂		XY to poles in 1st	Schaffer, '17	Biol. Bull., 32, p. 407
s. Diptera						
a. Anthomyiidae (Part I, p. 21)						
Fucellia marina............	12 som				Metz, '16	J. Exp. Zoöl., 21, p. 213
Homalomya sp............	12 som		6♂		Metz, '16	J. Exp. Zoöl., 21, p. 213
Ophyra leucostoma........	12 spg	6♂			Metz, '16	J. Exp. Zoöl., 21, p. 213

a². Asilidae

Asilus lecythus } Asilus notatus }	14 spg		7♂	XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
Asilus sericeus	10 spg			XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
Dasyllis thoracica	10 spg	5♂	5♂	XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
Deromyia winthemi	12 spg	6♂	6♂	XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
Erax rufibarbis	10 spg		5♂		Metz, '16	J. Exp. Zoöl., 21, p. 213
Leptogaster badius	10 spg		5♂	XY	Metz, '16	J. Exp. Zoöl., 21, p. 213

a³. Bombyliidae

Anthrax lateralis	12 spg				Metz, '16	J. Exp. Zoöl., 21, p. 213
Anthrax sinuosa	18 spg		9♂	XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
Spogostylum simson	12 oog			From figure	Metz, '16	J. Exp. Zoöl., 21, p. 213

d. Culicidae (Part I, p. 21)

Culex pipiens	6 spg 6 oog		3♂	3♂	Metz, '16	J. Exp. Zoöl., 21, p. 213
Culex pipiens	6 spg 6 oog 6 som	3♂		3♂ Possibly X attached to one spg chrom	Whiting, '17	J. Morph., 28, p. 523
Culex pipiens	6 som				Hance, '17	J. Morph., 28, p 579
Culex pipiens	6 som.			Multiple groups (12-72) in pupal intestine	Holt, '17	J. Morph., 29, p. 607
Culex pipiens	6 cl 3 (double) som				Taylor, '17	Q. J. M. S., 62, p. 287

II. ARTHROPODA—Continued

d². Drosophilidae (see under f. Muscidae acalyptratae, Part I, p. 22)

SPECIES	DIPLOID AND PARTHENO-GENETIC	1st-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Cla:lochaeta nebulosa...	8 oog					Metz, '16	Amer. Nat., 50, p. 587
Drosophila affinis.........	10 spg / 10 oog					Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila amoena.......	8 spg / 8 oog			XY		Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila ampelophila..	8 oog			XY		Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila ampelophila..	8 spg / 8 oog			XY		Bridges, '16	Genetics, 1, p. 1
Drosophila bromeliae....	8 spg					Metz, '16	Amer. Nat., 50, p. 587
Drosophila busckii.	8 oog	4♂				Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila carlini........	12 oog					Metz, '16	Amer. Nat., 50, p. 587
Drosophila caribes........	8 spg / 8 oog			XY		Metz, '16	Amer. Nat., 50, p 587
Drosophila dimidiata.....	8 oog					Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p 587
Drosophila earlei.........	6 oog					Metz, '16	Amer. Nat., 50, p. 587
Drosophila florae.........	8 oog					Metz, '16	Amer. Nat., 50, p. 587
Drosophila funebris.......	12 spg / 12 oog				Note i: '14 corrected	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila limbata (ne-bulosa)...............	8 oog					Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila melanica (ne-glecta)...............	10 spg / 10 oog		5♂			Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila modesta	12					Metz, '16	Amer. Nat., 50, p. 587

Species	Chromosome no. (germ cells)	♂	♂	Sex chromosomes / remarks	Authority	Reference
Drosophila obscura......	10 spg, 10 oog		5♂	XY	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila ornatipennis..	11 oog			XY. One supernumerary	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila pallida......	8				Metz, '16	Amer. Nat., 50, p 587
Drosophila procnemis....	8 oog				Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila ramsdeni.....	12 oog			=Sp. A of '14	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila repleta.......	12 spg, 12 oog			XY	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 58.
Drosophila robusta.......	8 spg				Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Drosophila saltans.......	8				Metz, '16	Amer. Nat., 50, p. 587
Drosophila similis.......	12 oog				Metz, '16	Amer. Nat., 50, p. 587
Drosophila tripunctata..	8 oog				Metz, '16	Amer. Nat., 50, p. 587
Drosophila virilis........	12 oog			=Sp. B of '14	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Scaptomyza adusta......	10 oog			XY	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
Scaptomyza graminum...	8 spg, 8 oog		4♂	XY	Metz, '16 / Metz, '16	J. Exp. Zoöl., 21, p. 213 / Amer. Nat., 50, p. 587
e. Muscidae (Part I, p. 22)						
Calliphora erythrocephala...	12 oog, 12 som	6♂			Metz, '16	J. Exp. Zoöl., 21, p. 213
Musca domestica.........	12 oog	6♂			Metz, '16	J. Exp. Zoöl., 21, p. 213
Phormia regina..........	12 spg	6♂		XY to poles in 1st	Metz, '16	J. Exp. Zoöl., 21, p. 213
e². Ortalidae (see under f. Musca acalyptratae, Part I, p. 22)						
Camptoneura picta........	12 spg	6♂			Metz, '16	J. Exp. Zoöl., 21, p. 213
Chaetopsis fulvifrons....	8 spg	4♂			Metz, '16	J. Exp. Zoöl., 21, p. 213

ii. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
				e³. Sapromyzidae			
Physegenua vittata......	12 spg					Metz, '16	J. Exp. Zoöl., 21, p. 213
				g. Sarcophagidae (Part I, p. 23)			
Ravinia peniculata........	12 oog					Metz, '16	J. Exp. Zoöl., 21, p. 213
Sarcophaga tuberosa sarraceniae........ Sarcophaga sp............	12 spg 12 oog 12 som	6♂	6♂		XY to poles in 1st. Multiple som groups with 24 and 48	Metz, '16	J. Exp. Zoöl., 21, p. 213
				g². Sciomyzidae			
Neuroctena analis........	12 spg		6♂		From figures	Metz, '16	J. Exp. Zoöl., 21, p. 213
				g³. Sepsidae			
Piophila casei............	12					Metz, '16	J. Exp. Zoöl., 21, p. 213
				g⁴. Stratiomyidae			
Ptecticus trivittatus.....	16				XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
				h. Syrphidae (Part I, p. 23)			
Eristalis bastardi....... Mesogramma marginata Volucella obesa..........	12 spg	6♂			XY	Metz, '16	J. Exp. Zoöl., 21, p. 213
				h². Trypetidae			
Euaresta melanogaster...	12					Metz, '16	J. Exp. Zoöl., 21, p. 213

4. *Hemiptera*
a. Hemiptera heteroptera
1. Belostomidae (Part I, p. 23)

Belostoma (Zaitha) fluminea...	24 spg	13♂		12♂	XY to poles in 2nd	Chickering, '16	Trans. Amer. Micr. Soc. 35, p. 45
2. Coreidae (Part I, p. 24)							
Anasa tristis...	21 spg, 21♂ cl / 21♂ som / 22 oog, 22♀ cl / 22♀ som				Some cells investing ovary have 44	Hoy, '16	Biol. Bull., 31, p. 329
Leptocoris haematoloma...	13 spg	7♂	7♂	6,7♂	X to pole in 2nd	Porter, '17	Biol. Bull., 33, p. 316
9². Nepidae							
Ranatra sp?...	40 spg	21♂	20♂	20♂	XY to poles in 2nd. Another type has 8 or 10 more chroms in spg	Chickering, '18	Trans. Amer. Micr. Soc. 37, p. 132
10. Notonectidae (Part I, p. 30)							
Notonecta glauca... / Notonecta irrorata...	24 spg / 24 oog	13♂	12♂	12♂	XY to poles in 2nd	Browne, '16	Jour. Morph., 27, p. 119
Notonecta indica... / Notonecta shooteri... / Notonecta undulata...	26 spg / 26 oog	14♂	13♂	13♂	XY to poles in 2nd	Browne, '16	Jour. Morph., 27, p. 119
Notonecta insulata...		13,14♂ (2 chroms may be fused or not)	12♂	12♂	XY to poles in 2nd. Wrongly tabulated in Part I	Browne, '16	Jour. Morph., 27, p. 119
14. Reduviidae (Part I, p. 35)							
Pselloides (milyas) cinctus...	28 spg / 30 oog	16♂	16♂		XY; X=3 elements	Goldsmith, '16	Biol. Bull., 31, p. 121

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
			5. Hymenoptera				
			b. Chalcididae (Part I, p. 43)				
Paracopidosomopsis floridaus............	8 spg 8♂ som		8♂	8♂	Pa. generation. 1st div. abortive	Patterson and Porter, '17	Biol. Bull., 33, p. 38
	16 cl 16♀ som	8♀	8♀		Fertilized egg	Patterson, '17	Biol. Bull., 33, p. 57
			7?. Odonata (See under Neuroptera, Part I, p. 51)				
Anax junius............	27 spg	13♂				Smith, '16	Biol. Bull., 31, p. 269
Libellula basalis............	25 spg	13♂	12, 13♂		X to pole in 1st	Smith, '16	Biol. Bull., 31, p. 26?
Sympetrum semicinctum	25 spg	13♂	13♂		X to pole in 2nd	Smith, '16	Biol. Bull., 31, p. 26?
			8. Orthoptera				
			a. Acrididae (Part I, p. 51)				
Acridium, see under Tetrigidae							
Chloealtis............	17 spg	9♂				McClung, '17	Jour. Morph., 29, p. 519
Chorthippus (Stenobothrus) curtipennis.........	17 sp?	9♂	8, 9♂		X to pole in 1st	Robertson, '16 Lewis and Robertson, '16	Jour. Morph., 27, p. 179 Biol. Bull., 30, p. 99
Chorthippus (Stenobothrus) curtipennis.........		9♂			X	Wenrich, '17	Jour. Morph., 29, p. 471
Circotettix lobatus...... Circotettix rabula.........	21 spg	11♂	10, 11♂		X to pole in 1st. Supernumeraries (1 or 2) may be present, to pole in 1st	Carothers, '17	Jour. Morph., 28, p. 445
Hesperotettix brevipennis...... Hesperotettix festivus...	23 spg	12♂				McClung, '17	Jour. Morph., 29, p. 519

		11♂ (=12)	11♂ (=11, 12)	11♂ (=11, 12)			
Hesperotettix pratensis } Hesperotettix speciosus }	22 spg (=23)	11♂ (=12)	11♂ (=11, 12)	11♂ (=11, 12)	X attached to another chrom, to pole in 1st	McClung, '17	Jour. Morph., 29, p. 519
Hesperotettix viridis	19-23 spg (=23)	9-12♂ (=12 or 12—18.)	10-12♂ (=11, 12)		X attached or free, to pole in 1st. Other chroms may be fused in pairs. Supernumerary present in one animal	McClung, '17	Jour. Morph., 29, p. 519
Mermeria bivittata	22 spg (=23) 22♀ som (=24)	11♂ (=12)	11♂ (=11, 12)	11♂ (=11, 12)	X attached to another chrom, to pole in 1st. Earlier account corrected	McClung, '17	Jour. Morph., 29, p. 519
Mermeria sp?	23 spg	12♂	11, 12♂			McClung, '17	Jour. Morph., 29, p. 519
Nomotettix } see under Paratettix } Tettigidae							
Phrynotettix magnus	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Wenrich, '16	Bull. Mus. Comp. Zool. Harfard, 60, p. 56
Stenobothrus, see Chorthippus							
Syrbula acuticornis	23 spg	12♂	11, 12♂		X to pole in 1st	Robertson, '16	Jour. Morph., 27, p. 179
Tettigidae (Subfamily of Acrididae) Acridium granulatus	13 spg 13♂ som 14♀ som	7♂	0, 7♂		X to pole in 1st	Robertson, '16	Jour. Morph., 27, p. 179
Acridium incurvatus	13♂ som	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Acridium obscurus	13 spg	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Acridium ornatus		7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Nomotettix cristatus	13 spg				X	Rayburn, '17	Kansas Univ. Sc. Bull. 10, p. 267
Paratettix cucculatus	13 spg	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Paratettix texanus		7♂				Robertson, '16	Jour. Morph., 27, p. 179

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Tettigidae (Subfamily of Acrididae) continued Tettigidea parvipennis	13 spg 13♂ som 14 oog	7♂	6, 7♂		X to pole in 1st. Supernumerary X present in 1 animal in som and ♂ cells. To pole in 1st, same pole as X or the other	Robertson, '16 Robertson, '17	Jour. Morph., 27, p. 179 Kansas Univ. Sc. Bull. 10, p. 275
Tettigidea parvipennis pennata..........	13 spg 14 oog	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Trimerotropis? fallax Trimerotropis? suffusa	23 spg 24♀ som	12♂	11, 12♂		X to pole in 1st	Carothers, '17	Jour. Morph., 28, p. 445
Trimerotropis suffusa....		12+1 super-numerary				Wenrich, '17	Jour. Morph., 29, p. 471

d. Gryllidae (Part I, p. 58)

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Gryllotalpa borealis	23 spg 24 oog	12♂	11, 12♂	11, 12♂	XY to poles in 1st. X=2 elements	Payne, '16	Jour. Morph., 28, p. 287
Gryllotalpa vulgaris from Freiburg..............	12 spg	6♂			XY	Payne '16	Jour. Morph., 28, p. 287
Gryllotalpa vulgaris from Naples	15 spg	8?♂				Payne, '16	Jour. Morph., 28, p. 287

e. Locustidae (Part I, p. 59)

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Jamaicana flava..........	35 spg	18♂	17, 18♂	17, 18♂	X to pole in 1st	Woolsey, '15	Biol. Bull., 28, p. 163
Jamaicana subguttata...	34, 35 spg	17, 18♂			2 chroms may be fused	Woolsey, '15	Biol. Bull., 28, p. 163
Jamaicana unicolor......	33, 35 spg		14, 15♂		2 pairs of chroms may be fused	Woolsey, '15	Biol. Bull., 28, p. 163 Tabulated in brief in Part I
Locusta viridissima......	29 spg 29♂ som 30 oog 30♀ som	15♂	14, 15♂	14, 15♂	X to pole in 1st	Mohr, '16	Liège, 1916

III. COELENTERATA
a. HYDROZOA
1. Leptolinae
3. Anthomedusae (Part I, p. 62)

Species					Remarks	Authority	Reference
Clava leptostyla.........	12♀	12♀	12♀			G. T. Hargit, '16	Jour. Morph., 27, p. 85

2. Trachylinae (Part I, p. 63)

Species					Remarks	Authority	Reference
Aglantha digitalis.........	8♀	8♀	8♀	16 oog		G. T. Hargit, '17	Jour. Morph., 28, p 593

PART II (1878–1918)
IV. ECHINODERMATA
a. ASTEROIDEA

Species					Remarks	Authority	Reference
Asterias forbesii.........	177♀	177♀	177♀			Wilson and Mathews, '95	Jour. Morph., 10, p. 319
Asterias forbesii.........	18♀	18♀	18♀	18? pa cl		Tennent and Hogue, '06	J. Exp. Zoöl, 3, p. 517
Asterias forbesii.........				36 cl / ca. 18 pa cl	May be A. vulgaris	Tennent, '07	Biol. Bull., 13, p. 309
Asterias forbesii.........	18♀	18♀	18♀	36 cl		Jordan, '07 / Jordan, '08	Anat. Anz., 31, p. 39 / Carnegie Inst. Pub. 102, p. 39
Asterias glacialis.........		8–9♂ pron				Field, '95	Jour. Morph., 11, p. 235
Asterias glacialis.........				18 cl / 18 pa cl (auto-regulation)		Delage, '01	Arch. Zoöl. exp. et gen. ser. III, vol. 9, p. 285
Asterias glacialis.........	18♀	18♀	18♀	36 pa cl	2nd p. b. +egg=36	Buchner, '11	Arch. Zellf. 6, p. 577
Asterias vulgaris.........	9♂	9♂	9♂	18 spg / 18 cl / 9 pa cl	May be A. forbesii	Tennent, '07	Biol. Bull., 13, p. 309
Cribrella sanguinolenta..				ca. 36 som		Jordan, '10	Arch. Zellf., 5, p. 394

b. CRINOIDEA

Species					Remarks	Authority	Reference
Antedon bifida...........				8? oog		Chubb, '06	Phil. Trans. Roy. Soc. London, 108 B, p. 447

IV. ECHINODERMATA—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
					c. ECHINOIDEA		
Arbacia punctulata......	ca. 40 cl					Jordan, '12	J. Exp. Zoöl., 12, p. 391
Arbacia pustulosa.........	40 cl					Baltzer, '10 .	Arch. Zellf., 5, p. 497
Echinus acutus...........	38 cl					Doncaster Gray, '11 and; Doncaster Gray, '13 and	Proc. Camb. Phil. Soc. 16, p. 415; Q. J. M. S., 58, p. 483
Echinus esculentus.......	32 cl	16♀	16♀	16♀		Bryce, '03	Q. J. M. S., 46, p. 177
Echinus esculentus.......	38 cl					Doncaster Gray, '11 and; Doncaster Gray, '13 and	Proc. Camb. Phil. Soc. 16, p. 415; Q. J. M. S., 58, p. 483
Echinus esculentus.......	18 cl					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Echinus microtuberculatus var. bivalens (Boveri '05)...............		18♀		18♀	In a few cases	Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
Echinus microtuberculatus var. bivalens........	30+cl; 16–18 pa cl	16–18♀			Pa eggs treated with strychnine	R. Hertwig, '88; R. Hertwig, '95; R. Hertwig, '96	Sitz. gesel. Morph. u. Physiol., München, 4, p. 99; Sitz. gesel. Morph. u. Physiol., München, 11, p. 41; Fest. Gegenbauer, 2, p. 21
Echinus microtuberculatus var. bivalens........	36 cl	18♀	18♀	18♂ in enucleated egg fragments	XY in ♂. From correction in '13	Stevens, '02	Arch. Entwick., 15, p. 421
Echinus microtuberculatus var. bivalens........	36 cl; 18 pa cl			18♂ in enucleated egg fragments 18♀		Baltzer, '09; Baltzer, '10; Baltzer, '13	Arch. Zellf., 2, p. 549; Arch. Zellf., 5, p. 497; Sitzungsber. Phy. med Ges. Wurzburg, 6, p. 90

Species	No.	♀♀	♀♀	Egg-fragment / sex notes		Authority	Reference
Echinus microtuberculatus var. univalens (Boveri, '05)	18 cl	♀♀	♀♀	♀♀ ♀♂ in enucleated egg fragments		Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
						Boveri, '05	Jen. Zeits., 32, p. 445 (Zellen-Studien V)
Echinus microtuberculatus var. univalens	18 cl					Stevens, '02	Arch. Entwick., 15, p. 421
Echinus microtuberculatus var. univalens (= Psammechinus)	20+ cl			9–12♂ in enucleated egg fragments		Krahelska, '05	Bull. intern. de l'acad. des Sciences de Cracovie, '05, p. 49
Echinus microtuberculatus var. univalens	18 cl					Godlewski, '06	Arch. Entwick., 20, p. 579
Echinus miliaris	22 cl. 8–11 cl, in enucleated egg fragments fertilized			10–12♂, in enucleated egg fragment		Morgan, '95	Arch. Entwick., 2, p. 268
Echinus miliaris	30–34 cl					Doncaster and Gray, '11 / Doncaster and Gray, '13	Proc. Camb. Phil. Soc., 16, p. 415 / Q. J. M. S., 58, p. 483
Echinus sphaera	18 cl			16–20♀	X or XY in ♂	Delage, '01	Arch. Zool. exp. et gen. III, 9, p. 285
Hipponoe esculenta	32? cl					Jordan, '08	Carnegie Inst. Pub. 102, p. 39
Hipponoe esculenta	32–34 cl					Pinney, '11	Biol. Bull., 21, p. 168
Hipponoe esculenta						Tennent, '12	Jour. Morph., 23, p.17
Moira atropos	46 cl					Pinney, '11	Biol. Bull., 21, p. 168
Parechinus miliaris	18 pa cl					Retzius, '10	Biol. Untersuchungen, 15, p. 1
Psammechinus, see under Echinus microtuberculatus							
Sphaerochinus granularis	16–18♀					R. Hertwig, '96	Fest. Gegenbaur, 2, p. 21
Sphaerochinus granularis	40 cl (probably)					Baltzer, '10	Arch. Zellf. 5, p. 497

IV. ECHINODERMATA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST CYTE	2ND CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Sphaerechinus granularis	18 pa cl					Godlewski, '12	Arch. Entwick., 33, p. 196
Strongylocentrotus livi-dus	30+cl / 16–18 pa cl	16–18♀			Pa eggs treated with strychnine	R. Hertwig, '96	Test. Gegenbaur, 2, p. 21
Strongylocentrotus livi-dus	18 cl / 18 pa cl (auto-regulation)			9♀		Delage, '99 / Delage, '01	Arch. Zool. exper., et gen. III, 7, p. 383 / Arch. Zool. exper., et gen. III, 9, p. 285
Strongylocentrotus livi-dus	36 cl			18♂ 18♀		Boveri, '02	Verh. phys. med. Gesel. Wurzburg, 35, p. 67
Strongylocentrotus livi-dus	36 cl					Petrunkewitsch, '04	Zool. Jahrb. suppl. 7, p. 77
Strongylocentrotus livi-dus	36 cl			18♂ 18♀	XY in ♂. From correction in '13	Baltzer, '09 / Baltzer, '10 / Baltzer, '13	Arch. Zellf., 2, p. 549 / Arch. Zelli., 5, p. 497 / Sitsungsber. Phys. Med. Gesel. Wurz-burg, 6, p. 90
Strongylocentrotus livi-dus	36 cl					Schaxel, '11	Arch. mikr. Anat., 76, p. 543
Strongylocentrotus pur-puratus	36 cl / 18 pa cl					Hindle, '11	Arch. Entwick., 31, p. 145
Toxopneustes variegatus.	14–24 cl					Selenka, '78	"Befruchtung des Eies von Tox. var." Leipzig.
Toxopneustes variegatus.	36 cl / 18 pa cl			18♂		Wilson, '95 / Wilson, '01	Jour. Morph.,11,p.443 / Arch. Entwick., 12, p. 529 / Arch. Entwick., 13, p. 353
Toxopneustes variegatus.	36 cl				XY (in ♀?)	Heffner, '10	Biol. Bull., 19, p. 195
Toxopneustes variegatus.	38? cl					Pinney, '11	Biol. Bull., 21, p. 168
Toxopneustes variegatus.	36 38 cl / 19 pa cl			18 or 19♂ in enucleated eggs	X or XY in ♂	Tennent, '12 / Tennent, '13	J. Exp. Zool, 12, p. 391 / J. Morph., 23, p. 17

					Authority	Reference
d. HOLOTHUROIDEA						
Stichopus regalis "and other Echinoderms"	28-36 spg	16-18♂		8-9♂	Field, '93 Field, '95	Anat. Anz., 8, p. 487 Jour. Morph., 11, p. 235
e. OPHUROIDEA						
Ophiocoma pumila		ca. 18♀			Jordan, '08	Carnegie Inst. Pub. 102, p. 1
V. MESOZOA						
Dicyemennea gracile		ca. 30♀			Hartmann, '07	Mem. pub. par Cl. d. Sc. Acad. Roy. de Belgique, (4°), 1, p. 1
Haplozoon armatun	100+som			3♀	Dogiel, '08	Zeit. wiss. Zool., 89, p. 417
Rhopalura ophiocomae	6 cl	3♀		3♀	Caullery et Lavallée, '08	Arch. Zool. exp. et ge. Ser. IV, t. 8, p. 421
VI. MOLLUSCA *a.* CEPHALOPODA						
Sepia officinalis		6♀				Arch. d'Anat. micros., 8, p. 239
b. GASTROPODA 1. *Euthyneura* a. Opisthobranchia 1. Nudibranchia						
Diaulula sandiegensis		12♀		16♀	MacFarland, '97	Zool. Jahrb., 10, p. 227
Doris bifida	32 cl	16♀		16♀	Smallwood, '05	Morph. Jahrb., 33, p. 87
Montagua gouldii / Montagua pilata	32 cl	16♀		16♀	Smallwood, '05	Morph. Jahrb., 33, p. 87
Phyllirhoe bucephala	32 cl	16♀		16♀ 16♂ pron	Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
Pleurophyllidia californica	20-24 cl	10-12♀			MacFarland, '97	Zool. Jahrb., 10, p. 227

VI. MOLLUSCA—Continued

2. Tectibranchia

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST CYTE	2ND-CYTE	♂ID	REMARKS	OBSERVER	REFERENCE
Aplysia depilans		16♀	16♀			Bochenek, '99	Bull. Acad. Sc. Cracovie 1899, p. 266
Aplysia limacina	24 cl (at least)	16♀				Carazzi, '05	Arch. ital. anat. e emb. 4, p. 231
Aplysia punctata			16♀	16♀		Janssens and Elrington, '04	La Cellule, 21, p. 315
Aplysia punctata	24 cl (at least)					Carazzi, '05	Arch. ital. anat. e emb. 4, p. 231
Bulla solitaria, see Haminea							
Creseis acicula	20 spg 20 oog 20 som 20 cl 10 pa cl	10♂ 10♀	10♂ 10♀	9, 10♂ (those with 9 not functional) 10♀	X to pole in 2nd	Zarnik, '11	Verh. d. deutsch. Zool. Gesell., 21, p. 205
Cymbulia peronii	32 cl	10♀				Nekrasof, '03 Nekrassof, '09	Anat. Anz., 24, p. 119, Arch. mikr. Anat., 73, p. 913
Cymbulia peronii	38 (not stated where)					Zarnik, '11	Verh. deutsch. Zool. Gesel., 21, p. 205
Haminea solitaria (= Bulla solitaria)		16♀	16♀		Accessory chrom	Smallwood, '04	Bull. Mus. Comp. Zool. Harvard, 45, p. 259
Ilyalaea tridentata	24 (not stated where)				X to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesel., 21, p. 205
Hyalocylis striata	24 (not stated where)	12♂	10+2X (fused) ♂	10, 10 + 2 X (fused) ♂. Those with 10 degenerate	2X (fused) to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesel., 21, p. 205
Tiedemannia neopolitana	28 (not stated where)				X to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesel., 21, p. 205

b. Pulmonata

Species					Notes	Author	Reference
Arion empiricorum		16–20♀				Platner, '86	Arch. mikr. Anat., 27, p. 32
Arion empiricorum		16–20♀				Garnault, '89	Zool. Anz., 12, p. 10
Arion empiricorum (or rufus)	32 cl	16♀				Lams, '10	Acad. roy. Belgique. Cl. d. Sc. Mém. Sér. 2, t. 2, no. 4, p. 1
Helix arbustorum	ca. 48 spg	24♂	24♂	24♂	Small heterochrom divides in 1st besides other 24. Fate undetermined	Soós, '10	Annales Mus. Nat. Hungarici, 8, p. 231
Helix arbustorum	40+spg (prob. 48)	24♂	24♂	24♂		Buresch, '11	Arch. Zellf., 7, p. 314
Helix aspersa		16–20♀				Garnault, '89	Zool. Anz., 12, p. 10
Helix hortensis (= Tachea h.)	48 spg (40–48)	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445
Helix nemoralis	48 spg (40–48)	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445
Helix nemoralis		22♂, one animal had 28–29♂	24♂	24♂		Baltzer, '13	Arch. Zellf., 11, p. 151
Helix pomatia var. bivalens (Murray, '98)	48 spg	24♂	24♂	24♂	From correction in '11	Bolles-Lee, '96; Bolles-Lee, '97; Bolles-Lee, '99; Bolles-Lee, '11	La Cellule, 11, p. 223; La Cellule, 13, p. 197; La Cellule, 16, p. 47; La Cellule, 27, p. 53
Helix pomatia var. bivalens	ca. 48 spg	24♂	prob. 24♂	ca. 24♂		Murray, '98	Zool. Jahrb., 11, p. 427
Helix pomatia var. bivalens	48 spg (= 24 double) 48 cl	24♂	24♂	2♂		Ancel, '02	Bibliogr. Anat., 11, p. 149
						Ancel, '03	Arch. Biol., 19, p. 389
Heli pomatia var. bivalens	30+som	24♂	24♂	24♂		Tschassownikow, '05	Anat. Hefte, 29, p. 313
Helix pomatia var. bivalens	48 spg	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445

VI. MOLLUSCA—Continued

SPECIES	DIPLOID AND PARTHENOGE ETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Helix pomatia var. bivalens...	ca. 48 spg	24♂	23, 24♂	23, 24♂. Those with 23 degenerate	One tetrad to pole in 1st	Demoll, '11; Demoll, '12	Zool. Anz. 38, p. 88; Zool. Jahrb. Suppl. 15, vol. 2, p. 107; Zool. Jahrb., Abt. Zool., 33, p. 40
Helix pomatia var. univalens (Murray, '98)....			24♂	12♂	Reduction in 2nd div.	Platner, '85; Platner, '89	Arch. mikr. Anat. 26, p. 599; Arch. mikr. Anat. 33, p. 134
Helix pomatia var. univalens........	24 spg				May be spc. no.	Zimmerman, '91	Verh. Anat. Gesel., 5, p. 187
Helix pomatia var. univalens	24 spg	12♂ (=48 el)	12♂ (=24 el)	12♂	Tetrads and dyads consist of separate elements	Vom Rath, '92	Arch. mikr. Anat., 40, p. 102
Helix pomatia var. univalens........	24 spg					Godlewski, '97	Bull. Acad. Sc. Cracovie, 1897, p. 68
Helix pomatia var. univalens........	24 spg	12♂ (=48 el)	12♂ (=24 el)	12♂		Prowazek, '02	Arb. Zool. Inst. Wien, 13, p. 197
Helix (sp. not given).....	16? spg					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Limax agrestis..........			16♂	8♂	Reduction in 2nd div.	Platner, '80	Arch. mikr. Anat., 33, p. 125
Limax cinereo-niger	16 spg	8♂ (=32 el)	8♂ (=16 el)	8♂	Tetrads and dyads consist of separate elements	Vom Rath, '92	Arch. mikr. Anat., 40, p. 102
Limax maximus.........		ca. 20♀				Washburn, '94	Amer. Nat. 28, p. 528
Limax maximus..........		16-20♀ (prob. 16)	16♀			Linville, '00	Bull. Mus. Comp. Zool. Harvard, 35, p. 211
Limnea elodes..........		16♀	16♀			Linville, '00	Bull. Mus. Comp. Zool. Harvard, 35, p. 211
Tachea austriaca (=Helix a.).........		25♂				Baltzer, '13	Arch. Zellf., 11, p. 151
Tachea hortensis (=Helix h.).........	44 spg; 40-48 pa (?) spg	22♂. (Perhaps 22, 23); 22 pa (?) ♂				Questions if pa. or self-fertilized	Baltzer, '13	Arch. Zellf., 11, p. 151

2. Streptoneura (=Prosobranchia)
a. Pectinobranchia
1. Heteropoda

Species						Author	Reference
Carinaria mediterrania...	32 cl	16♀	16♀	16♀	16♀ 16♂ pron	Boveri, '90	Jen. Zeits., 17, p. 314 (=Zellen-Studien III)
Columbella rustica.......		16+♂				Schitz, '17	Arch. Zool. exper. et gen. Notes et Rev., 56, p. 32
Enteroxenus oestergreni..	34 oog	17♀	17♀	17♀	17♀ 17♂	Bonnevie, '05 Bonnevie, '06	Anat. Anz., 26, pp. 374 and 497 Jen. Zeits., 34, p. 229
Enteroxenus oestergreni..	42 som	21♂ 21♀	21♂ 21♀	21♂ 21♀	21♂ pron 21♀ pron	Schreiner, '07	Vidensk-Selsk. Skr. Math.-Natur., 1907, no. 2, p. 1
Pterotrachea mutica......	32 cl	16♀	16♀	16♀	16♀ 16♂ pron	Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)

2. Platypoda

Species						Author	Reference
Bythinia tentaculata.....	22–28 spg	24–28♂			Fusion of chroms in oligopyrene	Von Kemnitz, '14	Arch. Zellf., 12, p. 567
Conus mediterranea.......		14♂				Kuschakewitsch, '13	Arch. Zellf., 10, p. 237
Crepidula plana..........	60 cl	30♀	30♀	30♀		Conklin, '02	Jour. Acad. Nat. Sc. Phila., 12, p. 1
Fulgur carica............	Prob. 16♀	Prob. 16♀				McMurrich, '96	Anat. Anz., 12, p. 534
Paludina vivipara	16 fuse to form 4 spg	4♂ (=16 el)	4♂ (=8 el)	4♂	4♂	Auerbach, '96	Jen. Zeits., 23, p. 405
Paludina vivipara........	14 spg	7♂	7♂	7♂	7♂	Meves, '01 Meves, '03	Verh. Anat. Gesel., 1901, p. 23 Arch. mikr. Anat., 61, p. 1
Paludina vivipara........	14 oog 14 som 14 cl	7♀	7♂	7♂	7♂	Popoff, '07 Popoff, '08	Arch. mikr. Anat. 70, p. 43 Biol. Centralb., 28, p. 555
Vulvata piscinalis.......	ca. 30 spg	10♂				Von Kemnitz, '14	Arch. Zellf., 12, p. 567
Vermetus gigas...........		14♂				Kuschakewitsch, '13	Arch. Zellf., 10, p. 235

VI. MOLLUSCA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
c. Lamellibranchia							
Cumingea tellinoides.....		18♀	18♀			Jordan, '10	Arch. Zellf., 4, p. 243
Cumingea tellinoides.....	Prob. 36 cl 18 pa. cl (2 p. b.'s) 50–60 pa. cl. (no p. b.'s)	18♀	18♀			Morris, '17 Morris, '18	J. Exp. Zoöl., 22, p. 1 Biol. Bull., 35, p. 260
Mactra.....	24 cl 12 pa cl	12♀	12♀	12♀	One or both p. b.'s may be retained in pa. causing variations in chrom no.	Kostanecki, '04 Kostanecki, '04 Kostanecki, '11	Arch. mikr. Anat., 64, p. 1 Bull. Acad. Sc. Cracovie, 1904, p. 70 Arch. mikr. Anat., 78, Abt. II, p. 1
Unio.....		16♀	16♀			Lillie, '01	Jour. Morph., 17, p. 227
VII. MOLLUSCOIDEA *a.* Brachiopoda							
Lingula anatina.....				8♂ pron 8♀		Yatsu, '02	Jour. Coll. Sc. Imp. Univ. Tokyo, 17, art. 4, p. 1
b. Bryozoa *1. Ectoprocta*							
Membranipora pilosa.....		11♀				Bonnevie, '06 Bonnevie, '07	Arch. f. Mathem. og Naturv., 27, no 13 Jen. Zeits., 35, p. 567
Plumatella fungosa.....	5 cl	6 or 7♂				Braem, '97	Zoologica, 10, Hefte 23, p. 1

2. Endoprocta

Pedicellina americana......	22 spg 22 oog 22 cl	11♂ 11♀	11♂ 11♀		Dublin, '05	Annals N. Y. Acad. Sc., 16, p. 1
Pedicellina echinata......		8♀	8♀		Lebedinsky, '05	Biol. Centralb., 25, p. 536
c. PHORONIDA						
Phoronis australis.........		12♂ 12♀			Ikeda, '03	Annot. Zool. Japan, 4, p. 141
Phoronis ijimai............		6♂ 6♀	3♂ 3♀	Reduction in 2nd div.	Ikeda, '01 Ikeda, '03	Jour. Coll. Sc. Imp. Univ. Tokyo, 13, p. 507 Annot. Zool. Japan, 4, p. 141
VIII. NEMATHELMINTHES *a. ACANTHOCEPHALA*						
Echinorhyncus acus.......	8 som	8♀	8♀		Hamann, '91	Jen. Zeits., 18, p. 113
Echinorhyncus gigas		4♂	4♂		Kaiser, '93	Bibliot. Zoöl., 7, Part II, p. 1
Echinorhyncus haeruca Echinorhyncus polymorphus		8♀	8♀		Hamann, '91	Jen. Zeits., 18, p. 113
Echinorhyncus proteus...	8 cl	4♀			Von Voss, '10	Arch. Zellf., 5, p. 430
Gigantorhyncus gigas....	6 spg	3♂	3♂		Noé, '10	Arch. ital. de Biol., 53, p. 315
Gigantorhyncus hirudinaceus..............	6 spg	3♂	3♂	3♂	Noé, '14	Mem. d. R. Accad. Lincei Ser. 5, vol. 10, p. 46

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
b. CHAETOGNATHA							
Sagitta bipunctata.........		8♂	8♂	4♂		Bolles-Lee, '89	La Cellule, 4, p. 105
Sagitta bipunctata.........	18 cl	9♀	9♀	9♀ / 9♂ pron		Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
Sagitta bipunctata.........	18 spg / 18 som	9♂ / 9♀	9♂ / 9♀	9♂ / 9♀		Stevens, '03 / Stevens, '10	Zool. Jahrb., 18, p. 227 / Jour. Morph., 21, p. 279
Sagitta bipunctata.........	18 cl	9♀		9♀		Buchner, '09 / Buchner, '10	Anat. Anz., 35, p. 433 / Fest. R. Hertwig 1, p. 235
Sagitta bipunctata.........	18 som	9♀				Elpatiewsky, '10	Biol. Zeits. Moskou, 1, p. 333
Sagitta bipunctata.........	18 spg	9♂	9♂			Bordas, '12 / Bordas, '14	La Cellule, 28, p. 165 / Mem. d. l. Real Soc. Espan. d Hist. nat. 10, p. 1
Sagitta elegans............	18 som	9♂ / 9♀	9♂	9♂		Stevens, '05 / Stevens, '10	Zoöl. Jahrb., 21, p. 243 / Jour. Morph., 21, p. 279
Sagitta inflata............		9♂	9♂	9♂		Stevens, '10	Joor. Morph., 21, p. 279
Sagitta inflata............	18 cl	9♀		9♀		Buchner, '10	Fest. R. Hertwig 1, p. 235
Sagitta minima............		9♂	9♂	9♂		Stevens, '10	Jour. Morph., 21, p. 279
c. NEMATODA *1. Gordioidea*							
Gordius affinis............	4 oog	1♀			4 chroms form 1 tetrad	Švábenik, '09	Sitz. Kon. Böhm. Gesel d Wissen Prague, 1009, art. 7
Gordius aquaticus.........	7-9 cl					N. Th. Meyer, '13	Zeit. wiss. Zool., 105, p. 125
Gordius aquaticus.........	4 cl					Muhldorf, '13 / Muhldorf, '14	Zool. Anz., 42, p. 431 / Zeit. wiss. Zool., 111. p. 1

Species					Remarks	Observer	Reference
Gordius gratianopolensis		8♀				Camerano, '90	Mem., R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
Gordius montenigrinus...	4 oog	1♀			4 chroms form 1 tetrad	Svábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
Gordius preslii...	4 spg			1♂		Vejdovsky, '94 / Vejdovsky, '12	Zeit. wiss. Zool., 57, p 642 / Kon. Böhm. Gesel. Wissen. Prague, p. 1
Gordius preslii...	4 oog	1♀			4 chroms form 1 tetrad	Svábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
Gordius tolosanus...		8♀				Camerano, '90	Mem., R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
Gordius tolosanus...	4 oog	1♀			4 chroms form 1 tetrad	Svábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
Gordius tolosanus...	4 spg / 4 oog	2♀				Vejdovsky, '12	Kon. Böhm. Gesel. Wissen. Prague, p. 1
Gordius villoti...		8♀				Camerano, '90	Mem., R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
Paragordius varius...	14 cl	7♀	7♀	7♀ / 7♂ pron		Montgomery, '04	Proc. Acad. Nat. Sc. Phila., 56, p. 735
e. Nematoidea							
Ancyracanthus cystidicola...	11 spg / 12 oog / 11♂ cl / 12♀ cl / 11♂ som	6♂ / 6♀	5, 6♂ / 6♀	5, 6♂ and ♂ pron. / 6♀ and ♀ pron.	X to pole in 1st	Mulsow, '11 / Mulsow, '12	Zoöl. Anz., 38, p. 484 / Arch. Zellf., 9, p. 63
Angiostomum nigrovenosum (=Ascaris nigrovenosa)...		6♀ (sometimes 5)	6♀ (sometimes 5)		Hermaphroditic generation	McDowall, '06 / McDowall, '08	Proc. Camb. Phil. Soc., 13, p. 309 / Proc. Camb. Phil. Soc., 14, p. 613

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Angiostomum nigrovenosum (= Rhabdonema nigrovenosa)	12 oog	7♂, sometimes 6, hetero-chroms united 6♀	7♂ 6♀	6♂. Later 5, 6, hetero-chrom dis-carded in the half cells. One case 5, 7, both hete-rochroms to same pole 6♀	Hermaphroditic generation. 2 heterochroms to opposite poles in 2nd ♂		
	11 emb. germ cells (♂?) 22 emb. som cells (♂?) 12 emb. germ cells (♀?) 24 emb. som cells (♀?)	11♂ 11♀			Separate generations	Schleip, '11 Schleip, '11	Ber. d. Naturf. Ges. Freiburg, 19, p. 1; or Arch. Zellf., 7, p. 87
Ascaris canis (A. mystax).	22 spg 22 oog 22 cl 11 (tetrads) prim. germ cells	11♂ 11♀	11♂ 11♀	11♂ 11♀	Chrom diminution in som cells. Walton '16 says this is A. triquetra	Marcus, '05 Marcus, '06	Sitz. Ber. Gesel. Morph. u. Physiol. Munchen 21, p. 39 Arch. mikr. Anat., 68, p. 441
Ascaris canis.	30 spg 36 oog 30, 36 som	18♂ 18♀	12, 18♂ 18♀	12, 18♂ 18♀	X (= 6 el) to pole in 1st. Fragmenta-tion in som cells into 60 (in ♂) or 72 (in ♀) monad chroms	Walton, '16 Walton, '16 Walton, '18	Jour. Parasitol., 3, p. 39 Biol. Bull., 31, p. 364 Jour. Morph., 30, p. 527
Ascaris clavata.		24♀	24♀	24♀	In 2 cases, no. is double	Carnoy, '86	La Cellule, 3, p. 229
Ascaris of dog (not A. mystax).		2 groups of 4♀	1 group of 4♀	4 (divided) ♂ pron		Carnoy, '86	La Cellule, 3, p. 1

		16 ♀	8 ♀	4 ♀			
Ascaris des Hundes.......		16 ♀			Uncertain if same as Carnoy's	Lukjanow, '89	Arch. mikr. Anat., 34, p. 397
Ascaris felis.		9♂	9♂	9♂	XY to poles in 1st	Edwards, '12	Arch. Zellf., 7, p. 309
Ascaris felis............		9♂	9♂	9♂	XY (or X attached to autosome) to poles in 1st	Walton, '16	Biol. Bull., 31, p. 364
Ascaris incurva	35 spg / 42 oog	21♂ / 21♀	14, 21♂ / 21♀		XY to poles in 1st. X=8 cl. Chromatin diminution in somatic cl.	Goodrich, '14 / Goodrich, '16	Biol. Bull., 27, p. 147 / Jour. Exp. Zool., 21 p. 61
Ascaris lumbricoides.....		20-24 ♀ (prob. 24)	24 ♀	24 ♀		Carnoy, '86	La Cellule, 3, pp. 1, 229
Ascaris lumbricoides......		24 ♀ (some-times 25)	24 ♀	24 ♀		Boveri, '86 Boveri, '87	Sitz. Ber. Gesel. Morph. u. Physiol. Munchen, 2, p. 101 / Jen. Zeits., 14, p. 423 (Zellen-Studien 1)
Ascaris lumbricoides......	48-50 cl			24♂ pron / 21♀ pron	Chromatin diminution and breaking of chroms in somatic cleavages	Bonnevie, '02	Jen. Zeits., 29, p. 275
Ascaris lumbricoides......	43 spg	24♂ / 24♀	19, 24♂ / 24♀	19, 24♂	X to pole in 1st. X=5 cl	Edwards, '10 / Edwards, '10	Science, 31, p. 514 / Arch. Zellf., 5, p. 422
Ascaris megalocephala var. bivalens (Hertwig '90)......		2 ♀		2 ♀		Schneider, '83	'Das Ei und Seine Be-fruchtung,' Breslau
Ascaris megalocephala var. bivalens.	4 sex cells / 4 cl	4♂ / 4♀	4 ♀		Diminution and fragmentation of chroms in som cl	Nussbaum, '84 / Nussbaum, '02	Arch. mikr. Anat., 23, p. 155 / Arch. mikr. Anat., 59, p. 647
Ascaris megalocephala var. bivalens..	1 spg / 4 oog / 1 cl	2♂ / 2♀	2♂ / 2♀	2♂ pron / 2♀ pron.		Van Beneden, '83 ('84) / Van Beneden and Julin, '84 / Van Beneden and Neyt, '87 / Van Beneden, '88	Arch Biol., 4, p. 265 / Bull. Acad. Roy. des Sc. de Belgique, Ser III, t. 7, p. 312 / Bull. Acad. Roy des Sc. de Belgique, Ser III, t. 14, p. 215 / Anat. Anz., 3, p. 104

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Ascaris megalocephala var. bivalens..........	4 cl	2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ pron / 2♀ pron		Carnoy, '86 / Carnoy, '86	La Cellule, 2, p. 1 / La Cellule, 3, pp. 1 and 229
Ascaris megalocephala var. bivalens..........	4 cl	2♀	2♀	2♂ pron / 2♀ pron / 3♂ pron (2 cases)	Diminution and fragmentation of chroms in som cl X sometimes fused with other chrom., sometimes separate	Carnoy and Lebrun, '97	La Cellule, 13, p. 61
						Boveri, '87	Sitz. Gesell. Morph. u. Physiol. München, 3. p. 71
						Boveri, '87 / Boveri, '87	Anat. Anz., 2, p. 688 / Jen. Zeits., 14, p. 423 (=Zellen-Studien I)
						Boveri, '88	Jen. Zeits., 15, p. 685 (=Zellen-Studien II)
						Boveri, '04	"Ergeb. Konstitution d. chrom. Substanz d. Zellkerns." Jena / Arch. Zellf., 3, p. 181 / Arch. Zellf., 4, p. 132 / Fest. R. Hertwig, III, p. 129
Ascaris megalocephala var. bivalens..........		2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ pron / 2♀ pron		Boveri, '09 / Boveri, '09 / Boveri, '10	
Ascaris megalocephala var. bivalens..........		2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ pron / 2♀ pron		Van Gehuchten, '87	Anat. Anz., 2, p. 751
Ascaris megalocephala var. bivalens..........	4 cl	2♀ (=8 el)	2♀ (=4 el)	2♂ pron / 2♀ pron	Also "parachromosomes"	Zacharias, '87 / Zacharias, '87	Anat. Anz., 2, p. 787 / Arch. mikr. Anat., 30, p. 111
						Zacharias, '12 / Zacharias, '12	Anat. Anz., 42, p. 353 / Zool. Anz., 40, p. 25
Ascaris megalocephala var. bivalens..........	4 cl		2 groups of 2 each ♀	2♂ pron / 2♀ pron		Dostoiewsky, '88	Anat. Anz., 3, p. 646
Ascaris megalocephala var. bivalens..........	4 cl	2 groups of 4 each ♀		2♀ / 2♂ pron		Kultschitzky, '88 / Kultschitzky, '88	Sitz. k. Akad. wissen. Berlin, 88, p. 17 / Arch. mikr. Anat., 31, p. 567
Ascaris megalocephala var. bivalens	4 spg 4 oog	2♂ 2♀	2♂	2♂		Hertwig, '90	Arch. mikr. Anat., 36, p. 1

Species	Cells					Notes	Author	Reference
Ascaris megalocephala var. bivalens	4 spg				2♂		Brauer, '93	Arch. mikr. Anat. 42, p 153
Ascaris megalocephala var. bivalens	4 sex cells						Von Wasielewski, '93	Arch. mikr. Anat. 41, p 324
Ascaris megalocephala var. bivalens	4 (or 2 double) cl. Sometimes 6. Sometimes fragmentation in primi. germ cells, giving 8–12+						Vom Rath, '94	Biol. Centralb., 14, p 449
Ascaris megalocephala var. bivalens	4 cl. Sometimes fragmentation or double fert., giving 5–6						Herla, '95	Arch. Biol., 13, p. 423
Ascaris megalocephala var. bivalens	4 cl / 2 cl (anomaly)			2♀		Fragmentation of chroms	Zoja, '96	Arch. mikr. Anat., 47, p. 218
Ascaris megalocephala var. bivalens	4 oog	2♀	2♀	2♀			Sabaschnikoff, '97	Bull. Soc. Imp. d. Nat. d. Moscou, 9, p. 82
Ascaris megalocephala var. bivalens		2♀	2♀		2♀ pron / 2♂ pron		Moszkowski, '02	Arch. mikr. Anat., 59, p. 388
Ascaris megalocephala var. bivalens	4 cl	2♀	2♀	2♀			Montgomery, '04 / Montgomery, '08	Biol. Bull, 6, p. 137 / Arch. Zellf., 2, p. 66
Ascaris megalocephala var. bivalens	4 cl	2♂ / 2♀	2♀	2♀			Tretjakoff, '05	Arch. mikr. Anat., 65, pp. 358 and 383
Ascaris megalocephala var. bivalens			2♀	2♀	2♀		Griggs, '06	Ohio Naturalist, 6, p 519
Ascaris megalocephala var. bivalens	5 spg / 5 cl					One small chrom in some eggs of most worms, due to fragmentation or a sex chrom	Boring, '09	Arch. Zellf., 4, p. 120

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Ascaris megalocephala, var. bivalens.	5 spg	3♂	3♂ or 2, 3	2, 3♂	X to pole in 1st or 2nd (3 worms)	Edwards, '10 Edwards, '10	Science, 31, p. 514; Arch. Zellf., 5, p. 422
Ascaris megalocephala, var. bivalens.	4 cl	2♀	2♀	2♀ pron 2♂ pron		Retzius, '11	Biol. Untersuchungen, 16, p. 21
Ascaris megalocephala, var. bivalens.	4 cl			2♂ pron 2♀ pron		Vejdovsky, '12	König. Böhm. Gesel. Wissen. Prag, p. 1
Ascaris megalocephala, var. bivalens.	27♂ (?) emb. 36♀ (?) emb. 1-8 cl			1-2♂ pron	Abnormalities in cl. (one animal).	Kautzsch, '12	Arch. Entwick., 35, p. 642
Ascaris megalocephala, var. bivalens.	5 cl (♂?) 6 cl (♀?) or 4 cl	3♀ or 2	3♀ or 2	3♀ or 2 or 3♂ pron	X separate or fused with another chrom	Frolowa, '12	Arch. Zellf., 9, p. 149
Ascaris megalocephala, var. bivalens.	4 spg 4 oog 4 cl	2♂ 2♀	2♂ 2♀	2♂ 2♀		Fauré Fremiet, '13	Arch. d'Anat. mikr., 15, p. 435
Ascaris megalocephala, var. bivalens.	4 oog	2♀	2♀			Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Ascaris megalocephala, var. bivalens.		2♀				de Saedeleer, '13	La Cellule, 28, p. 301
Ascaris megalocephala, var. bivalens.	2-7 cl	4♀, rarely 5 due to piece from 1 chrom. broken off	4♀, sometimes 8, no 1st p. b.; 5 or 6 due to fragments	2♀, also several due to fragmentation	One animal. Dyads instead of tetrads	Geinitz, '15	Arch. Zellf., 13, p. 588
	4 cl (no X) 5 cl (1 X) 6 cl (2 X) 7 cl (X and only 1 p.b.) ca. 52♂ emb (49-54) ca. 60♀ emb (58-62)	3♀	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	Three animals. X (double) to pole in 1st or 2nd, or divides in both. X = 8 el in embryos. Rarely X = 4 el		

Species	Cells			pron	Observations	Author	Reference
Ascaris megalocephala, var. trivalens (Zacharias, '12)......	3 cl			1♂ (?) pron 2♀ (?) pron	Probably cross biv. X univ. Sometimes small acces. chrom	Zacharias, '12	Biol. Centralb., 32, p. 718
Ascaris megalocephala, var. univalens (Hertwig, '90)....	2 cl	1♀	1♀	1♀ 1♂ pron	Chromatin diminution and fragmentation of chroms in som cells	Boveri, '87	Jen. Zeits, 14, p. 423 (Zellen-Studien I)
						Boveri, '88	Jen. Zeits., 15, p. 685 (Zellen-Studien II)
						Boveri, '92	Sitz. Gesel. Morph. u. Physiol. Munchen 8, p. 114
						Boveri, '99	Fest. Von Kupffer, p. 383
						Boveri, '04	"Ergeb. Konstitution d. chrom. Substanz d. Zellkerns," Jena
						Boveri, '09	Arch. Zellf., 3, p. 181
Ascaris megalocephala, var. univalens......	2 cl			1♂ pron 1♀ pron		Dostoiewsky, '88	Anat. Anz., 3, p. 646
Ascaris megalocephala, var. univalens......	2 cl					Kultschitzky, '88	Arch. mikr. Anat., 31, p. 567
Ascaris megalocephala, var. univalens......	2 spg 2 oog	1♂	1♂	1♂		Hertwig, '90	Arch. mikr. Anat., 36 p. 1
Ascaris megalocephala, var. univalens......	2 spg 2 oog 2 cl	1♂ 1♀	1♂ 1♀	1♂ 1♀		Schneider, '91	Arb. Zool. Inst.Wien, 9, p. 170
Ascaris megalocephala, var. univalens......	2 sex cells					Von Wasielewski, '93	Arch. mikr. Anat., 41, p. 324
Ascaris megalocephala, var. univalens......	2 spg	1♂	1♂	1♂		Brauer, '93	Arch. mikr. Anat., 42, p. 153
Ascaris megalocephala, var. univalens......	2 cl	4 cl♀	2 cl♀	1♀ 1♂ pron		Herla, '95	Arch. Biol. 13, p. 423
Ascaris megalocephala, var. univalens......	2 cl			1♂ pron 1♀ pron		Carnoy and Lebrun, '97	La Cellule, 13, p. 61
Ascaris megalocephala, var. univalens......	2 sex cells 2 cl				Diminution and fragmentation of chroms in som cl	Nussbaum, '02	Arch. mikr. Anat., 59, p. 647

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Ascaris megalocephala, var. univalens.........	2 spg	1♂	1♂	1♂		Tretjakoff, '05	Arch. mikr. Anat., 65, p. 383
Ascaris megalocephala, var. univalens.........		1♀				Bonnevie, '08	Arch. Zellf., 2, p. 201
Ascaris megalocephala, var. univalens.........	3 cl				Small chrom due to fragmentation or a sex chrom (in 1 or 2 eggs)	Boring, '09	Arch. Zellf., 4, p. 120
Ascaris megalocephala, var. univalens.........	3 cl				X in ♂. One worm	Edwards, '10	Arch. Zellf., 5, p. 422
Ascaris megalocephala, var. univalens.........	2 oog	1♀ (=4 el)				Blanckertz, '10	Arch. Zellf., 6, p. 1
Ascaris megalocephala, var. univalens.........				1♂ pron 1♀ pron		Zacharias, '12	Anat. Anz., 42, p. 353
Ascaris megalocephala, var. univalens.........	2 cl			1♂ pron 1♀ pron	.	Held, '12	Verh. Anat. Gesel., 26, p. 242
Ascaris megalocephala, var. univalens.........	2 spg 2 oog	1♀				Fauré Fremiet, '13	Arch. d'Anat. mikr., 15, p. 435
Ascaris megalocephala, var. univalens.........	2 oog	1♀ 1♂				Retzius, '14	Biol. Untersuchungen, 17, pp. 19 and 30
Ascaris mystax, see A. canis							
Ascaris nigrovenosa, see Angiostomum n.							
Ascaris triquetra.........	22 sex cells		4♀	2♀ 4♂ pron		Walton, '16	Jour. Parasitol., 3, p. 39
Coronilla scillicola (or robusta).........	8 cl	8♀				Carnoy, '86	La Cellule, 3, pp. 1 and 63
Cucullanus elegans.........	12 cl					Martini, '03	Zeit. wiss. Zool., 74, p. 501

Filaria papillosa	11? cl / 12? cl	6♀	6♀	5, 6♂	X to pole 1st	Meves, '15	Arch. mikr. Anat., 87, II, p. 12
Filaroides mustelarum	16 cl	8♀	4♀	2♀ (Prob. 4♂ pron)		Carnoy, '86	La Cellule, 3, pp. 1 and 63
Heterakis sp.?	9 spg	5♂ / 5♀	4, 5♂ / 5♀	4, 5♂ / 5♀	X to pole in 1st	Boveri, '09	Arch. Zellf., 4, p. 135
Heterakis dispar	9 spg / 10 oog	5♂ / 5♀	4, 5♂ / 5♀	4, 5♂ / 5♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
Heterakis inflexa		5♂ / 5♀	4, 5♂		X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
Heterakis vesicularis	9 spg / 10 oog	5♂ / 5♀	4, 5♂ / 5♀	4, 5♂ / 5♀	X to pole in 1st	Gulick, 11	Arch. Zellf., 6, p. 339
Ophiostomum muticoratum	12 cl	6 (double) ♀	6 (double) ♀	6♀ / 4+♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
Oxyuris ambigua	3-4? spg	1-3♀	1-3?	1-3?		Löwenthal, '89 / Löwenthal, '90	Inter. Monats. Anat u Physiol. 6, p 364 / Inter. Monats. Anat. u Physiol., 7, p. 375
Rhabditis aberrans	18 cl / 18 som	10♂ (= 8 biv. +2 univ.) ♀ Rarely biv. no univ. 18♀	10♂	9♂ / 18♀	Only 1 p. b. Sperm degenerate in egg, really pa. XY to poles in 2nd ♂. Occasionally one sex chrom discarded in Restkörper	Kruger, '12 / Kruger, '13	Zool. Anz., 40, p. 233 Zeit. wiss. Zool., 105, p. 87
Rhabditis nigrovenosa	11 cl (♂?) / 12 cl (♀?)	6♂ / 6♀	5, 6♂ / 6♀	5, 6♂ (sperm with 5 not functional) 6♀	Separate generations X to pole in 1st	Boveri, '11	Verh. phys. med. Gesell. Würzburg, 41, p 83
Rhabdonema nigrovenosa, see Angiostomum n	12 oog / 12 som	5 + 2 X♂ / 6♀	5 + 2 X♂ / 6♀	5, 6, 7♂ (sperm with 7 degenerate) 6♀	Hermaphrodite generation 2 X to pole in 2nd or X to each pole		
Sclerostomum (= Strongylus) edentatum Sclerostomum equinum Sclerostomum vulgare	11 spg / 12 oog	6♂ / 6♀	5, 6♂ / 6♀	5, 6♂ / 6♀	X to pole in 1st or 2nd	Kühtz, '13	Arch. mikr. Anat., 83, Abt II, p 191

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Spiroptera strumosa......	12 cl	8♀	4♀	2♀ 4♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
Strongylus, see Sclerostomum							
Strongylus filaria.........	12 spg 12 oog 12 cl	6♂ 6♀	6♂	6♂ 6♀		Struckmann, '06	Zool. Jahrb., 22, p. 577
Strongylus paradoxus....		6♀				Struckmann, '06	Zool. Jahrb., 22, p. 577
Strongylus paradoxus....	11 spg 12 oog 11 cl♂ 12 cl♀	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
Strongylus tenuis.........		6♂	6♂	5, 6♂	X to pole in 2nd	Gulick, '11	Arch. Zellf., 6, p. 339
Strongylus tetracanthus...		6♀		5, 6♂		Meyer, '95	Jen. Zeits., 22, p. 391

IX. NEMERTINEA
a. DIMYARIA

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Tetrastemma vermiculus.		4♀	2♀	2♀		Le bedinsky, '97 / Lebedinsky, '97	Arch. mikr. Anat., 49, p. 503 / Biol. Centralb., 17, p. 113

b. TRINYARIA

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Cerebratulus lacteus. ...			5♀ (in p. b.)			C. B. Wilson, '00	Q. J. M. S., 43, p. 97
Cerebratulus lacteus......	36 or 38 cl	18 or 19♀				Yatsu, '07 / Yatsu, '09	Biol. Bull., 13, p. 300 / Jour. Morph., 20, p. 353
Cerebratulus marginatus.	32 cl	16♀	16♀	16♀		Coe, '99	Zool. Jahrb., 12, p. 425
Cerebratulus marginatus.		16♂	16♀	16♀		Kostanecki '02	Bull. Inter. Acad. Sc. Cracovie 1902, p. 270

Species					Notes	Authority	Reference
Lineus gesserensis..........				8♀		Arnold '99	Trav. Soc.Imp.Nat.St. Petersburg no.9, p. 1
Lineus lacteus..........	32? cl		8♀	16?♀		Meek '13	Phil. Trans. Roy. Soc. London 203B, p. 1.
Lineus ruber..........	16 cl	8♀ (=32 el)		8♀ 8♂ pron		Nusbaum and Oxner, '13	Zeit. wiss. Zool.,107, p. 78
Micrura caeca..........	32 cl	16♀	8♀			Coe, '99	Zool. Jahrb., 12, p. 425

X. PLATHELMINTHES
a CESTODA

Species					Notes	Authority	Reference
Avitellina centripunctata		4?♀				Gough, '11	Q. J. M. S., 56, p. 317
Moniezia expansa....... Moniezia planissima .... }	12–14 ? som	6–9 ? ♂				Child, '07	Biol. Bull., 12, pp. 89, 191
Taenia serrata..........		6–10♀ (prob. 13–15♀)				Von Janicki, '07	Zeit. wiss. Zool., 87, p. 685

b. TREMATODA
1. Digenetica

Species					Notes	Authority	Reference
Bilharzia haematobia, see Schistosomum haematobium							
Brachycoelium salamandrae (=B. crassicole) ...	20 spg 20 oog 20 cl	10♂ 10♀				Von Kemnitz, '13	Arch. Zellf., 10, p. 470
Dicrocoelium lanceatum (=Distomum lanceolatum)..........	20 oog	10♀	10♀	10♀		Goldschmidt, '08	Arch. Zellf., 1, p. 232
Dicrocoelium lanceatum (=Distomum lanceolatum)..........	20 spg	10♂	10♂			Dingler, '10	Arch. Zellf., 4, p. 672
Diplodiscus temporatus..	16 pa cl	16♀			One p. b. for pa.	Cary, '09	Zool. Jahrb., 28, p. 595
Distomum hepaticum....		6–8♀				Henneguy, '06	Arch. d'Anat. micros., 9, p. 47
Distomum hepaticum, see Fasciola hepatica							

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Distomum lanceolatum, see Dicrocoelium lanceolatum							
Distomum turgidum.....	18 cl	9♀	9♀	9♀		Levy, '11	Arch. mikr. Anat., 85, Abt. II, p. 125
Fasciola hepatica (= Distomum hepaticum).....	8 cl	-	6♀	4♂ pron 4♀ pron		Schumann, '05	Zool. Jahrb., 21, p. 571
Fasciola hepatica.........	12 oog 12 cl	6♀, some cases 12♀, no reduction	6♀	6♀		Schellenberg, '11	Arch. Zellf., 6, p. 443
Fasciola hepatica.........	10 (pairs) spg 10 (pairs) oog	10 (pairs) ♂ 10 (pairs) ♀	5 (pairs) ♂	5 (pairs) ♂	5 pairs to each pole in 1st div.	Delorne, '11	Arch. Zool. exp. et gen. Ser. V, t. 9, p. 1
Schistosomum haematobium (= Bilharzia haematobia).............	14 spg	8♂ 8♀	6, 8♂	6, 8♂	2 X to pole in 1st	Lindner, '14	Arch. Zellf., 12, p. 516
Zoogonus mirus.........	10 spg 10 oog 10 cl 10 som	10♀	10♀	5♀ 5♂ pron	Reduction in 2nd div. 5 to each pole	Goldschmidt, '05 Goldschmidt, '08	Zool. Jahrb., 21, p. 607 Arch. Zellf., 2, p. 348
Zoogonus mirus.........	20+cl 22-26 som	11-13♀	11-13♀	11-13♀ 10-13♂ pron		Schreiner, '08	Skr. Vidensk-Selsk. Christiana, Math-Naturw., 1, no.3, p.1
Zoogonus mirus.........	12 spg 12-14 cl (prob. 12 cl) 12-14 som	6♂ 6♀	6♀	6♂ pron 6♀		Gregoire, '09	La Cellule, 25, p. 243
Zoogonus mirus.........	11-14 oog (prob. oog) 12 11-14 cl 12 som	6-7♀	6♀	7♂		Wassermann, '11 Wassermann, '12 Wassermann, '13	Sitz. Gesel. Morph. u. Physiol. München, 27, p. 128 Verh. Anat. Gesel., 26, p. 47 Arch. mikr. Anat., 83, Abt. II, p. 1

2. Monogenetica

			Prob. 8♀		Karyomerites		
Gyrodactylus elegans....	8 cl (sometimes 9)					Von Janicki, '03	Zool. Anz., 26, p. 241
Gyrodactylus elegans....	12 spg / 12 cl	8♀	8♀	4♀ / 4♂ pron	Reduction in 2nd	Kathariner, '04	Zool. Jahrb. Suppl., 7, p. 519
Gyrodactylus elegans....	12 cl	6♀	6♀	6♀ / 6♂ pron	Karyomerites	Gille, '14	Arch. Zellf., 12, p. 415
Polystomum integerrimum....	ca. 20 cl	10♀		10♀		Halkin, '02	Arch. Biol., 18, p. 291
Polystomum integerrimum....	8 cl	8♀	8♀	4♀		Goldschmidt, '02	Zeit. wiss. Zool., 71, p. 397

c. TURBELLARIA
1. Polycladidae

Cyloporus papillosus...	16 cl	8♀	8♀	8♀	Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 55, p. 5
					Francotte, '98	Arch. Zool. exper. et gen., Ser. III, t. 6, p. 189
Eustylochus ellipticus...	20 cl	8♀	10♀	10♀ pron / 10♂ pron	Van Name, '99	Trans. Conn. Acad. Sc. 10, p. 263
Leptoplana tremellaris...	16 cl	8♀	8♀		Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 55, p. 5
Oligoclades auritus...		8♀			Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 55, p. 5
Planocera inquilina...		10♀		9–10♀	Wheeler, '94	Jour. Morph., 9, p. 167
Planocera inquilina...		10♀			Patterson and Wieman, '12	Biol. Bull., 23, p. 271
Planocera nebulosa...	20 cl	10♀		10♀ / 10♂ pron	Van Name, '99	Trans. Conn. Acad. Sc. 10, p. 263
Prostheceraeus vittatus...	12 cl	6♀		6♀ / 6♂ pron	Klinckowstrom, '97	Arch. mikr. Anat., 48, p. 587

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Prostheceracus vittatus..		6♀	6♀	6♀ pron		Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 55, p. 5
						Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 189
		6♀				Gerard, '01	La Cellule, 18, p. 139
Prosthiostomum siphunculus...	16 cl	8♀	8♀	8♀		Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 189
Stylochus pelidium......		9♀				Gerard, '01	La Cellule, 18, p. 139
				2. Rhabdocoela			
Grafilla gemellipara.....	8 cl	4♀				Patterson, '12	Biol. Bull., 22, p. 173
Grafilla gemellipara, see Paravortex g.							
Mesostomum ehrenbergi.	ca. 7 cl				Probably refers to cleavage	Schneider, '83	"Das Ei und Seine Be-fruchtung." Breslau
Mesostomum ehrenbergi.	10 cl			5♀	Summer egg	Breslau, '04	Zeit. wiss. Zool., 76, p. 213
Mesostoma ehrenbergi...	10 oog	5 (pairs) ♀	5♀	5♀ 5♂ pron		Von Voss, '14	Arch. Zellf., 12, p. 159
Paravortex cardii........	4 cl		4♀(=8el)	2♀ (=4el)		Hallez, '08	C. R. Acad. Sc., 147, p 314
						Hallez, '08	Arch. Zool. exper. et gen., Ser. IV, t. 9, p. 429
Paravortex gemellipara (Grafilla g.).........	8 cl		4♀			Ball, '16	Jour. Morph., 27, p. 453
Polychoerus caudatus....	31 cl					Gardiner, '98	Jour. Morph., 15, p. 73
Vortex viridis.............	4 spz 4 som	2♂	2♂		Two chroms fuse in 2nd div.	Lepeschkin, '10	Biol. Zeits. Moscow, 1, p. 104

3. *Tricladida*

		8♀ (4-8), 4 in prophase	8♀ (4 in pro-phase)	4♀			
Dendrocoelum lacteum	16 cl				No. doubled in 1st and 2nd meta-phase; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Dendrocoelum lacteum		8♂	8♂			Schleip, '07	Zool. Jahrb., 24, p. 129
Dendrocoelum lacteum	14 spg	7♀				Gelei, '13	Arch. Zellf., 11, p. 51
Dendrocoelum lacteum, see Planaria lactea							
Planaria alpina	20-24 spg					Rappeport, '15	Arch. Zellf., 14, p. 1
Planaria gonocephala	16 spg 16 oog	8♂ 8♀	8♂	8♂		Schleip, '06 Schleip, '07	Zool. Jahrb., 23, p. 357 Zool. Jahrb., 24, p. 129
Planaria lactea (=Den-drocoelum lacteum)	16 oog	8♀ 8♂	8♂	8♂		Arnold, '09	Arch. Zellf., 3, p. 431
Planaria lactea, see Dendrocoelum lac-teum							
Planaria polychroa	16 cl	8♀ (4-8), 4 in prophase	8♀ (4 in pro-phase)	4♀	No. doubled in met-aphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Planaria simplissima	8 spg 6 cl 6 som	3 or 4♂ 3, 4, 6♀	3 or 4♂ 3-6♀		May be two kinds, 3 and 6 reduced	Stevens, '04	Proc. Acad. Nat.Sc. Phila., 56, p. 208
Planaria torva	16 cl	8♀ (4-8), 4 in prophase	8♀ (4 in pro-phase)	4♀	No. doubled in met-aphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Polycelis nigra		8♂	8♂			Schleip, '07	Zool. Jahrb., 24, p. 129
Procerodes gerlachei	12 spg 12 som	6♂	6♂			Böhmig, '07	Arch. Biol., 23, p. 1
Thysanozoon brocchi	18 spg 18 cl	9♂ 9♀	9♀	9♂ pron 9♀		Van der Stricht, '97 Van der Stricht, '98	Verh. Anat. Ges. 11. p. 92 Arch. Biol. 15, p. 367
Thysanozoon brocchi	18 cl	9♀	9♀			Schockaert, '02 Schockaert, '05	La Cellule, 20, p. 101 La Cellule, 22, p. 1

XI. PORIFERA

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Clathrina coriacea	ca. 16 collar cells					Robertson and Minchin, '10	Q. J. M. S., 55, p. 611
Grantia compressa	8-10 spg / 8-10 oog	8-10♀				Dendy, '14	Q. J. M. S., 60, p. 313
Sycandra raphanus	32 cl			16♀		Maas, '99	Anat. Anz., 16, p. 290
Sycandra raphanus	8 oog / 16 cl	8♀	8♀	8♀		Jörgensen, '09	Arch. Zellf., 4, p. 163

XII. ROTIFERA

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Hydatina senta		10 or 12♀ pa egg / 5♂ pa egg / 12 winter egg			No. p. b.'s / 1 p. b. / Prob. 2 p. b.'s	Lenssen, '98 / Lenssen, '98	La Cellule, 14, p. 419 / Zool. Anz., 21, p. 617
Hydatina senta		22-25♀ pa egg / 11-13♂ pa egg / 14 winter egg (fert)			1 p. b. / 2 p. b.'s / 2 p. b.'s	Whitney, '00	J. Exp. Zoöl., 6, p. 137

B. PROTOCHORDATA
I. ACRANIA (CEPHALOCHORDA)

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Amphioxus lanceolatus			10?♀		1 count	Van der Stricht, '95 / Van der Stricht, '96	Bull. Acad. roy. Belgique, Ser. 3, t. 30, p. 528. Same as / Arch. Biol., 14, p. 469
Amphioxus lanceolatus	24 cl	12?♀	10-15♀ (prob. 12)			Sobotta, '97	Arch. mikr. Anat., 50, p. 15
Amphioxus lanceolatus	24 oog	12♀	12♀	12♀		Cerfontaine, '05 / Cerfontaine, '05	Acad. roy. Belgique, Cl. de Science, '05, p. 643. Same as / Arch. Biol., 22, p. 229

II. UROCHORDA

Species						Authority	Reference
Ascidia mentula.........		9♀				Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
Cionia intestinalis.......	18 cl			9♂ pron 9♀ pron		Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
Distaplia occidentalis ..			12?♀			Bancroft, '99	Bull. Mus. Comp. Zool. Harvard, 35, p. 57
Phallusia mammillata......	16 cl (13-16)	8♀	8♀	8?♀ (4-9) 8♂ pron (8 or 9)		Hill, '95; Hill, '95	Rep. Brit. Assoc. Adv. Sc. Ipswich, p. 474; Q. J. M. S., 38, p. 315
Styelopsis grossularia....	4 spg 4 oog	4♂ 8♀	2♂ 4♀	1♂ 2♀	Chroms of spermatid probably divide in two in fert	Julin, '93	Bull. sc. Fr. et Belgique, 25, p. 93

C. VERTEBRATA
I. AMPHIBIA
a. ANURA

Species						Authority	Reference
Alytes obstetricans......	32 spg	16♂				Jansens et Willems, '09	La Cellule, 25, p. 151
Bombinator igneus.......			6-7♀			Lebrun, '01	La Cellule, 19, p. 315
Bufo calamita		12+♀				Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, p. 6, t. 101
Bufo lentiginosa.........	24 spg 24 oog	12♂ 12♀	12♂ 12♀	12♀		King, '02; King, '07; King, '08	Anat. Anz., 21, p. 411; Amer. Jour. Anat., 7, p. 315; Jour. Morph., 19, p. 369
Bufo vulgaris		8-10♀	8♀			Carnoy and Lebrun, '00; Lebrun, '01	La Cellule, 17, p. 199; La Cellule, 19, p. 315
Bufo vulgaris....	18-24 oog		12♀			Della Valle, '07	Atti R. Accad. Sc. di Napoli, Ser. 2a, no. 13, vol. 13, p. 1
Bufo vulgaris............		8-9♀				Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, t. 6, p. 101
Pelodytes punctatus......		6♀				Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, t. 6, p. 101

I. AMPHIBIA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Rana catesbiana.........	26 oog					Swingle, '17	Biol. Bull., 33, p. 70
Rana esculenta 'Gruner Frosch'.........	24 som					Schottländer, '88	Arch. mikr. Anat., 31, p. 426
Rana esculenta.........	24 (not stated where)					Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
Rana esculenta.........	16 spg					Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
Rana esculenta.........	ca. 25 spg	13♂	12, 13♂	12, 13♂	X to pole in 1st	Levy, '15	Arch. mikr. Anat., 86, II, p. 85
Rana fusca (?).........	24 spg	12♂	12♂	12♂		Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
Rana fusca.........	12 pa cl	12♀				Bataillon, '10	Arch. Zool. exp. et gen. Ser. V, t. 6, p. 101
Rana fusca.........	20+ pa som				Regulation to normal no.	Brachet, '11	Arch. Biol., 26, p. 337.
Rana pipiens.........	25 spg 26 oog	13♂	12, 13♂		X to pole in 1st	Swingle, '17	Biol. Bull., 33, p. 70
Rana temporaria.........		8♂				Bertacchini, '96	Inter. Monats., 13, p. 409
Rana temporaria.........		8-10♀	10♀			Carnoy, et brun, '00 Lebrun, '01	Le-La Cellule, 17, p. 199 La Cellule, 19, p. 315
Rana temporaria (or fusca).........	24 cl ca. 12 pa cl					Levy, '13	Arch. mikr. Anat., 82, II, p. 65
Rana 'Frosch'.........	16 som				Leucocytes	Dekhuyzen, '91	Anat. Anz., 6, p. 220
'Grenouille'.........	12 (pairs) som 6 pa som			6♂ 6♀		Dehorne, '10 Dehorne, '11	C. R. Acad. Sc. Paris, 150, p. 1451 C. R. Acad. Sc. Paris, 152, p. 1123
'Leopard frog'.........	20+pa spg.				Adult pa frog. Observation of Goldschmidt	Loeb, '18	Proc. Nat. Acad. So., 4, p. 60

b. URODELA

					Author	Reference
Amblystoma 'Siredon'.	12 cl				Kölliker, '89	"Geweblehre des Menschen"
'Axolotl'.	ca. 16 cl	4–10♀ (prob. 8)	8♀		Fick, '93	Zeit. wiss. Zool., 56, p 529
'Axolotl'.	ca. 30 cl	15♀ (14–16)	15♀ (14–16)		Jenkinson, '04	Q. J. M. S., 48, p. 407
'Siredon (Amblystoma)'.	24 som				Muckermann, '13	La Cellule, 28, p. 231
Amblystoma	24 som				Mack, '14	Kansas Univ. Sc. Bull. 9, p. 119
Amphiuma.		12♂	12♂	12♂	McGregor, '09	Jour. Morph.,15, Suppl. p. 56
Aneides lugubris (Autodax). . .	28 spg (23–30)	14♂ (in 2 cases 15)			Snook and Long, '14	Univ. California Pub. 11, p. 511
Batrachoseps attenuatus.	24 spg	12♂	12♂	12♂	Eisen, '00	Jour. Morph., 17, p. 1
Batrachoseps attenuatus.	24 som	12♂	12♂	12♂	Janssens et Dumez, '03; Janssens, '05	La Cellule, 20, p. 419; La Cellule, 22, p. 377
Cryptobranchus alleghe-niensis.		12♀ (prob.)			Smith, '12	Jour. Morph., 23, p. 61
Desmognathus fuscus. . . .		12♂	12♂	12♂	Kingsbury, '99; Kingsbury, '02	Zool. Bull., 2, p. 203; Amer. Jour. Anat., 1, p. 99
Desmognathus fuscus. . . .	24 spg	12♂	12♂		Montgomery, '03	Biol. Bull., 4, p. 259
Diemyctilus torosus.		12♀ (10–12)	10–12♀		Lebrun, '02; Lebrun, '02	Biol. Bull 3, p 1; La Cellule, 20, p. 1
Geotriton fuscus.	24 spg	12♂	12♂	12♂	Terni, '10; Terni, '11; Terni, '11	Monit. Zool. Ital., 21, p. 160; Arch. ital. Anat. e Emb., 10, p. 1; Arch. Zellf., 12, p. 1

I. AMPHIBIA—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1st-CYTE	2nd-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Molge pyrrhogastra......	24 spg					Muckermann, '13	La Cellule, 28, p. 231
Necturus maculosus......		12♂			X or XY attached to another chrom, to pole in 1st. Free or attached in 2nd. Detached pieces from chroms = supernumerary, equally distributed in 1-t	King, '12	Anat. Record., 6, p. 405
Plethodon cinereus......	24 spg	12♂				Montgomery, '03	Biol. Bull., 4, p. 259
Salamandra atra.........	16 spg	12♂				Champy, '13	Arch. Zool. Exp. et gen., 52, p. 13
Salamandra maculosa....	24 som	12♂	12♂	12♂		Flemming, '82 Flemming, '82 Flemming, '87	Arch. mikr. Anat., 20, p. 1 'Zell-sub-stanz, Kern und Zelltheilung' Arch. mikr. Anat., 29, p. 389
Salamandra maculosa....	24 som cn.16 testisepithelium and egg follicle cells					Rabl, '85 Rabl, '89	Morph. Jahrb., 10, p. 214 Anat. Anz., 4, p. 21
Salamandra maculosa...	12 (double) spg 12 (double) oog 24 som (sometimes 12 double)	1'' (=48 el)	12♂ (=24 el)	12♂	Tetrads and dyads = separate elements	Vom Rath, '93 Vom Rath, '94	Zeit. wiss. Zool., 57, p. 97 Biol. Centralb., 14, p. 449
Salamandra maculosa....	24 spg 24 oog 24 som	12♂	12♂			Meves, '95 Meves, '97 Meves, '11	Anat. Anz., 10, p. 635 Arch. mikr. Anat., 48, p. 1 Arch. mikr. Anat., 77, II, p. 273

Species					Authority	Reference
Salamandra maculosa	24 spg	12♂ 12♀	12♂ 12♀		Janssens, '00 / Janssens, '01 / Janssens, '02 / Janssens, '04	Anat. Anz., 17, p. 520 / La Cellule, 19, p. 5 / Anat. Anz., 21, p. 129 / Anat. Anz., 24, p. 648
Salamandra maculosa	24 spg	12♂	12♂	12♂	Schreiner, '07	Arch Biol., 22, p. 419
Salamandra maculosa	4 43 blood cells / 19–27 larval peritoneum				Della Valle, '09 / Della Valle, '11	Archivio Zoologico, 1, p. 1 / Archivio Zoologico, 5, p. 119
Salamandra maculosa	12 (pairs) spg / 12 (pairs) som				Dehorne, '10	C. R. Acad. Sc. Paris, 150, p. 1451
Salamandra maculosa	16 spg				Dehorne, '11	Arch. Zellf., 6, p. 613
Salamandra maculosa	24 spg / 24 som				Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
'Salamandra'	24 som				Marktrmann, '13	La Cellule, 28, p. 231
'Siredon,' see under Amblystoma					Von Erlanger, '96	Zool Anz. 19, p 11
Triton alpestris		12♀	12♀		Carnoy et Lebrun, '99 / Lebrun, '01	La Cellule, 16, p. 203 / La Cellule, 19, p. 315
Triton alpestris	24 spg	12♂ 12♀	12♂ 12♀		Janssens, '00 / Janssens, '01 / Janssens, '02 / Janssens, '04	Anat. Anz., 17, p. 520 / La Cellule, 19, p. 5 / Anat. Anz., 21, p. 129 / Anat. Anz., 24, p. 648
Triton alpestris	18–24 spg	12♀	12♀		Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
Triton cristatus		12♀	12♂ 12♀		Carnoy et Lebrun, '99	La Cellule, 16, p. 203
Triton cristatus	24 spg	12♂ 12♀	12♂ 12♀		Janssens, '00 / Janssens, '01 / Janssens, '02 / Janssens, '04	Anat. Anz. 17, p. 5 / La Cellule, 19, p. 5 / Anat. Anz., 21, p. 129 / Anat. Anz., 24, p. 648
Triton cristatus	24 som / 12 regenerating blood cells				Jolly, '04	Arch. d'Anat. micros. 6, p. 455

I. AMPHIBIA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Triton cristatus.........	18-24 spg	.				Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
Triton cristatus.........	24 spg	12♂	12♂˙			Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Triton palmatus.........	18-24 spg					Champy, '13	Arch. Zool. exp. et gen 52, p. 13
Triton punctatus.........	ca. 12-16 som					Retzius, '81	Biol. Untersuchungen, '81, p. 109
Triton punctatus.........	24 spg	12♂ 12♀	12♂ 12♀			Janssens, '00; Janssens, '01; Janssens, '02; Janssens, '04	Anat. Anz, 17, p. 520; La Cellule, 19, p. 5; Anat. Anz., 21, p. 129; Anat. Anz., 24, p. 648
Triton taeniatus.........		12-14♀				Born, '94	Arch. mikr. Anat., 43, p. 1
Triton taeniatus.........		12♀	12♀			Carnoy et Lebrun, '99	La Cellule, 16, p. 203
Triton vulgaris.........	18-24 spg					Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
Triton vulgaris.........	12 pa som				Sperm destroyed with radium	Hertwig, O., '13	Arch. mikr. Anat., 82 II, p. 1
Triton.........	20 + som (prob. 24)					Rabl, '85	Morph. Jahrb., 10, p. 214
Triton sp.........	24 spg	12 (gemini)♂				Moore and Embleton, '05; Moore and Arnold, '05	Proc. Roy. Soc., London, 77, p. 555; Proc. Roy. Soc., London, 77, p. 563

II. AVES
a. ANSERES

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Anas boschas......... Aythya ferina......... Cairina moschata......... Lampronesa sponsa......... Mareca penelope.........	ca. 16 spg.	8♂	8♂			Schöneberg, '13	Arch. mikr. Anat., 83, Abt. II, p. 321

b. COLUMBAE

Columba livia domestica	16 spg	8♂	4♂ (occasionally 8)		Second pairing of chroms before 2nd	Guyer, '00 / Guyer, '02	Dissertation Univ.Chicago Univ. Cincinnati Bull. 21, Ser. II, vol. II, p. 1
Columba livia domestica	16 cl	8♀	8♀	8 sperm nucleus		Harper, '04	Amer. Jour. Anat., 3, p. 349
Columba, 'Pigeon'	ca. 16 spg	8♂	4♂			Smith, '12	Q. J. M. S., 58, p. 159
Turtur risorius	16 spg	8♂	4 (occas. 8) ♂		Second pairing of chroms before 2nd div.	Guyer, '00 / Guyer, '02	Dissertation Univ.Chicago Univ. Cincinnati Bull. 21, Ser. II, vol. II, p. 1

c. GALLINAE

Gallus domesticus		6? ♀				Loyez, '06	Arch. d'Anat. micros. 8, p. 239
Gallus 'Huhn'		8-16 (pairs) ♀ (prob. 12)				Sonnenbrodt, '08	Arch. mikr. Anat., 72, p. 415
Gallus gallus domesticus (= common fowl; Langshan, Plymouth Rock, Rhode Is. Red, and chick embryos)	18 spg 18♂ som	9♂	4, 5♂ (fusion in pairs, may be incomplete giving 6, 7, etc.)	4, 5♂. Those with 4 prob. degenerate	X to pole in 1st. X=2 elements in spg and ♂ som; X=1 element in oog and ♀ som. From correction in '16	Guyer, '09 / Guyer, 16	Anat. Anz., 34, p. 573 / Biol. Bull., 31, p. 221
Gallus domesticus	12? ps cl 12 som				Prob. no reduction	Lecaillon, '10 / Lecaillon, '10	C. R. Soc. Biol., 69, p. 34 / Arch. d'anat. micros., 12, p. 511
Gallus 'Gold Campine fowl'	18-20 spg	8-10♂			Clumping in 2nd spc.	Cutler, '18	Jour. Genetics, 7, p 155
Numida meleagris dom. (=domestic guinea)	17 spg	9♂	8, 9♂	4, 5♂ (bivalent) (few with 6)	X to pole in 1st. Second pairing of chroms before 2nd div.	Guyer, '09	Anat. Anz., 34, p 502
Phasianus 'Pheasant'	20-22 spg	10-11 ♂	5 - 6♂		Secondary pairing and fusion in 2nd div., forming 1-8 masses	Cutler, '18	Jour. Genetics, 7, p. 15

III MAMMALIA

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
a CARNIVORA							
1 Canidae							
Canis..........	64? som (8, multiple, in some cells)					Vom Rath, '91	Biol. Centralb., 14, p. 410
Canis Dog..........	21 spg 22♀ som	11♂	10, 11♂	10, 11♂	X to pole in 1st	Malone, '18	Trans. Amer. Micr. Soc., 37, p. 97
2 Felidae							
Felis 'Chat'..........	35 spg 36 oog (24-43) 36 som	18♂ 12♀ ('09)	17, 18♂	17, 18♂	1 heterochrom in ♂ to pole in 1st; 2 heterochrom in ♀	Von Winiwarter et Saintmont, '09; Von Winiwarter, '11	Arch. Biol., 24, p. 165; Arch. Roy. Belgique. Bull. Cl. Sc., no. 4 p. 221
'Chatte'..........	12♀ (at least)					R.VanderStricht, '11	Arch. Biol., 26, p. 365
'Cat, domestic'..........	11-17?	14-16?				Longley, '11	Amer. Jour. Anat., 12, p 139
3 Viverridae							
Herpestes 'Mongoose'..........	ca. 24♂				No X	Jordan, '11	Carneg. Inst.Pub.,182, p. 165
b. CHIROPTERA							
Rhinolophus hipposideros..........	16♀	16♀				Athias, '12	Arch. R. Inst. Bacter. Cam.Pest.Lisbonne, 3, p. 287
Vesperugo noctula..........	9-10♀	9-10♀				Van der Stricht, '10	Acad. Roy. Belgique. Cl. d. Sc. Mem. Ser. 2, t. 2, no. 2, p. 1

				'Heterochromosomes?'		
Vesperugo 'Bat'	24 spg (at least)				Jordan, '12	Anat. Anz., 40, p. 513
Vesperugo serotinus		15-22 ♀	18-24 ♀		Athias, '12	Arch. R. Inst. Bacter Cam. Pest. Lisbonne, 3, p. 287

c. EDENTATA

Tatu novemcinctum (= 9-banded Armadillo)	31? spg, 32 oog	16 ♀ (14-19)		X ? in ♂	Newnan and Patterson, '10; Newman, '12	Jour. Morph., 21, p. 339; Biol. Bull., 23, p. 100

d. MARSUPIALIA

Didelphys aurita	17 spg, 17 som	12? ♀	12? ♀		Hill, '18	Q. J. M. S., 63, p. 91
Didelphys virginiana (= Opossum)		9 ♂	4, 5♂ (=8, 9, univalents)	4, 5♂ (=8, 9, univalents)	X to pole in 1st. Second pairing of chroms after 1st div.	Jordan, '11	Arch. Zellf., 7, p. 41
Marsupialia 'Beuteltiere'		8♂		4 (often) ♂	Von Bardeleben, '98	Jen. Zeits, 24, p. 475
Perameles, Phalangista					Benda, '06	Semon Zool. Forsch. Australia u. Malay Archipel., p. 439

e. MONOTREMATA

Echidna, Ornithorhynchus		8-12♂			Benda, '06	Semon Zool. Forsch. Australia u. Malay Archipel., p. 415

f. PRIMATES

Homo sapiens 'Mensch'	22-28 som (prob. 24)			Cornea	Flemming, '82; Flemming, '98	Arch. mikr. Anat., 20, p. 1; Anat. Anz., 14, p. 171
'Mensch'	18-40 som			'Normal tissue'	Hansemann, '91	Virch. Arch., 123, p. 356

III. MAMMALIA—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Homo sapiens—continued 'Mensch'............	8 spg (= 16 el)	8♂	4♂	4♂	3 men, 21, 39 and 46 yrs. old	Von Bardeleben, '92 Von Bardeleben, '97 Von Bardeleben, '98	Verh. Anat. Gesel. Wien, p. 202 Arch. Anat. u. Phys. (Anat. Abt) Suppl. p. 193 Jen. Zeits., 24, p. 475
'Man'..		18♂ (15–19)			Man 54 yrs. old	Wilcox, '00	Anat. Anz., 17, p. 316
'Mensch'.	32?				Material not described	Fick, '05	Arch. Anat. u. Phys. iol. (Anat. Abt) Suppl, p. 179
'Man (Homo)'..........	32 spg	16♂ (gemini)				Moore and Arnold, '05 Moore and Walker, '06	Proc. Roy. Soc. London, 77B, p. 563 Univ. Liverpool Reports, 106, p. 1
'L'homme'...........	ca. 24 spg	12♂			Material not described	Duesberg, '06	Anat. Anz., 28, p. 475
'Man'........	22 spg	12 ♂, few have 14	5, 7♂ (=10, 12 univalents)	5, 7♂ (=10, 12 univalents)	Negro 30 yrs. old 2X to pole in 1st. Second pairing of chrom. after 1st div.	Guyer, '10	Biol. Bull., 19, p. 219
'L'homme'...........	24 som	ca. 12♂	ca. 18+♂			Branca, '10 Branca, '11 Branca, '12	C. R. Assoc. Anat., 12, p. 5 Bibl. Anat., 21, p. 233 C. R. Assoc. Anat.
'Mensch'............		ca. 12♂			Man 23 yrs. old. No X chrom	Gutherz, '12	Arch. mikr. Anat., 79, (2), p. 79
'L'homme' .	47 spg (46–49) 48 oog	24♂ (23–25)	23, 24♂	23, 24♂	♂ count from 4 men 21, 23, 25, 41 yrs. old. ♀ count from 4 mo. embryo. X to pole in 1st	Von Winiwarter, '12	Arch. Biol., 27, p. '41
'Man'.......		12♂	10, 11 or 12♂	10, 11 or 12♂	2 X to pole in 1st or 2nd. Each divides once. Negro aged 50	Montgomery, '12	Jour. Acad. Nat. Sc., Phila., 15, p. 1

Species						Author	Reference
'Man'	24 spg 33-38 som, mostly 34	12♂			From 9 mm. embryo, also negro and white (age 37) adults. XY to poles in 2nd	Wieman, '13 / Wieman, '17	Amer. Jour. Anat., 14, p. 461. Amer. Jour. Anat., 21, p. 1
'Man'		12+♂			Double X ?	Jordan, '14	Carneg. Inst. Pub. 182, p. 165
o. RODENTIA							
Cavia 'Meerschweinchen'	16 spg			8♂		Von Bardeleben, '92	Verh. Anat. Gesel., '92, p. 202
'Meerschweinchen'	Prob. 24 som					Flemming, '98	Anat. Anz., 14, p. 171
'Guinea-pig'	32 spg	16♂ (gemini)	16♂			Moore and Walker, '06	Liverpool Univ. Rep., '06, p. 1
'Guinea-pig'	56? spg	28♂			XY to poles in 1st	Stevens, '11	Biol. Bull., 21, p. 155
Cavia porcellus		24-28♀	24?♀			Athias, '12	Arch. R. Inst. Bacter. Cam. Pest. Lisbonne, 3, p. 287
'Cobaye'	16 som	8♀	8♀	8♀		Lams, '13	Arch. Biol., 28, p. 229
Eliomys quercinus		16♀	16 (10-16)♀			Athias, '09 / Athias, '12	Anat. Anz., 34, p. 1. Arch. R. Inst. Bacter. Cam. Pest. Lisbonne, 3, p. 287
Lepus 'Kaninchen'	24? som					Flemming, '98	Anat. Anz., 14, p. 171
'Lapin'	41-43 oog 36-46 som (mostly 42)	10-12♀ (from Honoré)				Von Winiwarter, '00 / Von Winiwarter, '01	Arch. Biol., 16, p. 685. Arch. Biol., 17, p. 33
'Rabbit'	28-36 spg	14-18♂				Barrat, '07	Proc. Roy. Soc. London, 79B, p. 372
'Rabbit'	22 spg	12♂ (=11)	11♂		XY to poles in 1st. XY sometimes double in 1st	Bachhuber, '16	Biol. Bull., 30, p. 294
Microtus incertus		28-34♀	28-34♀			Athias, '12	Arch. R. Inst. Bacter. Cam. Pest. Lisbonne, 3, p. 287

III. MAMMALIA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Mus decumans (see also Mus rattus) 'Rat'...............	32 spg	16♂ (gemini)			Earlier accounts of Moore corrected in '05	Moore, '93 Moore, '94 Moore and Arnold, '05 Moore and Walker, '06	Anat. Anz., 8, p. 683 Intern. Monatschr., 11, p. 129 Proc. Roy. Soc., London, 77B, p. 563 Univ. Liverpool Reports, '06, p. 1
'Ratte'..............		12♂ (8-12)				Lenhossék, '98	Arch. mikr. Anat., 51, p. 215
'Wanderratte'..........	16? spg	8♂	8♂ (8-16)			Von Ebner, '99 Von Ebner, '02	Sitz. Ber. d. k. Akad. Wissen. Wien, 108 (3), p. 429 Kölliker's 'Gewebelehre des Menschen,' III
'Rat'..............	20-30 spg	ca. 12♂				Regaud, '01 Regaud, '01 Regaud, '09	C. R. Soc. Biol., 53, p. 406 Arch. d'Anat. micros., 4, p. 231 Arch. d'Anat. micros., 11, p. 291
'Mus decumans var. albinos'.........	24 som (prob)	12♂ (prob)	12♂ (prob)			Duesberg, '06	Arch. Zellf., 1, p. 399
'Weisse Ratte'.........		16♀ (10-20)	16? ♀ (8-16)			Sobotta u. Burkhard, '10	Anat. Hefte, 42, p. 433
'Mus decumans albinos'............	More than 24 spg	16♂	Prob. 16♂			Van Hoof, '11	La Cellule, 27, p. 289
Mus musculus 'Mus musculus, var. blanche et noire'......	20? cl	20♀	20♀			Tafani, '89	Atti R. Accad. Lincei, Rendiconte, Ser. 4, vol. 5, p. 119 (also in Pub. Inst. St. sup. Firenze, Med-chir-urg. = Arch. Anat. norm. e path., 5, p. 1
'Maus'.....		16♂				Hermann, '89	Arch. mikr. Anat., 34, p. 58

Name					Remarks	Authority	Reference
'Grau Maus'	24? og	6 groups of 4 ♀				Holl, '93	Verh. Anat. Gesel. Göttingen, '93, p. 122 Sitz. Ber. d. k. Akad. Wissen. Wien, 102 (3), p. 249
'Maus, weisse, grau und Tanz'	30+cl	16♀ (10-19)	16♀		Earlier accounts corrected in '07	Holl, '93	Verh. Anat. Gesell. Göttingen, '93, p. 111
						Sobotta, '93	Arch. mikr. Anat., 45, p. 15
						Sobotta, '95	Anat. Hefte, 35, p. 493 Verh. phys-med. Gesell. Wurzburg, 39, p. 241
						Sobotta, '07 Sobotta, '08	
'Souris blanche'	12 spg (10-12)	12♂ (double). Some cells 16 (double)	12♂ (single). Some cells 16	6♂. Some cells 8		Lukianow, '98	Arch. Sc. Biol. St. Petersburg, 6, p. 285
'Mouse'	24 spg					Moore and Arnold, '05 Moore and Walker '06	Proc. Roy. Soc. London, 77B, p. 563 Univ. Liverpool Reports, '06, p. 1
'Mus musculus'		12♀	12♀	12♀		Gerlach, '06	'Uber die Bildung der Richtungskorper bei Mus musculus,' Wiesbaden
'Mus musculus=souris blanche'		12♀ (12-15)	12♀	12♀		Lams et Doorme, '07	Arch. Biol. 23, p. 259
'Mus musculus var. alba'		8♀	8♀			Melisinos, '07	Arch. mikr. Anat, 70, p. 577
'White mouse'		12♀ (12-24 due to precocious division)	12♀ (=24 univ)	12♀		Coe and Kirkham, '07 Kirkham, '07 Kirkham, '08	Science, 25, p. 778 Biol. Bull., 12, p. 259 Trans. Connecticut Acad. Arts and Sc., 13, p. 65
'Mouse, white, black and hybrid white×gray'		20♀	20♀	20♀		Long, '08 Long and Mark, '11 Kingery '14	Science, 27, p. 443 Carnegie Institute Pub. 142, p. 2 Biol. Bul., 27, p. 240
'White mouse'		12-24♀	12-30♀				
'House mouse'		20♂	20♂	1♀, 20♂	X to pole in 2nd	Yocum, '17	Univ. California Pub. 16, p. 371

III. MAMMALIA—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Mus norvegicus albinus (=white rat)	40 (pachytene threads of oocyte)				X to pole in 1st	Pratt and Long, '17	Jour. Morph., 29, p. 441
Mus norvegicus albinus	37 spg 37♂ som	19♂	18, 19♂			Allen, '18	Jour. Morph., 31, p. 133
Mus rattus, see also Mus decumans							
'Mus rattus albus'		8♀	8♀			Melissinos, '07	Arch. mikr. Anat., 70, p. 577
'Mus rattus albinos'	More than 24 spg	16♂	Prob. 16♂			Van Hoof, '11	La Cellule, 27, p. 289
Sciurus 'Ecureuil'	24 + som	ca. 16♂				Van Mollé, '07	La Cellule, 24, p. 257
b. UNGULATA							
Bos 'Stier'	16 spg			8♂		Van Bardeleben, '92	Verh. Anat. Gesell, '92, p. 202
'Taureau'	24 spg (20-25)	12♂	12♂			Schoenfeld, '02	Arch. Biol., 18, p. 1
'Taureau'	Prob. 24 spg (20-24)	12♂				Van Hoof, '13	La Cellule, 30, p. 7
Equus 'Pferde'		10-16♂				Kirillow, '12	Arch. mikr. Anat., 79, II, p. 125
'Horse'	37 spg	19,?	9, 10♂ (quad-rivalent)	9, 10,? (biva-lent)	X to pole in 1st. Chroms pair in tel-ophase of 1st div.	Wodsedalek, '14	Biol. Bull., 27, p. 295
'Mule'	51 spg	34-40♂ (mostly 40-45) some univalent			X. Cells disinte-grate, no 2nd spc	Wodsedalek, '16	Biol. Bull., 30, p. 1-38

Sus 'Pig'	18 spg 18 som♂ 20 oog 20 som♀, sometimes 10 by pairing	10♂	8, 10♂	4, 6♂ (autosomes univalent)	2 X to pole in 1st	Wodsedalek, '13 Wodsedalek, '13	Science, 38, p. 30 Biol. Bul., 25, p. 8
Sus scrofa	40 spg (1 giant cell 74) 40-58 som (1 cell 74)	20♂			Variation in no. due to fragmentation	Hance, '17 Hance, '18	Jour. Morph., 30, p. 155 Biol. Bull., 35, p. 33

IV. PISCES
a. CYCLOSTOMATA

Bdellostoma burgeri	487 spg					Schreiner, '08	Arch. Zellf., 1, p. 152
Myxine glutinosa	ca. 50 som					Retzius, '90	Verh. d. biol. Vereins Stockholm, 2, p. 80
Myxine glutinosa	ca. 52 spg ca. 52 som	26♂ (possibly 27)	26♂			Schreiner, '01 Schreiner, '04	Anat. Anz., 24, p. 561 Arch. Biol., 21, p. 183

b. DIPNOI

Lepidosiren paradoxa	Prob 36 som (34-37)					Murray, '06	Anat. Anz., 28, p. 203
Lepidosiren paradoxa	38 som	19♂ gemini				Agar, '11 Agar, '12	Q. J. M. S., 57, p. 1 Q. J. M. S., 58, p. 285

c. ELASMOBRANCHII

Pristiurus melanostomus		30-50♀				Kastschenko, '90	Zeit. wiss. Zool., 50, p. 428
Pristiurus	ca. 36 spg 30-36 oog 30-36 som	ca. 18♀	ca. 18♀	ca. 18♀		Ruckert, '92	Anat. Anz., 7, p. 107
Pristiurus Raja macrorhynchus Raja maculata	24 spg	12♂	12♂	12♂		Moore, '95 Farmer and Moore, '04	Q. J. M. S., 38, p. 275 Q. J. M. S., 48, p. 489
Scyllium canicula		30-50♀				Kastschenko, '90	Zeit. wiss. Zool., 50, p. 428

IV. PISCES—Continued

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Scyllium canicula, catulus	24 spg	12♂	12♂	12♂		Moore, '94; Moore, '95; Farmer and Moore, '04	Anat. Anz., 9, p. 547; Q. J. M. S., 38, p. 275; Q. J. M. S., 48, p. 189
Scyllium canicula		20-24♂	14-16♂			Rawitz, '00	Arch. mikr. Anat., 53, p. 19
Scyllium canicula		17-19♀				Cerruti, '08	Atti real. Accad. Sc. fis. e math., Napoli, II a, vol. 13
Spinax niger	60-70 spg					Schreiner, '07	Arch. Biol. 22, p. 419
Torpedo ocellata		30-50♀				Ksatschenko, '90	Zeit. wiss. Zool., 50, p. 428
Torpedo	24 spg	12♂	12♂	12♂		Moore, '95; Farmer and Moore, '04	Q. J. M. S., 38, p. 275; Q. J. M. S., 48, p. 489
d. TELEOSTII							
Ctenolabrus adspersus	38-48 cl					Pinney, '18	Jour. Morph., 31, p. 225
Fundulus heteroclitus	36 cl					Moenkhaus, '04	Amer. Jour. Anat., 3, p. 29
Fundulus heteroclitus	45 cl					Pinney, '18	Jour. Morph., 31, p. 225
Menidia notata	36 cl					Moenkhaus, '04	Amer. Jour. Anat., 3, p. 29
Salmo fario (='Forelle')		ca. 12♀	ca. 12♀	ca. 12♀		Böhm, '91	Sitz. Gesel. Morph. u. Physiol. Munchen, 7, p. 63
'Forelle'	12 cl (prob.)				Sperm treated with radium. Not pa	Oppermann, '13	Arch. mikr. Anat., 83, Abt II, p. 307
Trutta fario (='Forelle')	24 cl	12♀	12♀	12♀		Behrens, '08	Anat. Hefte, 10, p. 227
Trutta lacustris	At least 24♀	At least 24♀	At least 24♀			Blanc, '94	Ber. Naturf. Gesel. Freiburg, 8, p. 163 (= Festschr. Weismann)

V. REPTILIA
a. CHELONIA

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Chrysemis marginata....		17♂			X to pole in 1st	Jordan, '14	Science, 39, p. 438
Cistudo carolina.........		16♂				Jordan, '14	Science, 39, p. 438

b. LACERTILIA

SPECIES	DIPLOID AND PARTHENO-GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Anguis fragilis 'Orvet'.............		12 ♀				Loyez, '05	Arch. de l'Anat. mikr., 8, p. 69
Anguis fragilis.............	36? som	18? ♀				Trinci, '08	Mem. R. Accad. Sc. Bologne, Ser. VI, vol. 5, p. 167
Lacerta agilis............. viridis	Prob 24 spg (20-28)	Prob. 12♂ (10-15)				Tellyesniezky, '97	Math. u. Naturw. Ber. Hungarn, 13, p. 303
Lacerta stirpium.........	24 oog	8-12 ♀				Loyez, '05	Arch. de l'Anat. mikr., 8, p. 69

III. HISTORICAL AND CRITICAL

The first attempt to count chromosomes in the Metazoa was made in 1878 by Selenka, in his "Befruchtung des Eies von Toxopneustes." He gave the number in the cleavage cells as varying between 14 and 24, a rather wide range and not very close to the mark (36). Retzius[2] in 1881 gave the number for Triton punctatus as between 12 and 16 in the somatic cells, also somewhat far from correct (24). The next observation, by Flemming[3] on the salamander in 1882, was accurate and correct, and to him therefore belongs the credit of establishing a definite number of chromosomes for a definite species. He was also the first to attempt a count on human cells, given in the same publication. Very shortly after this, Strassburger,[4] '82, gave definite and correct numbers for several species of plants. Then came three papers giving the chromosome numbers in Ascaris, Anton Schneider's 'Das Ei und seine Befruchtung' in 1883, Nussbaum's[5] paper in 1884 and the very thorough and brilliant work of Van Beneden[6] which was published in 1883, although it did not appear till April 1884. Soon after, still in the '80's, came Carnoy's and Boveri's papers on the nematodes and other works on the nematodes, molluscs and vertebrates. Since then, chromosome counts have been made by many observers on about 960 different species of animals.

Several lists of chromosome numbers have appeared previously, the first by Wilson in 'The Cell' in 1900, a partial list which included about fifty species of animals and a few plants. In 1905, Enriques[7] gave an incomplete list of numbers in animals, expressing the different numbers in mathematical formulae, as powers of 2 and 3. Montgomery in 1906 gave a list which was supposed to be very nearly complete, but there are many omissions and a good many inaccuracies in the list. Montgomery's

[2] G. Retzius. 1881. Biol. Untersuchungen, p. 109.
[3] W. Flemming. 1882. Arch. mikr. Anat., 20, p. 1.
[4] E. Strasburger. 1882. Arch. mikr. Anat., 21.
[5] M. Nussbaum. 1884. Arch. mikr. Anat., 23, p. 155.
[6] E. Van Beneden. 1883. Arch. de Biol., 4, p. 265.
[7] Paolo Enriques. 1905. Archivio di Fisiologia, 2, p. 258.
[8] T. H. Montgomery. 1906 Trans. Amer. Philos. Soc., 21, p. 97–162.

general conclusion was that chromosome number should be considered as an important factor in taxonomy and that animals having widely different numbers should be placed in different genera. McClung has also been a strong advocate of the value of chromosome numbers in taxonomy. Della Valle's[9] list in 1909 is of little value, as it is a prejudiced one, given entirely with the object of showing that chromosome numbers are inconstant and of little importance. Two comprehensive lists of chromosome numbers in plants have appeared recently, Tischler's[10] and Ishikawa's[11] in 1916. The latter is exclusively a list of numbers, his general conclusions being reserved for a further publication. Tischler's list is accompanied by able discussions and criticisms, his general conclusion being that it is still too soon to solve any large phylogenetic problems on the basis of chromosome investigations. It may be of interest as a comparison with the work on animals to give some of his statements concerning numbers in plants. The Asco- and Basidiomycetes have very small numbers, the mosses and Gymnosperms in general small numbers, whereas the Algae, Pteridophytes and Angiosperms have species with both small and large numbers. The Magnoliaceae and Nymphaceae (Angiosperms) and the Ophioglossacea, Equisitaceae and Lycopodiales (Pteridophytes) have very high numbers, although not a great many species have been studied cytologically. Finally Winge[12] in 1917 has given an additional list in plants and has concluded from that and from Tischler's list that the numbers in related species are in arithmetical progression,— e. g. the chrysanthemums with 9, 18, 27, 36, and 45,—these arising by hybridization of species with like numbers; and that in general numbers occur in factors of 2 and 3 (an idea similar to that of Enriques), the numbers 8 (2.2.2) and 12 (2.2.3) occurring most frequently.

A cursory survey of Tischler's or Ishikawa's list of numbers in plants and of my own list in animals is sufficient to show that very

[9] P. Della Valle. 1909. Archivio Zologico, 4, p. 1–177.

[10] G. Tischler. 1916. Progressus Rei Botanicae, 5, p. 164–260.

[11] Mitsuharu Ishikawa. 1916. The Botanical Magazine, Tokyo, 30, p. 404–448.

[12] O. Winge. 1917. C. R. Travaux du Laboratoire de Carlsberg, 13.

closely related species may have widely different numbers, and that
the numbers in related species are not usually in arithmetical ratio
although occasionally they are, especially in plants. It is also
apparent that numbers which are resolvable into factors of 2 and
3 are of frequent occurrence, as one would expect since nearly half
of the numbers between 2 and 20 (the most frequently occurring
numbers) are resolvable into these factors. However, it is equally
apparent that other numbers not resolvable into these factors are
also of frequent occurrence.

In using the present tabulation for any generalizations or con-
clusions, several facts must be taken into consideration. Many
of the observations recorded are of too early a date to be of much
value. Other observations are contradictory and in many cases
it is impossible to judge which is correct; this is largely due to
difficult material and is especially true for the mammals, where
for man the number of chromosomes varies between 8 and 48
(diploid) according to different authorities.

IV. CHROMOSOME NUMBERS

In looking over the fore-going list, there can be no doubt to
an unprejudiced mind that the constancy of chromosome
numbers for a species is a fact, and that any variation in number
for a definite species is an exception to the general rule. Such
variations occur regularly in Notonecta insulata, Jamaicana uni-
color and J. subguttata, and Hesperotettix viridis where two or
more chromosomes may be united or separate; in species with
supernumeraries (see p. 66); in cases where multiple groups occur
(e. g. Culex pipiens, Notonecta, Anasa), and where fragmenta-
tion has taken place (e. g. Ascaris, pig). A few sporadic varia-
tions occur in certain species owing to the lack of conjugation of
two univalents (e. g. Lygaeus turcicus, Coenus delius, Euschistus)
and a few which have not been explained (e. g. Trichopepla,
Lygaeus reclivatus which is now under investigation). When a
range of numbers is given instead of one definite number, it is
usually due either to the early date at which the observation was
made or to difficult material rendering accurate counting
impossible.

There is however a great range of numbers among the different forms of animals. As is well known, there is only one chromosome in the haploid groups of Ascaris megalocephala univalens; some species of Gordius (Nematode) also are reported as having only one chromosome in the reduced groups and Styelopsis (Ascidian) as having only one in the spermatid. Indeed, according to Moore, '93, there is only one chromosome in the oogonia of Apus (Phyllopod crustacean). Animals having only two chromosomes in the haploid groups are: Ascaris megalocephala bivalens, Cyclops viridis brevispinosus, Pediculopsis graminum (arachnid), Icerya purchasi (Homoptera), Tetrastemma vermiculus (Nemertean), Vortex viridis and Paravortex cardii (Rhabdocoels). At the other end of the series are: two species of Cambarus (Decapod), with 104 and 100 (reduced), Artemia (Phyllopod) with 84, Cancer and Hippa (Decapods) with 60, Astacus (Decapod) with about 58 and Nyssia (Moth) with 56. The number occurring most frequently among the forms investigated is 12; other numbers occurring very frequently are 6, 7, 8, 9, 10, 11 and 16.

There is also often a considerable range in number among different forms belonging to the same class, e. g. Nematoidea (1–24 reduced), Aphidae (3–20), Copepoda (2–17). The classes showing the greatest constancy are the Acrididae (Orthoptera) and the Urodeles (Amphibia). The Diptera and the Nematodes have, in general, low numbers whereas the Decapods and Lepidoptera have high numbers.

A chromosome is really a compound structure, carrying many characters or genes which are themselves the elements of heredity. However genes may arise, it is conceivable that in some cases one or more new genes may be placed in a chromosome without disturbing its integrity, the number of chromosomes in related species thus remaining the same. On the other hand such additional genes may disturb the existing complex and cause the whole mass of genes to be entirely redistributed, thus causing a change in chromosome number in nearly related species. Should a certain group of genes be placed in one chromosome in one species and in two in another, there would not be necessarily any difference in these two species. It would seem, however,

that there might be a tendency in any large group of related animals for the genes to segregate out according to some definite pattern.

If, therefore, we make a list of all the chromosome numbers which have been reported for all the species of a certain class[13] of Metazoa, leaving out of account results which are conflicting or are too old to be accurate, we find that a certain number of chromosomes is characteristic of that class; that is, there are considerably more species having that number of chromosomes than any other number. This I will call the 'type number.' The type number of a class of animals is the most frequently occurring number and may be considered tentatively as the fundamental chromosome group. One or more chromosomes of this group or of a group derived from it may split into two (or more) parts, or they may fuse, thus causing the differences in number which occur in related forms. Whether the double groups which occur in closely related forms in many plants (e. g. Oenothera gigas and O. lamarckiana, Drossera longifolia and D. rotundifolia, Spiranthes cernua and S. gracilis etc.) and in some animals (e. g. the bivalens and univalens varieties of Ascaris megalocephala, Helix pomatia, Echinus microtuberculatus, Artemia salina; Cyclops viridis and C. gracilis, Anopheles sp? and Anopheles punctipennis etc.) are derived in all cases by a splitting of all the chromosomes of the simple group, it is difficult to say. It may be, as suggested by Gates and supported by Strassburger that the double groups are derived in some cases at least, by a failure of cell division after the division of the chromosomes. A slight change in number may also be obtained by the disappearance of a whole chromosome, but this must be rare. All numbers referred to hereafter are the haploid numbers, and X, when present, is counted as one chromosome, even when it consists of several elements.

The type number for the Coelenterates cannot be determined yet, as the data are too scanty and the results conflicting (e. g. Hydra). Possibly it is 12. For the Nemathelminthes, the type

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[13] The term "class" is used loosely to include related families, orders or classes of ordinary classification.

number is 6, for the Echinoderms 18, for the Amphibia, the only class of Vertebrates satisfactory for generalizations, it is 12. For the Plathelminthes, the type number is 8, for the molluscs 16, for the Annelids 16. As one might expect in a group with so many distinct subgroups, the Arthropods have several type numbers. For the Crustacea it is 8 (the Malacostraca have higher numbers), for the Hemiptera 7, Orthoptera 12, Coleoptera 10, Diptera 6, Lepidoptera 31. It is of interest that the type numbers in the enterocoelous series are all multiples of 6 or 6, whereas those of the teloblastic series (except the tracheates) are multiples of 8 or 8. It is also of interest that the molluscs and Annelids which are so closely related have the same type number 16. The subgroups which are degenerate or highly modified (e. g. Trematodes, Acanthocephala) usually do not have the type number of their groups. The data for the insects are the fullest and the most reliable and these offer the best study of changes in chromosome numbers.

The type number for the Hemiptera is 7 (haploid), including an XY pair or an X. Other numbers occur, all of which can be attributed to the fusion or splitting of chromosomes of the type group. The two Thyantas have been a puzzle, owing to their likeness in form and the wide divergence in chromosome number. Thyanta custator has a diploid number of 16, which would mean 8 haploid including X or Y. Thyanta calceata has 28 in the ♀ diploid, 27 ♂ (owing to X being of two parts, Y of one), which would mean a haploid number of 14 ♀, 13 ♂. These numbers may be explained on the supposition that in Thyanta custator one chromosome of the type group has split in two, whereas in Thyanta calceata all of these have split except Y. Evidence of this splitting is given by X, which is of two parts, not always separate in T. calceata, each about the same size as Y and about half as large as the X in T. custator. This explanation may be expressed as follows:

	TYPE	THYANTA CUSTATOR	T. CALCEATA
♀	6+X	6+1+X	6+6+2X
♂	6+Y	6+1+Y	6+6+Y

The same explanation holds for the two Banasas, one of which, B. dimidiata has a haploid number of 8 (like Thyanta custator) and the other, B. calva has a haploid number of 13 (like T. calceata, except that X is single). Euschistus crassus differs from five other species of the same genus which have the type number, in having one less. A comparison of Foot and Strobell's figures of this species with their figures of the other species would indicate that a union of two large chromosomes has taken place. That the chromosome number does change by the fusion or splitting of chromosomes is shown by the Notonectidae. In three species (Browne, '16) there are 13 chromosomes, including two small ones, and in two species there are 12 chromosomes including only one small one. In a sixth species, N. insulata, the second small chromosome may be seen attached to another chromosome in the first division of some cells, while in other cells it is free, whereas in the second division it is permanently fused with the other chromosome. The d-chromosome of Nezara may also represent a stage in splitting or fusion, as suggested by Wilson. The fact that the X chromosome may consist of two or more parts, as in Syromastes, Phylloxera and some Reduvioids, would indicate that other chromosomes whose identity is not so easily established, may also split into two or more parts.

The type number for the Diptera is 6, including XY which are, however, not always distinguishable. There is a decided tendency for the chromosomes to fuse, especially among the Drosophilas, most of which have 4 chromosomes, and the Culicidae, most of which have 3. In Anopheles punctipennis, (Stevens, '11), the X and Y are seen to be attached to another pair in the diploid groups. Metz, '16, has made a careful study of the chromosomes in the genus Drosophila, and has shown that many groups having a larger number of chromosomes contain rod shaped ones which are represented in groups with a smaller number by half as many V-shaped ones, two rods uniting to form a V. When the linkage groups in other species of Drosophila have been worked out as extensively as in D. melanogaster, it may be possible to establish the relation between chromo-

somes of different species and to determine whether fusion of chromosomes has actually taken place.

The Orthoptera have been carefully studied by McClung and his students, and they have found a great constancy, especially among the Acrididae. The type group for the Orthoptera is 12 including X. In Stenobothrus (Chorthippus), which has 9, Robertson has shown that three of these are really compound. In Chortophaga, there is a union of chromosomes in the diploid groups (McClung, '14). In two species of Hesperotettix (McClung, '17), X is fused with another chromosome, while in another species, H. viridis, it may be fused or free, and fusion may occur among other pairs, correspondingly decreasing the number of chromosomes. Also in Mermeria bivittata (McClung, '17) X is fused with another chromosome, while in other species it is free. Among the Locustidae, Jamaicana (Woolsey, '15) shows steps in change of number. Some individuals of J. unicolor have 31 rod-shaped chromosomes and 2 Vs. in the diploid groups; some individuals of J. subguttata have 33 rods and 1 V: other individuals of these two species and all of J. flava have all rods and a diploid number of 35. Robertson ('16) has suggested that the 2 V-shaped chromosomes of Steiroxys are represented by 4 rods in Decticus, giving a total of two more in the diploid group of the latter. An unpublished account of Mohr agrees with this in showing the two Vs in Steiroxys and he also shows them in Locusta viridissima (diploid number 29) whereas no Vs are present in several other genera whose diploid number is 31. Robertson also points out that several Vs are present in Gryllus domesticus, which, if counted as twice as many rods, would give the number of chromosomes in G. assimilis; as no figures are given of the latter, this cannot be verified.

There is therefore considerable evidence from the Orthoptera, Diptera and Hemiptera that chromosome numbers change by the splitting and fusion of chromosomes. The splitting of the sex chromosomes, which occurs in many other groups than the insects (see p. 66) indicates the probability that a similar process may take place among the other chromosomes.

Sex chromosomes in insects

SEX CHRO-MOSOMI	GROUP	FAMILY	EXCEPTIONS	TO POLE IN	EXCEPTIONS
X	Orthoptera	Acrididae Blattidae Gryllidae Locustidae Phasmidae	Gryllotalpa; XY Forficulidae?	1st	
X	Hemiptera homoptera	Aphidae Cercopidae Fulgoridae Jassidae Membracidae	Enchenopa bi-nottata (X or XY?)	1st	Enchenopa bi-nottata (X, 2nd; or XY, 1st?)
X	Hemiptera heteroptera	Coreidae Hydrometridae Pyrrochoridae	Some Metapo-dius (XY)	2nd	Archimerus (1st)
X	Coleoptera	Elateridae Lampyridae One Carabidae Some Chryso-melidae One Silphidae		1st	Photinus, 2 sp. (2nd)
XY	Diptera	Anthomyidae Asilidae Bombyliidae Culicidae Drosophilidae Muscidae Sarcophagidae Statiomyidae Syrphidae		1st	
XY	Hemiptera heteroptera	Belostomidae Galgulidae Lygaeidae Nabidae Nepidae Notonectidae Pentatomidae Reduviidae	Oedancala (X)	2nd	Tingis (Tingiti-dae) (1st)

Sex chromosomes in insects—Continued

SEX CHROMOSOME	GROUP	FAMILY	EXCEPTIONS	TO POLE IN	EXCEPTIONS

XY	Coleoptera	Buprestidae
		Cerambycidae
		Cincindellidae
		Coccinellidae
		Lucanidae
		Melandryidae
		Meloidae
		Scarabaeidae
		Staphylinidae
		Tenebrionidae
		Some Carabi-
		    dae
		Some Chryso-
		    melidae
		One Silphidae
				1st	

V. HETEROCHROMOSOMES

The most conspicuous heterochromosomes are the sex chromosomes, an unpaired X or an unequal XY pair, which occur most characteristically in certain groups of insects. As may be seen from the accompanying table, an unpaired X occurs in practically all the Orthoptera and Hemiptera homoptera and in some families of the Hemiptera heteroptera and Coleoptera; an XY occurs in practically all the Diptera and in some families of the Hemiptera heteroptera and Coleoptera. In some families of the Coleoptera, an X is found in some genera and an XY in others. The sex chromosomes undergo their differential division in the *first* maturation in the Orthoptera, Hemiptera homoptera (except Enchenopa?), Diptera and Coleoptera (except Photinus), and in the *second* maturation division in the Hemiptera heteroptera (except Archimerus and Tingis). Sex chromosomes have not been described in any Hymenoptera, and are not of general occurrence in the Lepidoptera, though here an equal XY in the ♂ has been described in several species, an XY in the ♀ in Phragmatobia and an X in the ♀ in Abraxas. Among the other Arthropods, an X

has been described in some Myriapods, most Arachnids (Araneida) and a few Copepods, but in no other Crustacea and not in Peripatus. Sex chromosomes have not been described in any Annelids, Coelenterates, Nemertines, Porifera, Rotifera, Protochordates or fishes and for only one Plathelminth. A few cases of their occurrence have been reported in the Echinoderms, molluscs, Amphibia, Birds, Reptiles and mammals. In the Nematodes they are of frequent occurrence. In all cases it is the ♂ which is heterozygous except in the Lepidoptera and birds and the genetic evidence agrees with the cytological. Although X and XY are typically single elements, the X consists in some cases of two or more elements closely or loosely associated. An unpaired X of two elements has been described for Syromastes, two species of Phylloxera, Leptinotarsa, Agalena (spider), Hyalocylis (mollusc), Schistosomum (Trematode), Rhabditis, fowl, and man and pig; an X of two elements accompanied by a single Y for Thyanta calceata, Gryllotalpa borealis, Cincindella and some of the Reduvioids. In other Reduvioids, Galgalus, Notonecta indica and Ascaris incurva, the X of an XY pair consists of three or more parts—of 8 in the last named, and in Ascaris lumbricoides and A. canis, an unpaired X consists of five and six elements respectively. A Y consisting of several elements has been described for Phragmatobia, and a Y of two elements in one testis of Odontota (Coleoptera). In a few cases the sex chromosomes are attached to another chromosome during part at least of their history: Ascaris megalocephala, Leptynia, Dixippus, Hesperotettix, Mermeria bivittata, Anopheles punctipennis and Necturus.

Another set of heterochromosomes are the supernumerary chromosomes, which typically accompany sex chromosomes and divide in only one division, but are distributed irrespective of X and Y; they are constant in number in an individual, but differ in different individuals of the same species. They have been found in Metapodius, Euschistus variolarius, Banasa calva, Diabrotica, Ceuthophilus, Drosophila ornatipennis, Circotettix, Trimerotropis, Hesperotettix viridis, Tettigidea parvipennis, some spiders and Necturus.

A third set of heterochromosomes are the m-chromosomes, small chromosomes which remain condensed in the growth period and conjugate late. These are characteristic of the coreid Hemiptera and occur in some of the Lygaeidae; they accompany an unpaired X except in Metapodius and Ichnodemus where they accompany an XY.

Finally there are those chromosomes which are normal in behavior but consist of unequal parts. To this class belong the d-chromosome of Nezara hilaris and the compound chromosome of Notonecta insulata, which divide equally in the two divisions, and the unequal tetrads of some of the Orthoptera which divide into unequal parts in one division: Schistocerca (Hartmann, '13); Acridium granulatus, Tettigidea parvipennis (Robertson, '16); Arphia, Dissosteira, Brachystola (Carothers, '13); and Phrynotettix (Wenrich, '16).

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HUMAN TYPES AND GROWTH REACTIONS

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THREE FIGURES

The following article is presented as an introduction to a series of studies now under way which bear on the origin and development of certain well marked types found among the mammals.

In a previous communication, '21, the significance has been pointed out of the influence of modifications in developmental rate upon embryonic structure. It has also been shown in the case of chemically treated male mammals how certain questions of inheritance are involved in analyzing modified structural reactions. These considerations may now be extended to many problems of fetal and post-natal growth and to an interpretation of several peculiar structural patterns and conditions seen in man and other animals. In order to make clear the situation extreme structural peculiarities may be briefly reviewed and from these we shall attempt to determine the factors concerned in regulating the less marked and more usual human types. The growth rate of the individual depends upon both internal and external factors—hereditary composition and functional activities—the latter being modified largely by surrounding conditions.

To illustrate the problem we may begin with the familiar condition presented by the thyroid cretin. Is the absence of the thyroid hereditary or due to an arrest in development or to both? Such individuals so far as is known may apparently arise from a parentage with subnormal or poorly functioning thyroid glands. However, no one has yet analyzed this condition sufficiently to determine exactly its genesis. It is not known, for example, whether the subnormal thyroid of either parent affects the germ-

cells so as to modify those genes which determine thyroid develop-
ment and structure. We may have reasons for believing that
the thyroid of the embryo and fetus is capable of developing
independently of the thyroid hormones in the blood of the
mother, but there has not been sufficient experiment to make it
certain that the mammalian embryo does not need the thyroid
stuff in the mother's blood for its proper growth functions.

We may simply state that the cretin is a thyroid dwarf which
has attained an incomplete degree of development and cannot go
further without the application of thyroid stuff. This we know,
but why the condition arises we have yet to determine. It is
obvious that the origin of the condition is the more important
thing to know and the mere treatment of the abnormal end pro-
duct can supply little knowledge of its cause.

The one instructive thing to us at present is that the human
child without a thyroid gland can only develop to the stage
shown by the typical cretin. This stage might be called the
early larval condition of man. The most definite and clear cut
experiments done on the influence of the internal secretions in
development are those which show that the thyroid is essential
for the metamorphosis of the amphibian larva into the adult stage.
The human cretin without thyroid will not metamorphose or
develop into an adult. Also the human individual with a sub-
normal amount of thyroid may be expected to be more child-like
and less adult than the individual with a normal supply of the
thyroid secretion. There is a great bulk of evidence, impossible
to mention within the limits of this introductory article to show
that the amount or quality of thyroid secretion present in the
developing individual is an enormously important element in
determining the rate of its growth. The thyroid effects the
growth rate by primarily determining or affecting the rate of
metabolism. The significance of this we have pointed out in
the articles cited above by showing that the rate of development
is the most profound factor in determining the quality and type
of structural production.

The cretin is an abnormal or pathological individual. Its
conditions would preclude the breeding of a race of such speci-

mens. It is not in itself a type, it is an arrested child stage but the cretin furnishes an extreme growth condition which is most helpful in fully appreciating the influence of the thyroid gland on the growth of the so-called normal types of men.

If space permitted we might review a number of peculiar specimens that could in some manner be attributed to the unusual action of several other glands of the body which modify growth by effecting the rate of metabolism. Many striking pathological growth reactions are familiar to all, so we may proceed at once to consider certain strange and unusual individuals constituting real or actual types, which should not properly be classed as pathological.

The various dwarf types of man are instructive in a study of growth reactions and structure. Many of the dwarfs are in most respects normal and they simply differ from the common type as the small breeds of several domestic animals differ from the large. The cause of such types we can determine by considering both the genetic and developmental modifications possibly concerned in their production. The statement that this may be the same large problem as the origin of any species or type should not be discouraging since certain peculiar types may be more likely to reveal their causes than the commoner long existing ones.

The simplest dwarf type of man from our present growth standpoint is the small African pygmy. These are childish Africans of very low intelligence as well as small size. When one examines pygmies closely, particularly their physiognomy, a striking similarity of features is found between them and the thyroid cretin, in both the mouth, nose, forehead and brow are closely similar in form and proportions. On administering thyroid to the cretin these features become rapidly modified by progressive development and approach the normal picture. One can scarcely resist the temptation to suggest the experiment of administering thyroid to the young pygmy. The pygmy may be looked upon as a not fully metamorphosed large negro, a mild and slow growing condition of cretinism. They are not true dwarf types but certainly people with general growth arrests.

They are quite cretinous in their behavior which is certainly unlike the fully grown African of the coast countries surrounding them. The pygmy is the most primitive of African savages in his behavior, building no huts and having only the crudest tribal organization. The fact that they are capable of reproduction does not argue against their partial cretinism since arrests are so commonly known in which all organs are not equally affected. With our present knowledge it is impossible to say whether the pygmies are a true genetic breed or whether they simply live in an environment unfavorable to complete development. On removal to the coast regions they might within a short time become taller and better developed.

There are other true human dwarfs, however, about which we may speak with a greater degree of certainty since they have been studied for a long time and a considerable mass of data exists regarding them. Much of the data has been well presented by Rischbieth and Barrington of the Francis Galton Laboratory for National Eugenics in England. These workers, however, discarded one of the most valuable means of understanding such human types by deciding that they show only an apparent resemblance to the similar types found among lower animal breeds. This attitude I believe is a vital mistake since certain animal breeds not only closely resemble the human dwarfs but very probably arise and developed in an exactly similar way. The understanding of the origin and development of the one is certainly of the highest value in a biological consideration of the other.

Among the true dwarfs the achondroplastics may be discussed first since other dwarfs also often show some degree of achondroplasia. The typical achondroplastic dwarf is very short and stocky, the head and trunk are large being often as big as those of an ordinary individual, but the extremities are short and somewhat twisted, on account of the peculiar development of the long bones. The muscles are short and thick, standing out in a knotty fashion, particularly on the extremities. They are very active, often acrobatic and quite bright and intelligent. Their head growth and face shape is characteristic. The base

of the skull is short. The ossification and cessation of growth in the basal cartilage between the occipital and sphenoid bones frequently occurs before birth, instead of after twenty-two years as is the usual case. This failure to continue the growth of the skull base after birth renders the head short and consequently disproportionately wide. Such dwarfs are, therefore, brachycephalic. The lack of growth at the base of the skull also causes a failure to push forward the nasal septum. The nose bridge remains low and often actually sunken in below the overhanging forehead. The upper jaw likewise is not carried the usual distance forward so that the fully developed mandible projects beyond the maxilla, the teeth do not properly lock, and the 'undershot' jaw condition prevails. The entire face is flat, vulgarly termed a 'dish-face.'

Achondroplastic dwarfs have been accounted for in many ways on the basis of disturbance in the glands of internal secretion which regulate growth. Several have attempted to associate their entire peculiar make up with an unusual action of the hypophysis. But many of these dwarfs seem to show some peculiarity in the structure of the thyroid and for this reason they have been thought to be the result of unusual thyroid function. Sir Arthur Keith, '22, in his very suggestive Herter Lectures at Baltimore, for instance, has referred to them as a "minus-thyroid" condition.

Be the condition of the thyroid as it may, the primary cause for these dwarfs is more fundamental and deep-seated than the glands and the probable effects of the glands on growth are secondary even though their secretions may induce the peculiar forms. We know from the monograph by Rischbieth and Barrington that the condition is already present far back in the early fetus. The extremities are originally far too short for the trunk size and length and the early bones are typically peculiar, not as in rickets or any other known condition. This definitely peculiar growth continues throughout development until the adult dwarf condition is attained. The question may arise as to whether their dwarf condition may be due to peculiar glands in the mother. That this cannot be true is shown clearly in the pedigree tables of Rischbieth and Barrington where the

condition is transmitted by the father. The male could only transmit such a state to its offspring through the spermatozoon, and if this be done the condition is truly hereditary. Rischbieth and Barrington record the case of a typical achondroplastic dwarf man married to a normal woman. Two children resulted from this combination, a boy and a girl that lived to become adult, and both were completely achondroplastic dwarfs closely resembling the father. There are other such cases on record as well as similar dwarfs produced by achondroplastic mothers. Ordinary parents may sometimes produce a typical achondroplastic dwarf along with several normal sisters and brothers.

One strange fact regarding these dwarfs is that they frequently die at birth from no known cause. This and their sporadic occurrence in families make it seem possible that this complex may arise as a Mendelian dominant that is only viable in the heterozygous form. When there is a double dose of the dominant and the zygote is, therefore, homozygous a lethal expression follows and the child is incapable of living after birth. Morgan and others have pointed out several cases of this homozygous dominant lethal in their genetic studies. Such cases show why although a character may be dominant it is unable to establish itself in a homozygous condition and may never become abundant in the race.

There are only isolated facts from human cases of achondroplasia but in the light of our present knowledge of inheritance and development they leave little doubt that the fundamental or primary cause of the complete achondroplastic dwarf condition is a germinal mutation or sport and the condition is definitely hereditary. The expression of this condition is probably due to an inherited modification of a gland regulating growth, the hypophysis.

Turning from man to the domestic animals, we have on hand an abundance of material for the study of achondroplastic dwarf conditions. Achondroplasia occurs in various degrees among several of the domesticated species, but the most varied and remarkable examples of the condition exist among the fancy breeds of dogs. With these animals nature and man have per-

formed an enormous experiment lasting in some cases for hundreds of years and today they present the investigator with material of remarkable value for a real analysis of the processes of inheritance and growth concerned in mammalian achondroplasia. There are breeds of dogs, such as the small French bull, which show a complete achondroplastic condition exactly duplicating the typical human achondroplastic dwarf. The head form, extremity and trunk conditions are all closely comparable. Other dogs show a marked skull and head type without fully developed achondroplasia of the extremities. Still others show the most pronounced condition in the extremities with the usual head form, as seen in the Dachshund, Scottish terrier, Bassett and others. Finally there are several dog breeds, Pekingese and some peculiar terrier forms, combining various degrees of achondroplasia with an ateliotic or midget condition. This combination with achondroplasia is also very often seen among human midgets.

If we choose either the best type British or French bull-dog we find on close study and comparison point for point a resemblance to the stocky human dwarf. All have the short wide head with flat muzzle giving the sunken 'dish-face.' The base of the skull has failed to attain its usual length through failure in growth of the basi-sphenoid and more especially the basi-occipital bone. A shortened disproportionately wide condition necessarily follows. The nasal septum is not carried fully forward and the root of the nose is flat or actually sunken giving a marked depression below the forehead. The maxilla is also for associated reasons in an unusually posterior position and the unaffected mandible, therefore, projects in front of the maxilla. The teeth fail to meet in the normal biting position and the common undershot jaw condition exists. The entire facial expression and head carriage of the bull-dog and the stocky human dwarf are strikingly the same and are due to the same structural background. The condition of the extremities in these dogs and in the dwarf are also structurally alike. The proximal segment in all four extremities is very short and somewhat bowed or bent being the most modified segment of the limb. The fore-arm and leg seg-

ments are also short and somewhat bowed though not so short-
ened as the arm and thigh. The hands and feet are very much
of the ordinary shape and proportions and are about as large
as those of the common large breeds of the species. The muscles
of the extremities are short and knotty and the entire animal
makes a most muscular and stocky appearance. The trunk
as well as the head may be about as large as in the ordinary ani-
mal. These points are more evident in the British than in the
French bull, the latter being more of a terrier. The external
genitalia are generally of normal size but the extremely short
thighs give the male genitalia by contrast an abnormally exag-
gerated appearance.

A most striking and convincing comparison is obtained on
holding a fine pointed French bull dog up on its hind feet by the
side of a human achondroplastic dwarf, or the identity of the
types is equally well shown by placing both the man and the dog
down on all fours.

Breeders of various achondroplastic varieties of dogs often
admit that a considerable mortality occurs among the pups at
birth and also recognize that they are difficult to feed and rear
during the first few weeks after birth. Following this period
the survivors are strong, long-lived and hardy. These observa-
tions scarcely warrant in themselves scientific consideration, but
they suggest the desirability of obtaining comparative early
mortality records, since the high birth mortality of human achon-
droplastic dwarfs seems well established as well as the record of
hardy long lives for those that do survive. As mentioned before
such records may indicate that the survivors in these breeds are
possibly heterozygous for a dominant achondroplastic condition
and that homozygous achondroplastics with the two dominant
doses have some lethal complex that causes their elimination or
death about the time of birth or before.

It may be possible, however, that the double dominant condi-
tion in man would give a lethal effect, while among the achon-
droplastic dogs this would not necessarily be the case, and the
bull dogs might thus be a pure homozygous breed. Yet their
out-crosses do not indicate them to be homozygous, provided
achondroplasia is a dominant condition.

The general attempt to explain achondroplasia as due to peculiar actions of the glands of internal secretion, either the hypophysis, thyroid or any other, leaves out of account the numerous examples of partial or localized achondroplasia so commonly seen in man and the lower animals. If the internal secretions in the blood produce these peculiar growths, why do they not always act on all similarly growing parts in a similar manner? How is it possible that one humerus may be typically short and twisted and all other long bones unaffected? How does it happen that the dachshund and the bassett hound have the most pronounced achondroplastic legs and yet the heads of these dogs are like the ordinary head of the common large hounds. Numerous other examples of partial and localized achondroplasia could be cited which make it difficult to account for the condition as simply due to unusual internal secretions. Yet we might imagine that certain tissues or parts such as one humerus could inherit a peculiar sensitiveness to certain internal secretions which would cause this one bone to respond differently from all others, though such an hypothesis is difficult to conceive.

All that we know from the breeding records of dogs of the above varieties and the fragmentary data from human dwarfs indicates most strongly that these are true varieties or breeds of animals that probably have arisen by some mutation or sport from the ordinary form. They breed fairly true to type but not perfectly so since great variations in the condition constantly arise and for this reason most careful selection is necessary to maintain a high standard stock. Such selection is of course entirely unnecessary in maintaining the stock of a wild species of the wolf or fox, for example.

The point of much importance to recognize is that these strange forms, in spite of some apparent evidence to the contrary, may be transmitting peculiarly acting glands of internal secretion. This type of gland is being inherited, and possibly causes the production of the strange structural type. The types are definitely the result of clean cut growth reactions and these might be secondarily brought about by the primarily strange type of internal gland. There is much argument both for and

against such a position on the basis of apparent facts available at present. It must be recognized that these problems are still largely unsolved and the remarks on the cases above, as well as what is to follow, are given as a conception of the situation, a conception which we hope to either prove or modify by the detailed data now being accumulated in our studies.

ATELIOSIS

A final dwarf type of interest from the standpoint of growth and structure is the tiny ateliotic midget. Some of the most celebrated dwarfs have been of this type. They differ from the achondroplastics in having the bodily proportions and general outline of the normal large individual. Their trunk and arms and legs may all be of the proper proportionate length. The head is small and the body is often delicately and gracefully formed. A sexual infantilism with poorly developed genitalia is very common in the ateliotic, yet many are normally developed sexually and there are numerous records of their having produced offspring.

A midget full grown may resemble in head and body size a child of six. Yet they are frequently bright and very intelligent. They seem to grow normally for five or six years after birth and then stop. The wrist and ankle cartilages fail to ossify, the epiphyses of the long bones do not fuse with the shaft and the skeleton is about like that of a child of seven. In nature this is clearly a genetic condition. One or more midgets may be born from perfectly full grown parents along with full size brothers and sisters. We are investigating a case of three midget sisters who claim to have two large brothers and two large sisters who were born alternately with them from large parents, the father being Dutch and the mother German.

Although the ateliotic condition may occur in the simple form it very frequently has associated with it a certain degree of achondroplasia. When one examines a great number of midgets it will be found that many of them have unusually short arms, the fingers reach only to the hip joint or great trochanter instead

of striking almost half way down the thighs. The faces of these are closely alike and usually sunken at the nasion just as in the achondroplastic, though not so pronounced; but the undershot jaw condition is rarely present in the midget.

Again we find dogs of exactly similar type, for example, the head and face of the King Charles Spaniel is in shape, outline and expression almost a picture of the human midget. The psychology of the two is much the same for general behavior reactions. The extremities and head of the King Charles Spaniel are also quite achondroplastic as is so frequent in the human midget. There are several other tiny breeds of dogs which are slender and have a sharp muzzle, real ateliotics, with no achondroplasia. Many of these tiny dog breeds also seem to have some infantilism and individuals are frequently sterile.

Bantam chickens and other miniature animal forms are probably the same in origin and type as the human ateliotic dwarf.

All of these dwarf forms give us valuable suggestions for an interpretation of many usual types of growth and development. And when a study of them is associated with an investigation of the opposite extreme, the giants, a remarkably complete series of growth and structural conditions is supplied.

EXCESSIVE GROWTHS

The giants and acromegalic individuals are so well known from many recent studies that only passing mention need here be given. I simply wish to point out certain features of these types before attempting a final estimate of what may be considered the usual human types from a growth and development standpoint.

The fine youthful giant is a properly proportioned lithe and active individual often with a well chiseled prominently featured face. This is simply what might be recognized as a splendid human specimen of gigantic size. The giant forms of lower animals are frequently of this type of supernormal growth with proper proportions. Among the dogs, the Great Dane is a

splendid example of this kind, alert and youthful with no acromegalic symptoms.

Just as most human midgets show some achondroplasia, so do most human giants present some degree of acromegaly. Achondroplasia and acromegaly are apparently opposite reactions in bony growth and both conditions are probably associated with definite hypophyseal states. It may safely be added that the usual or so-called normal bone growth and the ordinary hypophyseal condition stand just between the two. This strikingly illustrates the delicacy of the growth balance in the normal individual, if the scales waver in one direction a countenance mildly suggesting acromegaly accompanies a large framed body, whereas a tip the other way gives a flattened face on a small person with a somewhat long body and short extremities.

The usual giant is particularly apt to become acromegalic as he grows older. It should be recognized, at the same time, that most normal people, as well as other animals, also show various slight degrees of acromegaly as they approach middle life. The general thickening up and increased body weight after the age of thirty-five is something of this nature though complicated in other ways. Many pronounced acromegalics were short and stocky as young men. The diseased condition of the hypophysis may come on gradually after the long bones have ossified their growth cartilages so that no further increase in height or length can then take place. The bones merely become larger and heavier and the features thicken to greatly exaggerated size. Early photographs of patients taken before acromegaly had developed generally show a face which, of course, would and did pass as normal but which has a very decided touch of those symptoms which later develop in so pronounced a fashion. In other words, as a rule an uncertain hypophysis that is later to become deranged fails originally to give an extremely delicately modeled face.

There are certain human types presenting what might be called anatomical or normal rather than pathological acromegaly. These types may be pronounced in a certain family or even in

a localized region where pathological acromegaly is not unusually abundant. This is also true of lower animals. There are some really acromegalic species. Several of the large dogs, the St. Bernard, the mastiff and others, show recognized symptoms of acromegaly along with gigantism. But in the dogs as in man acromegaly may exist apart from gigantism. The blood-hound is a splendid example of the acromegalic type without gigantism and his facial expression and general appearance is closely similar to the human acromegalic.

Many breeds of dogs have been selected and perpetuated for centuries. The breeders selected those specimens having certain well pronounced characters and features that they desired to preserve. They also thought that these characters were the things primarily inherited but we may be certain for many breeds at least that the visible marks for which they are selected are simply the external symptoms or expressions of a particular quality or action of glands of internal secretion. These peculiar glands are the fundamental things that the breeders have unconsciously been selecting, since when they choose animals with the desired structural symptoms they also blindly choose the glandular cause. Thus a certain gland type may be inherited and the dog breeders have produced the experimental proof of this over and over again.

ACUTE STRUCTURAL REACTIONS

Extremely abnormal or pathological cases could be cited in great numbers to show the acute action of the glands of internal secretion on growth and structural responses, but many of these are so well known as to be taken for granted in following the present discussion. However, before we pass to a brief consideration of the normal human being, one of the most striking experimental cases will be cited to illustrate the remarkable possibilities of these glands for determining personal types.

In the breed of fowls known as the Golden Seabright Bantam the plumage of the cock is almost exactly like that of the hen in both coloring and feather form. The rooster's head, however,

is decorated with the usual large fully developed comb and wattles of the male. Morgan, '19, has studied the conditions found in these chickens and has obtained most striking and valuable results. He finds that when both testicles are completely removed from the hen-feathered rooster, a marked revolution in its appearance takes place at the next molt. The castrated male now develops the fine plumage of the rooster with plume-like hackles and saddle feathers and long sickle plumes in the tail. In plumage and color the capon no longer resembles the hen but is a perfectly feathered male. This is not all that has taken place. The large comb and wattles have now degenerated in size until the head is much like that of the hen in appearance. The removal of the gonads has induced a sudden and extensive structural change. Morgan states, "Feathers that may have started their development at the time of the operation show the old influence at the tip of the feathers and the new one in the rest of the feather. The change is abrupt, although the transition is perfect." We could have no more striking evidence of the important influence of the gonads on bodily structure and appearance, nor of the changed action of something else in the body which calls forth the fine plumage after the gonads have been removed. We might suppose that since the comb and wattles were large before castration, their condition was due to some action of the testicles, but the subsequent feather development following castration is certainly not the result of gonadal secretion; it is very probably due to a change in hypophyseal action after removal of the gonads since this gland seems to modify the growth of hair and feathers in many individuals after the gonads begin degeneration.

Morgan has shown that the hen-feathered condition of the male is inherited in a perfectly definite way and is due to two dominant Mendelian genes. In other words this means that the strange gland condition which underlies the peculiar plumage of these birds is definitely inherited. The character of the plumage is merely the symptom indicating the presence of the unusual gland action.

This experiment is of further interest to us in connection with certain general changes that occur during the life of human individuals. It will be found on observing a group of men that at about the age of thirty-five or a little later a coarse growth of hair begins on parts which in youth were not so hairy or on which the hair was fine. Strange coarse hairs grow in the eyebrows, on the pinna of the ears and at the entrance to the external meatus. The beard becomes coarser and heavier, and coarser hairs develop on the trunk and extremities. The man now possesses a more pronounced male hairiness than he did when under thirty. On first thought one might consider him to have fully arrived at the completely developed male state. This is not correct, however, since the gonads of such an individual have actually begun to decrease in their sexual power. The coarse hair growth is a plumage expression resulting from a decline in the male gonadal activity rather than the attainment of its zenith. The change is gradual but of exactly the same nature as that suddenly produced in the Seabright bantam by castration. In man this is truly to be recognized as an early indication of senility. It is, however, peculiar that in man the hair growth reaction is not called forth by early castration but only follows a gradual degeneration of the gonads after middle life.

It is also known that castration generally favors an extra accumulation of fat in mammals. The ox is more readily fattened than the bull. Here again in man at about the same time the coarse hair growth appears an accumulation of fat also takes place. The anterior abdominal wall often becomes prominent and a decided increase in waist measure occurs. These mild symptoms seem to accompany the physiological castration which is gradually taking place. The structural changes occurring at the menopause in women are similar in character but more pronounced and rapid in their development than the above changes in men, and very probably because the physiological castration in women is much more complete at this time.

Thus we see that all normal human beings are experiencing developmental and growth changes which are noticeably due to

the usual fluctuations in function of the organs of internal secretion. The existence of these very evident changes serve to illustrate the fact that if we study closer the entire developmental history of the individual it will be found that growth and expression are constantly being influenced and molded by the amount and quality of the internal secretions that have been inherited in the breed to which the individual belongs.

NORMAL HUMAN TYPES

When the reader reviews his personal acquaintances or any group of human beings it is evident that many of them show slight degrees of one or another of the peculiar structural conditions which we have surveyed above. There are those with short arms and flattened faces, others with heavy features and large wide hands, others with long narrow faces and delicate hands, some with rough hairy skin and others with smooth, until we may simply admit, that each is sufficiently peculiar to be distinguished from all the rest and addressed by name. We also recognize certain family and racial resemblances which aid us in classing or grouping our acquaintances. Such resemblances have been more or less taken as a matter of course and simply explained as being inherited, but actually to what are they due? Anthropologists have never explained why some heads are long and others wide, although they lay great stress on the fact that such is the case. Would it be possible for any baby to grow either a long or a wide head?

Does a great heterogeneous population lend itself to a system of classing or grouping from the structural standpoint? If such is possible to what cause or causes is this division into structural groups due? The first question is very old and almost endless fanciful groups and classes have been arranged by various students of the subject. As a rule each has begun with a few classes, but later these have been divided until in the end a return to confusion resulted.

One premise we may depend upon, namely, that all structural form in animals results from processes of unequal growth. Equal growth in all directions from the original spherical egg would

result in a sphere. Spheres may differ in size but in form all are alike. Should the growth processes be exactly the same in two specimens their final structures will also be exactly alike. Whenever the growth processes of the two differ, the resemblance is modified. Thus the problem of human types is a problem of growth and all individuals that may be grouped together under one type are individuals with closely similar growth histories. In previous articles the writer has pointed out that in the embryo and the fetus the type of structure largely depends upon the rate of growth. A rapid growth and development gives one result and a slow growth produces, even in a twin individual, an entirely different result. The peculiar human adult and animal forms that have been described were also interpreted as due to modification of the usual growth processes by the actions of substances which affect metabolism and, therefore, growth rates. Certainly from the time of birth numerous growth affecting substances are being produced in the body and the action of these stuffs regulate and modify the rate and type of growth. Their usual effects are simply to increase or decrease the rate of metabolism and to cause the individual to grow faster or slower than another, thus giving rise to the variations above and below the mean.

It is necessary to recall at this point several propositions discussed in connection with embryonic development (Stockard '21) in introducing the present conception of human types. In the first place initial growth and rapid growth tend to produce linear structure. In all plants and animals there is this primary tendency to form an axis or line of growth. Following this a lateral growth in width takes place. Crudely stated there is a tendency first to attain length and later width. Secondly, there is a certain degree of competition between these two tendencies so that as a rule the growth in width only expresses itself after the length growth has worn itself down and become slower.

It follows that any organ capable of affecting the rate of metabolism or oxidation would necessarily affect the growth rate and must likewise affect the form and structure of the individual. The one organ in the vertebrate body which seems above all others to affect the rate of metabolism is the thyroid gland and

we actually know from convincing experimental proof that this gland also greatly affects the rate of growth and structural development. It is a fact that the cretin without a thyroid gland is incapable of growth and development. The tadpole without thyroid does not metamorphose into a frog. The thyroid is essential for growth from babyhood to maturity. Very probably the thyroid is not alone in its action, it may be affected by many other things and so may growth rate, but the point of primary importance is that the thyroid is the central body tending to control the rate of oxidation, and therefore growth rate in the individual. An active thyroid gives fast growing rapidly differentiating structures and linear rather than large lateral type individuals.

It is impossible here to go into questions of the interactions among the internal glands and it is only necessary for our present purpose to state that if a substance such as the secretion of the thyroid does regulate growth rate it must also tend to determine structural types. And since the rate of growth is the important thing in structural type, we should expect not more than two extreme types which will grade regularly into each other. The thyroid is so delicate in its response and is so certainly different in its action in different environments that the two types may be quite well separated, the one due to highly active thyroid and the other due to a low acting thyroid The intermediate ideally balanced or indifferent condition would be the most difficult to attain on the part of the sensitive gland.

The two groups into which almost all ordinary persons fall more or less exactly may, therefore, be termed the *Linear Type* and the *Lateral Type*. The linear type is the faster growing high metabolizing thin but not necessarily tall group, while the lateral type is slower in maturing and is stocky and rounder in form.

Taking the tip of the nose as the extreme anterior point of the body and viewing the figure laterally, as seen in figure 1, we may draw a line which would indicate the morphological lateral line. This line on each side of the body separates the truly dorsal from the truly ventral surface regions. When these lines

Fig. 1 A side view of the human figure to indicate the superior **tip of the body**, the tip of the nose, and the general direction of the lateral line.

on the two lateral surfaces of the head and body are thought
of in space we may imagine that the nearer they come together
the more linear is the individual, and the wider apart they diverge
the less linear and more lateral the individual type will be.
Figure 2 illustrates this in the growth and development of the
two types from the infant condition.

Examining figure 2B it is seen that when the lateral lines are
near together the head is of course narrow or dolichocephalic.
The interpupillary distance is short and the eyes are close
together, the nose bridge is narrow and therefore generally high,
the mouth arch is narrow and for the same reason generally high,
the lower jaw is small and narrow and usually not strongly de-
veloped. The teeth are usually crowded and somewhat ill-set.
The neck is long and small in circumference, the shoulders are
square, high and angular, the extremities are long and slender
with long slender muscles and slender bones, the trunk is short
and narrow tapering to the waist. The intercostal angle is
quite acute. The stomach in such a person is long and narrow
and rather vertical in position, extending to low in the abdomen,
and the liver is generally small.

The shape of the eye in this type is such that it is usually
physiologically far-sighted though not pathologically so. They
need no glasses on the street unless for astigmatism or some patho-
logical condition. They are under weight for height according
to the crude average tables now in use, and are often so as
children. They arrive at puberty rather early than late and
differentiate rapidly so that the males develop a large strong
larynx and a low pitched bass or baritone voice. Their skin
is thin and sensitive as is also the epithelial lining of their ali-
mentary tracts. They are as a rule active, energetic and nervous,
quite self-conscious and thus constantly exerting considerable
nervous control. When in normal health they rarely laugh
aloud and when suddenly shocked they resist the reflex jump and
never scream. In this way they pass for cool, calm individuals
with steady nerve, but as a matter of fact the body is almost
constantly held under nerve control and they are actually
nervous, usually suffering more after a shock than on the occasion.

The lateral type when fully expressed is the antithesis of the linear type in all of the respects mentioned. The lateral lines are far apart and the head grows wide and not long (Brachycephalic), the interpupillary distance is wide and the eyes are
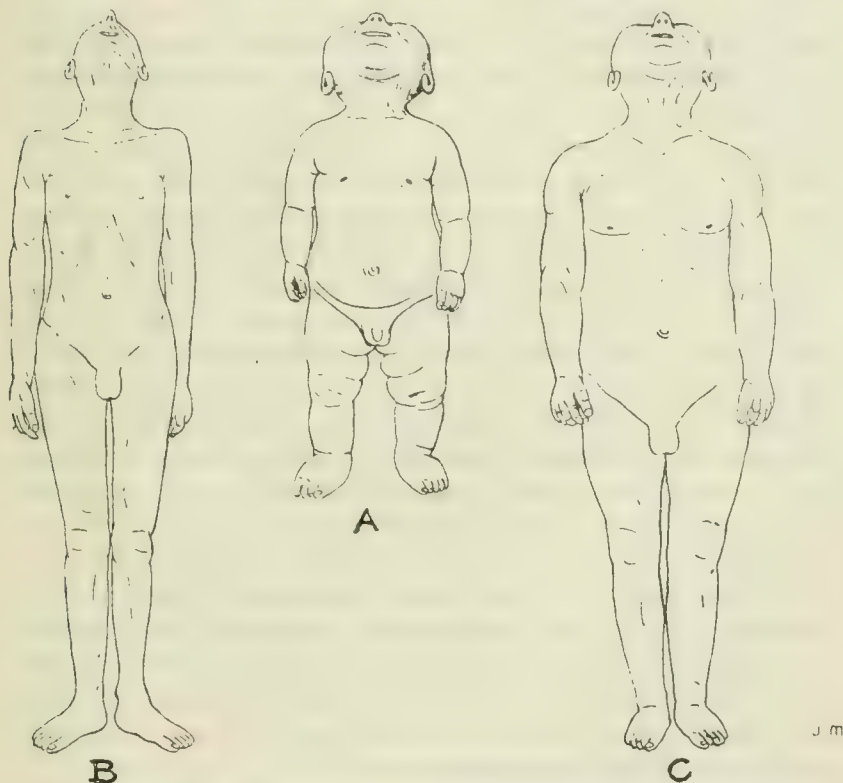
Fig. 2 The ventral aspects of three figures to indicate the degree of divergence of the lateral lines in A, the infant; B, the 'Linear Type'; and C, the 'Lateral Type.'

far apart, the nose bridge is wide and often, though not necessarily, low. The mouth arch is wide and low the teeth are not crowded and are usually smoothly set. The lower jaw is large and strongly developed. The neck is short and large in circumference. The shoulders are round and sloping. The extremities are not long and are stocky with large bones and thick short

muscles. The trunk is inclined to be long and full, not constricted but bulging at the waist. The intercostal angle is quite obtuse. The stomach in such a person is large and tends to be transverse and high in position, the liver is generally large.

The eye in the lateral type is so shaped as to be anatomically near-sighted instead of far and such persons frequently wear glasses on the street. This type is well rounded and over weight for height and also shows great fluctuations in weight, often gaining or losing as much as 15 or 20 pounds in a short space of time. Those of the linear type on the contrary do not experience rapid weight changes but maintain a very constant weight, and may during the twenty years from about nineteen to thirty-nine vary a small number of pounds. The lateral type arrives at puberty a little late and is slow differentiating, the larynx of the male does not develop so suddenly as in the linear type and does not usually grow so large. The voice is thus high or tenor instead of bass. When men are under thirty years old the heaviest bass voices are almost always found among the thin linear individuals and these are very rarely tenors. The finest tenor voices are those of the round lateral type. Everyone recalls that the fine tenor is a fat man while the heaviest bass is a tall thin man.

The two types are more clearly expressed in men than in women since the growth and glandular reactions are more decided in the male than in the female and are also freer from physiological disturbances. Many more physical points of difference and contrast could be cited for the groups but the above list is sufficient to make the differences clear.

The actual recognition of the two types is not new. They have long been recognized just as we should expect, since if the contrasting features are really of any significant value anyone studying the physical characters of men should have discovered the types. Numerous workers have realized the existence of the two types and they have been designated by many names. The most recent, and what seem to me about the best physical distinctions have been recorded by Bean and his data bring out in a statistical way a number of physical differences which may be added to those mentioned above. My linear group Bean

formerly termed hyperontomorph, meaning high developed
structure, but he has since changed the term to hyperphylo-
morph indicating a high phyletic origin. This change in terms
means that Bean considers these types largely as fixed phyletic
or hereditary entities rather than as ontogenetic or develop-
mental results as his former terms indicated. The lateral type
is close to his mesophylomorph. He arranges five types in all
in considering the world races. It is a usual procedure to think
of the races as grouped into separate types and this accounts
for the change of term from onto- to phylomorph. It is the
method of grouping end products to which I object from the devel-
opmental and growth standpoint. I do not question the value
of thoroughly analyzing the physical race differences but it
must be more clearly emphasized that there are frequently
extreme type differences within the race groups. These differ-
ences are often very probably of a genetic origin but they are
complicated in other ways, and in all cases they are directly the
result of definite growth and developmental reactions. The
hereditary type is transmitted but the expression or develop-
ment of this type depends upon numerous environmental influ-
ences, and although a feature may be definitely inherited it may
never be developed or expressed, just as the Seabright bantam
cock inherits the male plumage but is unable to develop such
plumage until his testicles are removed. Without this operation
a casual observer might conclude that the rooster plumage had
failed to be inherited when it has actually failed to be expressed.

In the same way the human types are modified and changed
by environment and the problem of expression or development
is equally as important in understanding them as are the involved
questions of heredity. The two types above outlined actually
occur among all races and nations of men, but some races or
groups show a great majority of one type and only a few of the
other. So that, in general, it may be said that one race is of the
linear type and another of the lateral, whereas the actual ancestry
or stock of the races may have been closely similar. The upper
classes of people in England and Germany illustrate the point.
Almost all of the Englishmen of this cast are linear in type, thin

and dolichocephalic, while almost all of the Germans are of the lateral type, stocky and brachycephalic. Yet some of these Englishmen are decidedly lateral and some of the Germans are decidedly linear in type. All in all, however, the English may be called a linear type race, and the Germans a lateral type race.

We may go still further and claim that these types among the English and Germans are more largely the result of the effects on growth of the environments in which they live rather than of hereditary differences in the stock. That is, the differences are more ontogenetic than phylogenetic in origin. This position will be borne out if we consider the types in conjunction with the geographical distribution of the peoples of the world. The linear types are usually found along the costal planes, in maritime climates where there is a good supply of iodine in the environment and where the thyroid gland is normally active or even hyperactive. The lateral types are largely central continentals living in an inland environment away from the iodine supply of the sea. The thyroid gland functions poorly in these central continental regions, colloidal goitre is common and cretinism occurs in the extreme situations. This is a brief general statement of type distribution, all that space permits, and at once a number of exceptional cases occur to the reader but these have also occurred to the writer and when they are fully considered and analyzed they may be fitted into the general scheme. The high volcanic islands often have in certain of their central valleys lateral type people but the environment may not be actually maritime though it is insular.

It is a well recognized fact that when the distribution of an animal species becomes very broad the species is frequently broken up into a number of varieties, each typical for a given geographic region. There are also certain similar characters developed in the varieties of different species in a given geographic region. The blue-jay has several geographic varieties as has also the bob-white and other birds in different parts of the United States. The northern varieties of these birds are as a rule larger than the southern, and the Florida types are often the smallest and frequently have longer feathers. The small mammals,

squirrels and others, also show numerous geographic varieties. There are reasons to believe that food and other environmental conditions are the primary factors that have brought about these varieties.

When we consider wider geographic ranges, it becomes rare indeed to find the same variety or even species of bird or mammal living on two different continents. The greater the degree and time of isolation for a given region the more the species differ from those of all other world regions, as is illustrated by Australia, New Zealand and other such regions of long isolation.

A species of such wide distribution as the primitive man of several thousand years ago with only limited means of world migration must necessarily have broken up into the geographic varieties which we now find. But the species has in all of its ranges either a tendency to produce a majority of the linear type, long narrow individuals or a majority of the lateral type, stocky round persons. Whichever the tendency may be, we attribute the result to the action of the environment on the function of the thyroid gland and the type is the result of either a fast or slow rate of development. It is a growth reaction.

In diagnosing these types it is very essential to take into consideration the age factor. The well expressed typical condition may only be depended upon during the twenties, at times younger than this the type may not be fully expressed and at periods above thirty it is becoming modified by age changes. It is of course possible to diagnose the type at any age but it is far more difficult and must be done in detail on the young and over mature, while during the twenties it may with practice be done very readily.

During the twenties the linear type is always rather thin, but after thirty-five many of this type become fat and rounder and may at first sight be mistaken for a lateral individual, a change in the pituitary condition having probably occurred, but on close examination the head shape, interpupillary distance, tone of voice and other characters that have not changed make the linear type recognizable in this fat individual.

The study made by Boas a number of years ago on the children of immigrants in New York City and later confirmed in Boston by Guthe, '18, furnishes most important data of the environmental modification of types. Central continentals from Europe with brachycephalic heads and lateral type characters when bred in a maritime environment for several generations tend to become dolichocephalic and of the linear type. The gland quality that produces the type is certainly inherited but the action of the gland itself is actually modified by the environment, and the race stock in the new environment is changed in spite of heredity, the possibilities of which Morgan's experiments on the chickens so beautifully illustrate.

Again there are persons who do not properly fall into either type, nor are they typical intermediates, or blends of the two types. These individuals may possess well marked fully expressed features of the linear type along with typically developed lateral features. They may be dolichocephalic with near-sighted eyes, wide palate arches, and tenor voices. These combinations are at once out of harmony. Such individuals are almost invariably found to be derived from parents of opposite types, and they are very common among the offspring of race mixtures. The fact that the purest type individuals are derived from two similarly pure type parents emphasizes the hereditary elements concerned. In spite of this there is much evidence to indicate that environment may modify the growth regulating mechanism and so tend to change the brachycephalic into the dolichocephalic head.

If two distinct types actually exist in a wide population we should fail to obtain a usually proportioned figure by averaging a large number of physical measurements. A striking illustration of this fact is now at hand. Accurate physical measurements were collected from a great number of men in the recent army

Fig. 3. A statue by Miss Davenport accurately constructed on the basis of average physical measurements obtained from the American army draft. The figure presents unusual proportions and probably indicates that the elements of the draft were not all simple variations about one common mean or single anatomical type.

I am indebted to the American Museum of Natural History for this photograph.

draft by Professor C. B. Davenport of the Carnegie Institution. From these data averages were obtained for each of the several measurements collected. A statue of the human figure was then built on the basis of these averages by Miss Davenport. This might be called a statue of the average young American male. Yet any anatomist examining the illustration shown by figure 3 will at once recognize a number of abnormal proportions. The abnormally long trunk renders the arms disproportionately short and throws the mid point of the figure entirely out of position. Other unusual details render the figure that of a person one would rarely ever see. In melting together the accumulated measurements an unusual or almost deformed figure is produced instead of a combination of commonly seen proportions. This is just such a result as would be expected when measurements from two or more distinct types are averaged. Failure to obtain a commonly seen figure from such measurements is very good evidence for the existence in the population of at least two distinct anatomical types. These are, I believe, those termed above the Linear and the Lateral growth types.

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199 (1946)

Morphology of cystic growths in the ovary and uterus of the guinea pig.

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The ovary in the guinea pig, as in most other mammals, frequently presents a cystic condition. The cysts are the result of a proliferation of the cuboidal epithelium which lines the epididymal portions of the embryonic Wolffian duct. The epididymal tubules are located towards one pole of the ovary and are connected with similar tubules lying outside the body of the ovary between it and the oviduct.

Under certain conditions the walls of these blind tubules begin to proliferate, apparently forming a number of new tubules. A fluid accumulates in the interior of the tubules and distends them into spheroidal shapes. They become greatly distended and break into one another or fuse, thus forming large "ovarian cysts" in the case of those tubules lying within the ovary or "parovarian cysts" in the tubules lying outside.

Thus the ovarian and parovarian cysts are similar in structure and their formation is of the nature of a tumor-like growth of the cuboidal epithelium which lines them. The accumulation of fluid which is essential to the formation of typical cysts is not to be considered their primary cause.

In studying a great many ovaries for cystic conditions during several years we have never observed a follicular cyst. Large atretic follicles may be confused at times with small cysts, but such follicles always begin to disappear or atrophy before attaining significant dimensions.

The uterine glands occasionally become cystic. Such cysts usually break into the lumen of the uterus when their epithelial lining becomes greatly distended. These are similar to the ovarian cysts in that both occur under identical conditions in tubules lined by epithelium. The fact that the uterine glands open directly into the lumen of the uterus makes the occurrence of such cysts exceptional.

200 (1947)

Experimental results bearing on the etiology of cystic growths in the ovary and uterus of the guinea pig.

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In experiments on underfeeding it was found that malnutrition readily gave rise to marked cystic conditions in the ovaries of healthy young guinea pigs. Such cystic conditions are, of course, frequently found in normal stock but here especially in old or unhealthy specimens.

The changed nutritive conditions in the reproductive organs of underfed animals cause circulatory congestion, and as was pointed out in a previous communication[1] such conditions suppress the œstrous changes and prevent ovulation in these animals. The congestion and the high pressure resulting therefrom seem to favor the proliferation of the epithelial lining of the epididymal tubules located near one pole of the ovary, and the accumulation of fluid within the lumen of the blind tubules.

The malnutrition expresses itself first within the ovary by a wholesale degeneration of developing follicles which seem to respond most delicately to changes in nutritive conditions. The congestion and follicular degeneration seem then to favor an overgrowth of the more resistant epididymal tubules which become distended and crowd out the parenchymatous portion of the ovary.

Uterine cysts seem to develop in the same way as those above as a response to the congestion resulting from malnutrition. The open mouths of the uterine glands make their cystic condition rare so that among hundreds of ovarian cysts of all sizes we have observed only one perfectly typical case of uterine cyst.

These experiments seem to indicate that ovarian and parovarian cysts represent growths of persistent embryonic tissue, and that an accompanying congestion and high pressure are necessary to the formation of typical cysts, and that these conditions may result from disturbed nutrition as is demonstrated by underfeeding the guinea pigs.

[1] G. N. Papanicolaou and C. R. Stockard, PROC. SOC. EXP. BIOL. AND MED., 1920, xvii, 143.

STUDIES ON THE GONADS OF THE FOWL

III. THE ORIGIN OF THE SO-CALLED LUTEAL CELLS IN THE TESTIS OF HEN-FEATHERED COCKS

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SEVEN FIGURES

The discovery by Boring and Morgan ('18) of cells identical
with the interstitial cells of the ovary in the testes of cocks of the
Sebright bantam breed, in which all the males are hen-feathered,
has promised to clear up one of the most interesting problems
in the field of endocrinology. Since castration of these hen-
feathered cocks is followed by the appearance of cock-feathering—
a result similar to that obtained after ovariotomy in the female
of other breeds—it seemed logical to suppose that the condition of
the plumage in such males depended upon the presence of inter-
stitial cells of ovarian type. The secretion from these cells was
thought to inhibit the development of the plumage character-
istic of the normal cock (Morgan, '19, '20) In this way a close
relation between a specific tissue and the development of one
of the most highly expressed secondary sexual characters would
be established. This would also confirm the view, so often ex-
pressed, that the interstitial tissue found in the gonads must be
regarded as an endocrine gland.

In a recent paper ('22) I described the origin of the interstitial
cells of the ovary, which have been regarded as luteal cells
by Pearl and Boring. As previously shown by Firket ('14),
these cells arise from the degenerating sexual cords of the first
proliferation and their epithelial origin seems well established.
The clusters of interstitial cells I regarded as the remnants of
the sexual cords infiltrated with fat. It was thought that if the
cells found in the testis of the Sebrights are homologous with those

in the normal ovary they must also arise from epithelium, either in the sexual cords of the early gonad or in the seminiferous tubules which represent their direct continuation. In the following pages I shall show the results of a study of developing testes which has amply confirmed this view.

In the present paper the misleading term 'luteal cells' will be avoided, since a true corpus luteum or luteal cells in the ovary of the hen probably does not exist or is at least extremely doubtful. Indeed, the mere presence of these cells in the testis is sufficient proof that whatever their function may be they are not primarily concerned in the formation of a corpus luteum. On the other hand, their secretory function has not yet been established beyond a reasonable doubt. The term 'interstitial' has been so often associated with endocrine activity that it might convey the idea that the cells under question are glandular elements. Until their true function can be demonstrated, it seems more convenient to speak of the clusters as 'remnants of the sexual cords,' since in both sexes they are derived from these structures, and we may refer to the cells themselves as the 'fat-laden cells' of the clusters.

MATERIAL

The material used in the embryological part of the work consisted of a brood of eight Sebright eggs, generously put at my disposal by Prof. T. H. Morgan. Five of the embryos were males and two were females; one of the eggs failed to develop. The gonads from the male embryos while still attached to the surrounding organs were preserved in Bouin's fluid at the tenth, seventeenth, eighteenth, twentieth, and twenty-first days of incubation. In addition to this material, I was able through the kindness of Prof. H. D. Goodale, of Massachusetts Agricultural College, to study testes of four- and eight-day-old Sebright chicks.

In order to ascertain whether remnants of the sexual cords also occur in chicks of breeds other than the Sebrights, the testes of four- and eight-day-old Rhode Island Red chicks were studied as well as a slide from a young Leghorn prepared by Professor Goodale.

THE REMNANTS OF THE SEXUAL CORDS IN THE SEBRIGHT TESTIS

a. Degeneration of the seminiferous tubules

As in the case of the ovary, the clusters of the so-called luteal cells found in the testis arise as a result of the degeneration of the sexual cords. The sexual cords are represented in late stages of development of the testis by the young seminiferous tubules, which are their direct continuation. Degenerative processes in some of the tubules were only found in the embryos about the time of hatching (twenty and twenty-one days) and in young chicks. They were absent in the younger embryos the testes of which appeared normal.

The seminal epithelium at the end of incubation and during the first weeks after hatching is made up of columnar cells with oval nuclei (fig. 1, n). Scattered among these cells, either toward the periphery of the tubule or near the center there are large, round cells, the spermatogonia. Transitional stages represented by columnar cells with round nucleus of large size are not uncommon. As reported by Firket ('20), secondary spermatogonia are produced by the growth and rounding up of some of the epithelial cells. This columnar epithelium is also the source of the Sertoli cells, which do not appear until spermatogenesis begins.

Since degeneration does not start simultaneously in all the tubules affected by this process, it was possible to establish a closely graded series of stages, some of which have been represented in figures 1 to 4. In the degeneration of the seminal epithelium not only the spermatogonia, but also a large number of the columnar epithelial cells are involved. While many of the latter shrink considerably, their cytoplasm becoming homogeneous and the nuclei pyknotic until no trace of structure can be recognized in them (figs. 1, 2 and 3, d), other epithelial cells are slowly infiltrated with fat as shown by the vacuoles contained in their cytoplasm. These fat-laden cells persist as the so-called luteal cells. The whole process is very similar to the changes observed in the degenerating tubules of the adult under certain conditions (ligature of the vas deferens, partial castration, etc.) which result in the disappearance of all but the Sertoli cells.
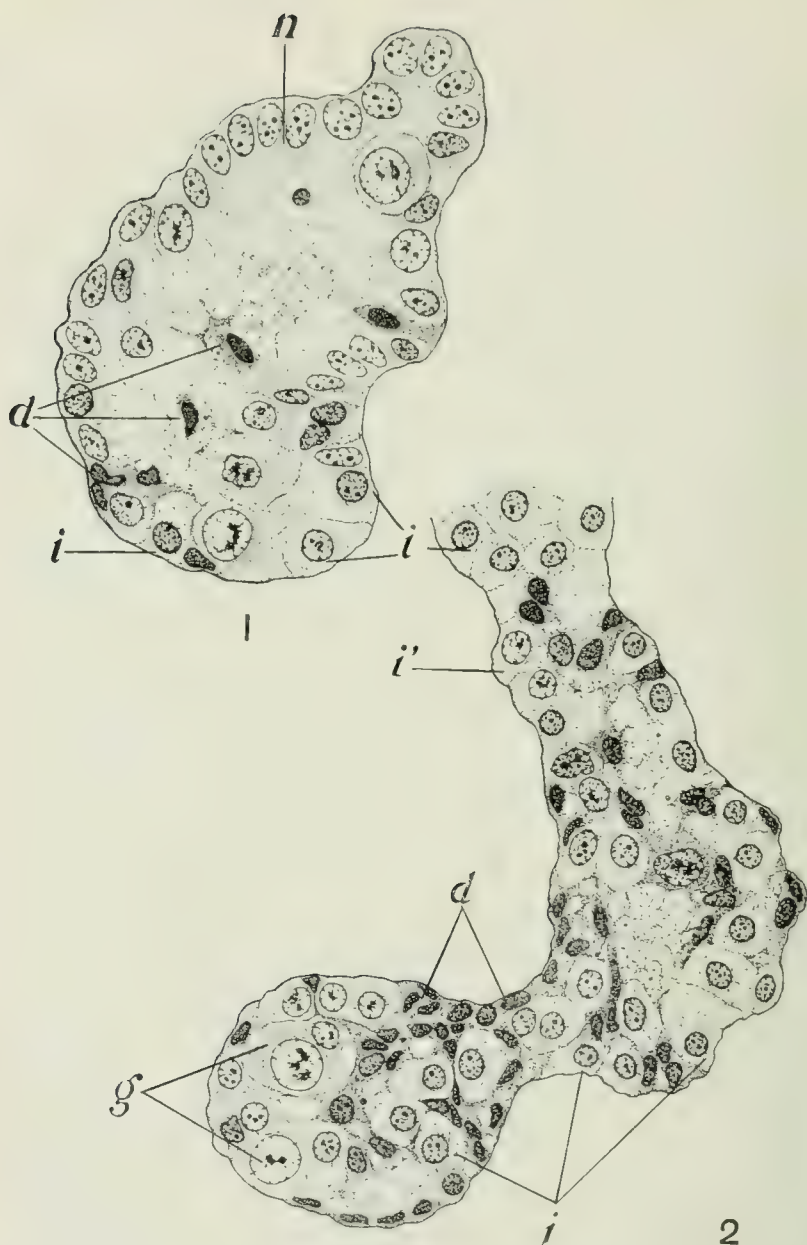
Fig. 1 Embryo of twenty days. Early stage in the degeneration of a semi-niferous tubule of the right testis. *d*, degenerating cells; *i*, fat-laden cells; *n*, normal portion of the seminal epithelium.

Fig. 2 Embryo of twenty days. A more advanced stage in the degeneration of a seminiferous tubule of the right testis. *d*, degenerating cells; *g*, spermato-gonia; *i*, fat-laden cells; *i'*, epithelial cells in early stages of fatty infiltration.

112
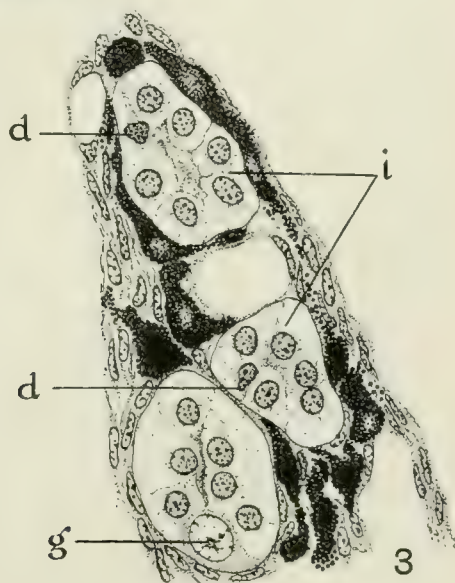
Fig. 3 Four-day-old chick. Clusters of fat-laden cells (*i*) still showing degenerated cells (*d*) and a normal spermatogonium (*g*).

Fig. 4 Four-day-old chick. Degenerating tubule in the periphery of the testis between two normal tubules. *a*, albuginea; *d*, degenerating cells; *i*, fat-laden cells; *t*, portion of the tubule undergoing degeneration.

In the upper portion of the tubule drawn in figure 1 the seminal epithelium (n) appears normal as in the other tubules of the testis. The lower portion shows the beginning of degenerative processes in some of the epithelial cells, whereas others (i) are already infiltrated with fat and resemble very closely the fat-laden cells of the clusters found in later stages. This infiltration takes place following the same steps described by Firket ('14) and more recently by the writer ('22) in the ovary. It begins with the appearance of vacuoles, which cause the cell to become round or polygonal; at the same time the nucleus undergoes a slight decrease in size. Instead of a conspicuous chromatin network, it exhibits scattered chromatin granules and one or two small nucleoli; the nuclear sap stains more deeply than in the undifferentiated cells. Owing to these peculiarities, it is easy to identify the fat-laden cells in the midst of degenerating cells; they appear as polygonal or round elements with very clear cytoplasm and rather deeply stained nucleus. In the figure a normal spermatogonium is still seen in the lower part of the tubule, and directly above it there is another with a shrunken nucleus, the beginning of degeneration. A few pyknotic nuclei (d) are also seen.

In figure 2 I have represented a seminiferous tubule in a state of more advanced degeneration. In this case a still larger number of pyknotic nuclei occur. The region corresponding to the lumen is filled with degenerating cells. In this tubule there are also more fat-laden cells (i) with highly vacuolar cytoplasm. Degenerating spermatogonia can also be detected; finally, two apparently normal spermatogonia (g) are seen in the figure.

A still more advanced stage in the degeneration of the tubules has been represented in figure 3. The seminal epithelium is reduced to fat-laden cells and in some parts of the tubules a few degenerated cells and a normal spermatogonium still occur.

As the tubules degenerate they become irregular in shape and shrink considerably, their epithelium disappearing as such a structure, while the fat-laden cells tend to crowd together. At the end of this process massive clusters are formed. That these clusters are the direct continuation of the seminiferous tubules

is best seen in figure 4, drawn from a section of the testis of a young chick (four days after hatching). In this figure a degenerating tubule has been represented between two normal tubules. The portion nearer to the albuginea (*a*) contains only fat-laden cells (*i*), whereas the opposite portion (*t*) situated towards the center of the testis still shows some slightly modified epithelial cells and two cells (*d*) undergoing degeneration. At a later stage, when all the epithelial cells which do not undergo fatty infiltration disappear, the tubule will appear as an elongated cluster of fat-laden cells, the remnant of a young seminiferous tubule.

The possibility of mesenchyme cells entering the tubules was not overlooked. Indeed, it might be possible that such cells could work their way through the basement membrane and after entering the tubule become infiltrated with fat released by the degenerating cells. From my own observations on testes in which some tubules are undergoing degeneration as a result of partial castration, it seems clear that the basement membrane is impervious and that neither leucocytes nor lymphocytes ever enter the tubules, although in some cases they may be very abundant in the vicinity. In the degenerating tubules of the embryo and young bird the basement membrane stains much deeper than in those which are normal, and owing to this fact it was possible to be certain that there was no break in its continuity, thus preventing the immigration of elements from the surrounding mesenchyme.

b. Developmental disturbances in the testis of embryos and young birds

Aside from the degenerative changes just described, some of the embryos showed in their testes certain features which I regard as abnormal. Since they might have some bearing on the abnormal shape and color of the testis often found in adult birds (Morgan, '19) and on the sterility of some of the individuals, I will describe them briefly.

In the embryo of twenty days circulatory disturbances in the right testis were very conspicuous. The left testis appeared on
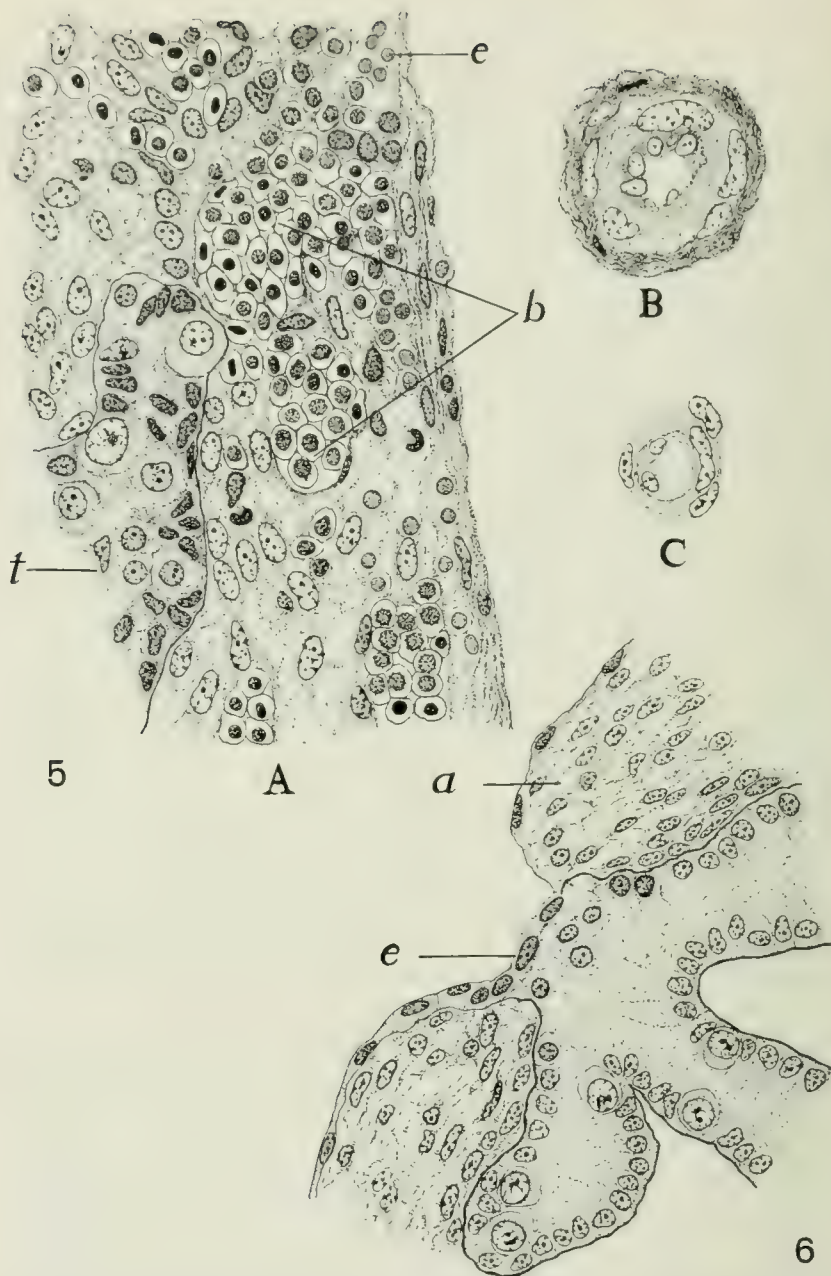
Fig. 5 Embryo of twenty days. Circulatory disturbances in the right testis. A, portion of the albuginea; B, genital artery of the right testis; C, genital artery of the left (normal) testis. b, blood vessels showing blood stasis and hemorrhage; e, degenerating erythrocytes; t, a portion of a degenerating tubule.

Fig. 6 Embryo of twenty-one days. A 'pit' in the albuginea of the left testis. a, albuginea; e, epithelium of the serous layer.

116

the whole entirely normal, but a slight degeneration in the peripheral tubules could be noticed. In the right testis degeneration was widespread in some areas, a third or even a half of the tubules in a given section showing regressive changes in their epithelium. This degeneration was accompanied by diffuse hemorrhagic processes in certain areas of the albuginea.

The study of the mesenchyme under the albuginea showed what at first sight might be regarded as a marked hyperemic condition accompanied with hemorrhage. In the normal albuginea the blood vessels are very thin and usually contain a few normal erythrocytes whose cytoplasm stains readily with eosin. In the portion of the albuginea near the degenerating tubules the capillaries were distended with blood and the erythrocytes were undergoing degenerative changes, their nucleus becoming round and spiny in appearance, while the cytoplasm no longer takes the eosin (fig. 5, A, b). The marked increase in the size of the capillaries and the degeneration of large numbers of erythrocytes within the vessels suggest that the condition just described is one of blood stasis. The walls of the vessels are not distinct, for the erythrocytes have passed into the surrounding mesenchyme, thus causing a hemorrhage. Indeed, it seems likely that the endothelial wall of the distended capillaries no longer exists, at least as a continuous structure; the presence of deeply stained nuclei in their vicinity suggests that there has been a proliferation of the endothelium—a feature not uncommon in blood stasis under pathological conditions in man.

The study of the right mesonephros showed that hemorrhage was also widespread in this organ, perhaps to a larger extent than in the testis, since the blood supply is more abundant. In some areas the mesonephric tubules appeared embedded in a continuous mass of degenerating erythrocytes. Hemorrhage was also noticed in the root of the mesentery.

A comparison of the arteries supplying the testis of each side showed that the right genital artery (fig. 5, B) was much larger and had thicker walls than the left genital artery (C). This points to degenerative changes in the former, and perhaps to this condition are due the circulatory disturbances described above.

In the embryo of twenty-one days degenerating tubules occurred only in the right testis, but they were less abundant than in the twenty-day embryo and were restricted to the periphery. In both testes the primitive relation of the sexual cords and the germinal epithelium (now the epithelium of the serous layer) had been retained. Under normal conditions the sexual cords lose their connections with the germinal epithelium when the albuginea begins to develop. In the embryo we are considering the albuginea developed normally, but mesenchyme cells failed to penetrate between the epithelium and the cords in the areas of proliferation. As a result of the thickening of the albuginea in certain places contact between the epithelium and the cords takes place at the bottom of depressions or pits, one of which has been represented in figure 6. The portion of the cords in immediate contact with the serous epithelium (c) showed slight degeneration in some of its cells.

These 'pits,' of variable depth, were very abundant in both testes; over seventy were found in the left only. The arrangement of the peripheral tubules, which almost invariably converge into the pits, I regard as the result of their formation by proliferation of the germinal epithelium in the restricted areas forming the bottom of the pits.

Inasmuch as similar conditions were not found in the twenty-day embryo and in young chicks, I believe that the condition described is abnormal. It represents the persistence of an early stage in the development of the gonad in an embryo ready to hatch. Whether the albuginea becomes of uniform thickness in later stages of development or whether the condition described persists in the adult is not known. I might state, however, that in both cases the chances of degeneration of the peripheral tubules are great, thus contributing to a further increase in the number of clusters of fat-laden cells.

In the four-day-old chick there were neither circulatory disturbances nor 'pits' in the albuginea, but an enormous amount of pigment appears within giant-cells in the intertubular spaces (figs. 3 and 4). The round shape of the pigment granules and their deep brown or black color suggest that this substance is

melanin. It appeared in both gonads, but was absent in adjacent organs, such as the adrenals and developing epididymis. The amount of melanin within the giant-cells was so large that their nuclei were almost invisible.

The presence of variable amounts of pigment in the testes of the Sebrights has been reported by Morgan ('19); in some cases both testes appear entirely black. The most striking feature of the pigmentary cells is their large size when compared with the ordinary cells of the mesenchyme. Pigmentary cells were not found in the late embryos, but in the embryo of ten days cells were observed in which pigment granules were beginning to develop. The similarity of these cells with the wandering hemoblasts is so striking that one might believe that they are modified hemoblasts. They were most abundant in the loose mesenchyme separating the testis from the mesonephros and also in the mesenchyme surrounding the adrenals. It seems likely that these cells are the forerunners of the giant pigmentary cells found in later stages and in the adult cocks. In some regions of the testis of the seventeen- and eighteen-day embryos pigmentary cells were also found; some were round and others showed well-developed branches.

The absence of pigmentary cells in the testes of some birds, their variable amount when present, and the unequal extent of their distribution suggest that the formation of pigment is an abnormal feature. The formation of melanin and related pigment in man is common in the melanoma, but in the testis it has never been found. On the other hand, in the Sebrights there is nothing suggesting neoplastic growth, unless the pigmentary cells themselves are regarded as a diffuse tumor which had invaded the gonad.

Pigment was also observed in the upper half of the right testis of an eight-day-old Sebright chick; the other testis appeared entirely normal.

THE REMNANTS OF THE SEXUAL CORDS IN THE TESTIS OF YOUNG CHICKS OF OTHER BREEDS

Clusters of fat-laden cells surrounded by a distinct basement membrane and identical in all respects with those described in the Sebrights were also observed in the testes of birds of other breeds in which they occur at least during the first days after hatching. Among my slides two series, one belonging to a four-day-old and another to an eight-day-old chick of the Rhode Island Reds breed showed typical clusters; they were absent in the adult cock of the same race. In the four-day-old chick degenerating tubules could be noticed in one of the poles of the testis and a few fat-laden cells in early stages of infiltration were seen in the midst of cells undergoing regression.

In a slide of a young Leghorn chick, prepared by Prof. H. D. Goodale, the testes showed an enormous amount of fat-laden cells forming large clusters surrounded with a conspicuous basement membrane (fig. 7). Judging from the appearance of these testes and the abundance of remnants of the sexual cords, a widespread degeneration of seminiferous tubules must have taken place at some stage in development. The seminal epithelium appeared normal and showed the structure characteristic of the stage preceding spermatogenesis.

From these facts it may be gathered that the formation of clusters of fat-laden cells persisting as the remnants of the sexual cords is by no means a characteristic feature of the Sebrights, but may also take place in the testis of birds of other races. That a few tubules may degenerate at early stages of their development prior to the full differentiation of the seminal epithelium is not surprising, and must be regarded as a result of the readjustment of the organ to functional conditions. In all probability, some portions of the sexual cords fail to become incorporated into the system of the seminiferous tubules, and under such conditions slowly degenerate, whereas normal tubules undergo the series of changes preceding spermatogenesis.

DISCUSSION

The most significant feature in connection with the presence of clusters of fat-laden cells in the testis of the Sebrights is their origin from the epithelium of the sexual cords as represented in late stages by the seminiferous tubules. The clusters found in
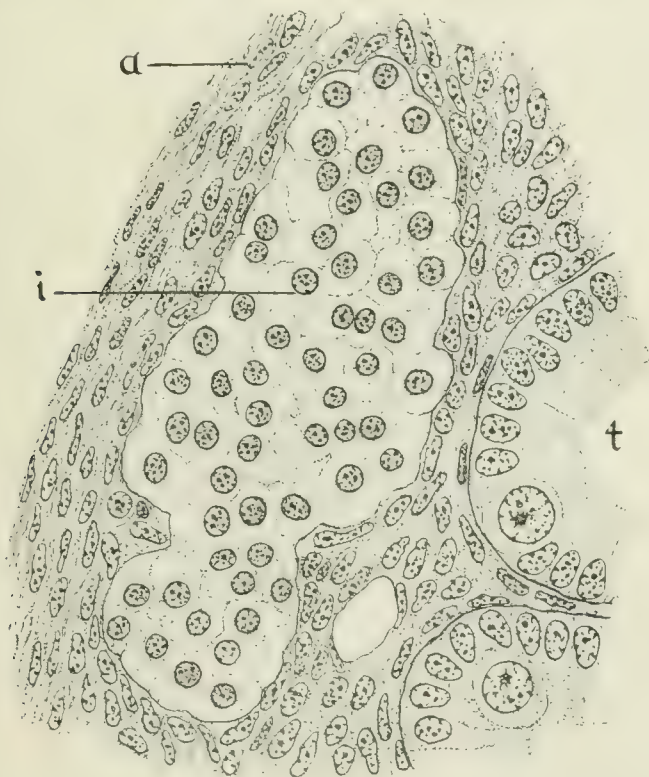
Fig. 7 A cluster of fat-laden cells in the periphery of the testis of a young Leghorn chick.   a, albuginea; i, fat-laden cells; t, normal tubules.

the ovary also arise from these structures at an earlier stage of development. There can be little doubt about the homology of the cells in both organs. They represent the same kind of element, coming from identical origin and showing similar characteristics. If they perform a definite function in the gonads, it must be very similar in both sexes. The question naturally

arises whether, as has been suggested, they are secretory ele-
ments playing the rôle of an endocrine gland or whether they
represent the result of fatty infiltration of degenerating cells.
Unfortunately, from a study of the slides, no definite conclusion
can be reached with regard to this important question. From
a purely histological standpoint, the deep regressive changes
undergone by these seminiferous tubules in which fat-laden cells
arise strongly suggest that the formation of the clusters in the
testis is an abnormal feature due to regressive differentiation of
immature elements rather than to progressive changes leading to
the formation of new morphological and physiological units.

As shown in the figures, the spermatogonia without exception
and many of the columnar cells which at the end of development
constitute the bulk of the seminal epithelium are readily affected
by degeneration, shrinking and finally disappearing without
leaving any trace. But other epithelial cells undergo fatty in-
filtration and persist, at least during the first weeks after hatch-
ing. The cause of this different behavior is unknown. It may
be due to the fact that they are elements of higher vitality or
else that they do not require the same optimum of environmental
conditions to survive as do the germ-cells.

Under certain conditions (partial castration, ligature of the
vas deferens, etc.), the seminal epithelium of adult tubules also
shows similar phenomena, the only difference being that the
elements which persist are fully differentiated and can be easily
identified. As is well known, in these cases the germ-cells in
the various stages of spermatogenesis degenerate and finally
disappear, but the Sertoli cells are left behind and persist un-
changed during a considerable time, if not throughout the life
of the individual. As yet there is no evidence that the elements
which in the embryo and young chick become fat-laden cells are
the forerunners of the Sertoli cells, but it is possible that, although
uniform in appearance, the epithelium of these early tubules
already contains two different kinds of cells which cannot be
distinguished from each other by any morphological characteris-
tic. If some of the cells in this epithelium are already potential
Sertoli cells, it is not surprising that their behavior may be differ-

ent, for this is precisely what happens in the adult in which full
morphological differentiation has already been attained.

With regard to the factors which cause degeneration in some of
the seminiferous tubules, very little can be said. As already
mentioned, young chicks of breeds other than the Sebrights may
show remnants of the sexual cords in the form of clusters of fat-
laden cells. It is easy to conceive this degeneration as the result
of the readjustment of the young gonad to functional conditions,
since at the late stages in development and first days after hatch-
ing there occur changes in the testis leading to the formation of
the system of seminiferous tubules. If portions of the sexual
cords have been cut off from this system by the increase in the
mesenchyme which precedes the formation of connective tissue,
their degeneration is only a question of time; they may disappear
as such structures before spermatogenesis begins, leaving clusters
of fat-laden cells, or they may persist until they become fully
differentiated tubules, undergoing degeneration while distended
by the spermatozoa, which are unable to leave the tubule on
account of its lack of an outlet.

In the late stages of the Sebright embryos degenerative changes
in the tubules were accompanied by other conditions which can
scarcely be regarded as normal. The existence of deep circulatory
disturbances, the persistence of connections between the sexual
cords and the germinal epithelium in one of the embryos, and the
abundance of giant pigmentary cells in the young chicks are
probably due to the influence of disturbing factors. Whether
these abnormal features are always found in the late stages of
embryos of this peculiar breed or whether normal development
is the rule rather than the exception is a point to be established by
further researches. I will, however, mention the fact that Mor-
gan ('19) has reported abnormalities in the gonads of adult cocks:
according to this investigator, the testis "was often more flattened
than is the testis of the typical male bird, that it was often some-
what pear-shaped, and that frequently it was in part or entirely
black" (p. 5). Sebright cocks are said to be often sterile. That
these conditions may be the outcome of disturbances during
development is extremely likely. Yet the factors causing the

abnormal development can only be surmised. Equally puzzling is the fact that degeneration of the tubules in the embryos studied was more conspicuous in the right testis than in the left, and that in the case of the eight-day-old chick pigment had only developed in the upper half of the right gonad. This is important in connection with the constant degeneration of the right ovary in birds.

CONCLUSIONS

1. The clusters of the so-called luteal cells found in the testis of Sebright cocks arise during degeneration of the sexual cords and early seminiferous tubules as the result of fatty infiltration of certain elements of the seminal epithelium. The spermatogonia and many of the columnar epithelial cells disappear without leaving any trace of their former existence.

2. Degeneration of the young seminiferous tubules was only found in late embryos and young chicks; in the former it was more marked in the right testis.

3. Aside from degeneration in some of the tubules, developmental disturbances, such as blood stasis, abnormal persistence of connections between the germinal epithelium and the seminiferous tubules, and excessive formation of pigment, were observed.

4. Remnants of the sexual cords in the form of clusters of luteal cells were also found in the testes of young birds of breeds other than the Sebright.

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THE FORMATION OF THE ASTER IN ARTIFICIAL PARTHENOGENESIS.*

By ROBERT CHAMBERS.

(*From the Cornell University Medical College, New York.*)

In normally fertilized eggs the development of the aster is attributed to a substance carried into the egg by the spermatozoon. The aster first makes its appearance in the form of diminutive radiations surrounding the neck-piece of the spermatozoon within a few minutes after it has entered the egg. The writer[1] has shown that the formation of the radiations is accompanied by a jellying of the cytoplasm of the egg. The jellying process extends more and more as the aster increases in size and the entire egg becomes involved when the center of the aster comes to occupy the center of the egg.

The formation of the aster is accompanied by an increase in size of a hyaline area in its center. This is Wilson's hyaloplasm-sphere[2] also called centrosphere and astrosphere by other investigators. The microdissection method has demonstrated that this sphere area is liquid in contrast to the surrounding jellied cytoplasm. The pioneer observers of mitotic division, such as Auerbach, Hertwig, Bütschli and Fol, described the accumulation of a hyaline plasma at the astral centers and suggested that the astral radiations are a result of protoplasmic currents. Later investigators, such as Morgan, Wilson and Conklin, considered this view as the most probable one.

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* The experiments, upon which this paper is based, were conducted in the Research Division of Eli Lilly and Company, at the Marine Biological Laboratory, Woods Hole. The experiments constitute a part of a joint research project in which Dr. G. H. A. Clowes and the writer are engaged.

[1] Chambers, R. Microdissection studies. II. The cell aster: A reversible gelation phenomenon, *J. Exp. Zool.*, 1917, xxiii, 483.

[2] Wilson, E. B., Experimental studies in cytology. I. A cytological study of artificial parthenogenesis in sea-urchin eggs, *Arch. Entwcklngsmcchn.*, 1901, xii, 529.

The movement of the egg nucleus is possibly also a case in point. As long as the egg nucleus is beyond the confines of the aster it is stationary. As soon, however, as the extending aster reaches it, the nucleus begins travelling toward the sphere in which it finally lies close beside the sperm nucleus. The existence of a centripetal current may be inferred also from the following experiment. In an egg one may occasionally see one or more oil-like droplets 2 to 4 microns in diameter. If one of these droplets be pushed by the needle from the liquid cytoplasm into the periphery of the aster the droplet will move along the rays toward the center.

In view of the above observations it is highly probable that the liquid which accumulates in the center of the aster streams into it from all sides during the jellying of the cytoplasm. It is this streaming which probably occasions the innumerable radiations characteristic of the aster. After the aster has attained its full size the radiations begin to fade from view as the jelly state reverts to a more fluid one. The liquid of the central sphere does not mix with the fluid cytoplasm but separates into two areas, one at each pole of the mitotic figure of the dividing nucleus. Astral radiations now appear about the two areas as the egg cytoplasm jellies again with the formation of two jellied masses instead of one, as heretofore. These grow at the expense of the fluid cytoplasm until all of the cytoplasm of the egg is taken up into two bodies, the two blastomeres of the segmenting egg.

During the rapidly succeeding cleavages of the egg there is always a cap of liquid on the nucleus of each blastomere. With each mitosis this liquid flows around the nucleus to accumulate in two areas at the poles of the mitotic figure. These areas are periodically augmented during the formation of an aster and the ensuing jellying process.

There is every evidence[3] that the mechanism of cell division depends upon a readiness of the cytoplasm to pass from a liquid to a

[3] Heilbrunn, L. V., Studies in artificial parthenogenesis. II. Physical changes in the egg of *Arbacia*, *Biol. Bull.*, 1915, xxix, 149; An experimental study of cell division. I. The physical changes which determine the appearance of the spindle in sea-urchin eggs. *J. Exp. Zool.*, 1920, xxx, 211; Chambers, R., Changes in protoplasmic consistency and their relation to cell division, *J. Gen. Physiol.*, 1919, ii, 49.

jellied state and *vice versa*. The protoplasm must have its phase relations in a delicately balanced state in order that this may occur. In the egg we have seen that the reversal to a jellied state is probably accompanied by a separating out of a liquid. Something in this liquid may possibly control, in periodic rhythms, the physical state of the protoplasm surrounding it. We may assume that as long as there is a quantity of this substance localized in the egg it can induce aster formation. The idea suggests itself that one purpose of the spermatozoon is to accumulate this substance. In the mature unfertilized egg there is no localized area from which the jellying process may spread. The entrance of a sperm furnishes a focus as it were. Around this focus an aster develops with a steady accumulation of the liquid in its center. This liquid area surrounds the nucleus and puts the egg in a condition similar to that of a blastomere. The process of cleavage then becomes the same in both.

An interpretation disconsonant with previous ones concerning the mode of aster formation in artificially parthenogenetic eggs has been recently put forward by Herlant.[4] Wilson[2] in *Toxopneustes*, had long ago shown that eggs treated insufficiently with a parthenogenetic agent may form monasters which disappear and reappear in several successive rhythms. Hindle[5] found this to be true also for the sea-urchin egg, if treated with butyric acid alone. A sufficient treatment, however, of a parthenogenetic agent results in the disappearance of the monaster followed by the appearance of an amphiaster. This results in cleavage of the egg. In the sea-urchin egg, the butyric acid treatment has to be followed by a bath of hypertonic sea water in order that this may occur. The hypertonic treatment often results in the formation of several cytasters in the egg. The cytasters produced by the hypertonic treatment Herlant claimed to be due to dehydrative effects producing spots within the egg cytoplasm about which the asters appear. Herlant assumed that one of these cytas-

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[4] Herlant, M., Comment agit la solution hypertonique dans la parthénogenése éxperimentale (méthod de Loeb). I. Origine et signification des asters accessoires. *Arch. Zool. exp. et gén.*, 1918, lvii, 511; II. Le mecanisme de la segmentation. *Arch. Zool. exp. et gén.*, 1919, lviii, 291.

[5] Hindle, E., A cytological study of artificial parthenogenesis in *Strongylo-centrotus purpuratus*, *Arch. Entwcklngsmechn.*, 1910–11, xxxi, 145.

ters connects in some way with the monaster, thus forming the amphiaster which initiates segmentation. The weakness in this interpretation is the lack of conclusive evidence for the union of the originally independent asters. Neither Wilson nor Hindle ever observed such a phenomenon. All my observations also indicate that the amphiaster in parthenogenetic eggs arises from a previous single aster just as it does in normally fertilized eggs.

My studies were mainly confined to the egg of the sand-dollar. In its behavior to parthenogenetic agents[6] the egg is almost identical with that of the sea-urchin which Herlant studied. The absence of pigment and the highly translucent nature of its protoplasm makes the sand-dollar egg an ideal object for observational study.

The mature eggs, normally shed by the female, are placed in butyric acid (2 cc. 1, 10 N in 50 cc. of sea water) for 35 seconds. During this treatment the eggs distinctly round up. They are then returned to sea water where, within a few minutes, the fertilization membrane lifts off. After 20 minutes the eggs are placed in hypertonic sea water (5 cc. 2.5 M NaCl in 50 cc. sea water). The eggs shrink slightly in this solution. After 20 minutes the eggs are transfered to a large quantity of normal sea water and the sea water is changed several times to free the eggs from any further action of the hypertonic solution.

Up to this time no change whatever is to be seen in the cytoplasm or in the nucleus. While in the hypertonic solution the cytoplasm appears more granular and opaque than that of an untreated mature egg. However, on the return of the treated eggs to sea water the cytoplasm reverts to its former appearance and to the eye the eggs differ in no respect whatever from unfertilized eggs except for the presence of a fertilization membrane.

It is not until the treated eggs have stood in sea water for several minutes that any cytoplasmic change is to be observed. The first sign of a change consists in the appearance of faintly defined vacuoles about the center of the egg. Within a few minutes they coalesce to form a central clear area of about one-tenth the diameter of the

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[6] Just, E. E., The fertilization reaction in *Echinarachnius parma*. III. The nature of the activation of the egg by butyric acid. *Biol. Bull.*, 1919, xxxvi, 39.

egg. The egg nucleus lies close to or within this area. Gradually rays begin to appear in the jellying cytoplasm about the area. These rays become more numerous and more pronounced until the entire egg is occupied by a large monaster which corresponds exactly with the fully developed sperm aster of a normally inseminated egg. From now on the process is entirely analogous to that of a sperm fertilized egg. During the development of the aster the hyaline central area increases in size and the microdissection needle shows it to be a liquid area characteristic of that of the sperm aster. When the monaster disappears the liquid central area flows around the nucleus now undergoing mitosis and accumulates at the two poles of the nucleus into two polar areas. A jellying process now sets in with these two areas as centers and results in the amphiaster preparatory to the first cleavage of the egg.

In the mode of aster formation the only difference between the sperm fertilized and the parthenogenetic egg consists in the manner in which a liquid separates out of the jellying protoplasm in connection with the formation of the preliminary single aster. In the fertilized egg radiations appear immediately about the sperm-head and the accumulation of the liquid substance is from the beginning through the agency of the ray-like channels of the growing aster. In the parthenogenetic egg several vacuoles first appear in the cytoplasm. These vacuoles collect in the center of the egg after which an aster appears.

The frequent irregularities which obtain in parthenogenetic eggs are apparently due to an incomplete fusing of the vacuoles and to a lack of polarity in the preliminary stages of the aster formation. In undertreatment, or when butyric acid alone is used, a monaster developes as usual. Upon the disappearance of the monaster, the persisting liquid centrosphere, instead of flowing to the two polar regions of the nucleus, remains a single body. With the return of the jellying period a single aster again forms and more fluid accumulates in the centrosphere which increases in size. This process repeats itself several times and segmentation of the egg never occurs.

Eggs treated with butyric followed by a prolonged treatment of the hypertonic solution become abnormal. In cases of this kind the eggs, when returned to sea water from the hypertonic solution,

exhibit vacuoles which, instead of being collected in the center of the egg, are scattered throughout the cytoplasm. Radiations appear about these vacuoles with the result that the egg becomes filled with many small asters. The longer the eggs have been left in the hypertonic solution the more numerous will be the asters, and most if not all of these asters develop independently of one another. Irregularities may occur, even when the vacuoles collect in the center of the egg. In such cases an apparently normal single aster first results. Upon its disappearance, the central liquid area, instead of flowing away from the center into two polar bodies, produces three or four irregular lobes. About each of these lobes radiations appear in the egg cytoplasm producing a multipolar aster. In one instance one such lobe separated itself from the main body and a complete aster formed about it while a multipolar aster formed about the rest of the hyaline area. When the periphery of a multipolar aster reaches the surface of the egg cleavage furrows form between each lobe of the aster so that such eggs may segment simultaneously into three or four or more blastomeres. Asters which form independently of the central area never seem to be large enough to bring about segmentation of the egg into considerable masses. When such asters lie close to the periphery of the egg, furrows often grow in from the surface of the egg enclosing the asters. In this way a superficial type of segmentation results with the pinching off of small masses of the egg. The development of cytasters resulting in a spurious segmentation has already been described by Wilson.[2]

The first aster appears at about the same time after the acid treatment, irrespective of whether the eggs have been subsequently treated with the hypertonic solution or not. However, with subsequent hypertonic treatment, the reappearance of the radiations following the fading away of the first aster occurs about more than one center. This results in segmentation of the egg. The reaction, therefore, which is peculiar to hypertonic treatment shows up only *after* the disappearance of the first aster. At that time the persisting central liquid area of the aster, instead of remaining as a single centralized mass, separates into two or more bodies with the result that the following reappearance of rays in the cytoplasm occurs as radiations about these bodies. This produces multiple asters. If there be

only two focal points the liquid collects into two bodies, a typical
amphiaster then develops, and the egg cleaves into two normal
blastomeres.

Aster formation not only consists in a jellying process but also in
the separating out of a liquid. The optically visible phenomenon
peculiar to the parthenogenetic egg consists in the manner in which
this liquid begins to separate out of the egg cytoplasm preparatory to
the formation of the preliminary single aster. In the sperm fertilized
egg both processes are rapid and occur together, radiations appear
immediately about the sperm-head, and the accumulation of the
liquid substance is from the very start through the agency of the
ray-like channels of the growing aster. In the parthenogenetic egg
the jellying process is apparently very slow, and the separating out
of a liquid takes place before the cytoplasm is stiff enough to exhibit
channels through which the liquid flows to the center. The liquid
first collects into several vacuoles and an optimum treatment is nec-
essary to cause these vacuoles to fuse into one body with the subse-
quent formation of a single aster. Overtreatment causes the appear-
ance of many vacuoles scattered throughout the egg resulting in
multiple asters. Undertreatment may result in the formation of a
single aster which, however, periodically disappears and reappears as
a single aster.

The parthenogenetic treatment, in order to be successful, must
not only bring about the separating out of a liquid from the egg
cytoplasm, but must also induce polarity within the resulting hyaline
area in order to enable it to form two centers about which an amphi-
aster may develop.

STUDIES ON THE ORGANIZATION OF THE STARFISH EGG.*

By ROBERT CHAMBERS.

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(Received for publication, July 19, 1921.)

The following is a preliminary record of operative work on the starfish egg which throws some light on the nature of the fertilization membrane, the interaction between nucleus and cytoplasm, and the relation of the cortex to the interior of the egg.

By means of the microdissection needle it has been possible to show that a morphologically definite membrane closely invests the unfertilized egg, and that it is this membrane which lifts off upon fertilization as the so called fertilization membrane. The description of two methods will suffice to demonstrate this. By carefully pressing an unfertilized mature egg between the surface of a cover-slip and the side of a slender glass needle the egg may be cut in two without tearing the investing membrane. This membrane now becomes apparent, bridging the gap between the two egg fragments and holding them together. Upon the addition of sperm this membrane lifts off as the fertilization membrane, in such a way that the two egg fragments come to lie within a single cavity.

The unfertilized egg can also be slipped entirely out of its investing membrane. Such an egg will undergo normal fertilization and cleave into blastomeres having no investing membrane whatever.

These two experiments definitely show that the normal unfertilized starfish egg is already surrounded by a membrane which, upon fertilization, becomes the fertilization membrane.

The difference in behavior towards sperm of an egg, which has been denuded not only of its jelly but also of its membrane, and one which has not is very striking. In an egg enclosed in its membrane

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* The experiments reported in this paper constitute a part of the joint investigation of the mechanism of fertilization in which Dr. G. H. A. Clowes and the writer are engaged.

the spermatozoa quickly crowd about the egg as they are trapped in the jelly surrounding the membrane. In a membraneless egg no crowding of spermatozoa is noticeable and heavy insemination is necessary to bring about fertilization. With such eggs, when a cloud of sperm has been blown upon them, one may frequently observe a spermatozoon swim toward an egg, wander over its surface and then swim away. On the other hand the empty membrane with its investing jelly immediately becomes covered with a halo of active spermatozoa.

The nucleus of the egg cell is a liquid drop surrounded by a morphologically definite membrane. The nucleus may be moved about within the egg with the needle, and can be considerably deformed by pressure. On removal of the needle the nucleus quickly resumes its spherical shape. Tearing the nucleus slightly causes the nucleus to shrink and the nucleolus to disappear; this is followed by a remarkable spread of a disintegrative process which involves the cytoplasm surrounding the nuclear area. In the immature egg, where the nucleus is large, the disintegrative process may extend throughout the entire egg. In the mature egg with a relatively small nucleus the destruction is restricted to a limited area.

The disappearance of the nucleus or germinal vesicle during maturation has been described by several investigators. The nuclear membrane breaks down spontaneously and the nuclear sap spreads slowly throughout the cytoplasm. So long as the nuclear area, aside from the definitive egg nucleus, has not yet mixed with the cytoplasm, I find that a puncture of the area starts up the disintegrative process. When the nuclear sap has entirely mixed with the cytoplasm, any part of the egg, with the exception of the minute egg nucleus, may be torn with impunity. The mere presence of the glass needle in the nuclear sap is not sufficient to start up the disintegrative process. This process occurs only when the nuclear sap is agitated by the needle while the sap is in direct contact with the cytoplasm.

Wilson[1] found in the Nemertine egg that any non-nucleated fragment, prior to the dissolution of the germinal vesicle, is non-fertilizable whereas, any fragment from a mature egg is capable of being fertilized and undergoing cleavage. This I have found to be true also for the

[1] Wilson, E. B., Experiments on cleavage and localization in the Nemertine egg, *Arch. Entwcklingsmechn.*, 1903, xvi, 411.

starfish egg. It is also of interest to note that the fertilizability of the egg fragments is directly connected with the extent of the mixing of the nuclear sap with the cytoplasm in the maturing egg. A non-nucleated fragment, taken from an egg in the early stages of the dissolution of the germinal vesicle, will admit sperm which will undergo several nuclear divisions with, at most, an abortive attempt on the part of the fragment to cleave. When the sap of the germinal vesicle has completely mixed with the cytoplasm, any fragment larger than a certain size limit is capable of being fertilized and undergoing cleavage.

It is well known that immature eggs can be kept in sea water at room temperature for 24 hours or more without disintegrating and that unfertilized mature eggs go to pieces under the same conditions within a much shorter time.[2] The writer has found that nucleated fragments of the two kinds of eggs behave similarly, while non-nucleated fragments act quite differently indicating that the substance which prevents the disintegration is distributed differently in the two eggs. Non-nucleated fragments of immature eggs last for about 4 hours only. Similar fragments of mature eggs last from 8 to 10 hours, or about as long as the mature, nucleated fragments. The substance which prevents the destruction of the egg is apparently in the nuclear sap which, in the immature egg, is confined within the large nucleus or germinal vesicle, while in the mature egg this sap has escaped from the nucleus and spread throughout the entire egg.

The following experiments indicate that the part of the starfish egg which is capable of development is chiefly confined to the cortex of the egg. It was long ago shown by Driesch,[3] Loeb[4] and others that starfish and sea-urchin eggs are highly fluid in that fragments quickly round up into spheres. That the cortex of the mature un-fertilized eggs is firmer in consistency than their interior has been

[2] Loeb, J., and Lewis, W. H., On the prolongation of the life of the unfertilized eggs of the sea-urchins by potassium cyanide, *Am. J. Physiol.*, 1902, vi, 305. Loeb, J., Maturation, natural death and the prolongation of the life of the unfertilized starfish eggs (Asterias forbesii) and their significance for the theory of fertilization, *Biol. Bull.*, 1902, iii, 295.

[3] Driesch, H., Entwicklungsmechanische Studien. Der Werth der beiden ersten Furchungszellen der Echinodermentwicklung, *Z. wiss. Zool.*, 1891, liii, 60.

[4] Loeb, J., Ueber die Grenzen der Theilbarkeit der Eisubstanz, *Arch. Physiol.*, 1895, lix, 379.

described by the writer.[5] If the surface of the mature starfish egg
be torn with a needle, and the egg then caught at the opposite side
and pulled to the edge of the hanging drop, the compression on the
egg produced by the shallow water at the edge of the drop will
cause the fluid interior to ooze out through the tear, forming a perfect
sphere. One may so manipulate the process as to cause the egg
nucleus either to remain behind in the cortex (the cortical remnant)
or to pass into the extruded sphere.

The cortical remnant is relatively solid and remains more or less
enclosed within the egg membrane and its jelly. If left long enough
it will eventually round up so as to present the appearance of a dimin-
utive egg surrounded by a collapsed and wrinkled egg membrane.

The material which has escaped from the egg into the sea water
is fluid and tends immediately to round up. On tearing with a needle
its surface behaves like that of a highly viscous oil drop. These spheres
adhere tenaciously to glass and, in the effort to remove them by
blowing a current of water against them, they sometimes leave a torn
off piece behind. The cortical remnant is readily fertilizable and
undergoes normal segmentation. On the other hand, the material
which has escaped from the interior of the egg whether nucleated or
not, is non-fertilizable. It remains inert until it finally undergoes
disintegration. As long as it possesses an intact surface it appears
exactly like an egg fragment and will undergo disintegrative changes
similar to those of entire eggs, on being torn with the needle. If even
a small part of the original cortex is allowed to remain continuous
with the sphere it is fertilizable and the more cortical material present
the more will the sphere approach normal cleavage.

It is significant that the fluid spheres which escape from the interior
of the mature unfertilized egg, whether nucleated or not, withstand
disintegration for a much longer period than do fragments, containing
cortical material, which have been produced simply by cutting an
egg into two or more pieces.

It follows from these facts that the part of the starfish egg chiefly
concerned in development lies in its periphery. The interior when
separated from the cortex is incapable of developing. On the other
hand, an egg containing cortical material alone is able to carry on its
usual life activities.

[5] Chambers, R., Microdissection studies. I. The visible structure of cell proto-
plasm and death changes, *Am. J. Physiol.*, 1917, xliii, 1.

46 (1793)

The effect of experimentally induced changes in consistency on protoplasmic movement.

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Agitation by means of a micro-dissection needle tends to cause the protoplasm of a living cell to pass from a more solid to a less solid phase.

In marine ova, where one can closely follow the solidifying of the protoplasm just prior to cell division, mechanical agitation will cause the protoplasm to revert to its original liquid state so that the egg reverts to the shape of a sphere. If the egg so treated be subsequently left undisturbed the solidifying process starts up again with the result that the egg undergoes normal cleavage.

In a previous communication[1] the writer has described the structural relations of changes in protoplasmic consistency of the *Amœba* to the formation of pseudopodia. The maintenance of pseudopodia depends upon a relatively solid state of certain parts of the *Amœba*.

A resting *Amœba*, with numerous slender pseudopodia all over its surface, is relatively solid. Upon mechanical agitation the pseudopodia are retracted as the *Amœba* becomes more liquid. Fresh pseudopodia in an agitated *Amœba* tend to be broad lobate and, if the agitation be continued, all of the *Amœba* liquefies. The entire body then becomes, as it were, a single pseudopodium with a peripheral current of granules flowing away from its anterior end and a central current flowing forward. An *Amœba* in this extreme state does not change in position as the back flow tends to equal the forward flow. *Amœbæ* which are experimentally brought into this state have, so far, not been observed to return to their previous condition. The rate of flow of the currents gradually slows down until the animal dies.

[1] Chambers, Robert, PROC. SOC. EXP. BIOL. AND MED., 1920, xviii, 66.

The protoplasm of an *Amœba* exists in a certain normal state of consistency from which it may deviate so as to solidify on the one hand or liquefy on the other. This normal state may be shifted not only by agitating the *Amœba* but also by injecting certain solutions. This I have been able to do with hydrochloric acid and with sodium hydrate.

A trace of acid throws the normal state to the more solid side, while the alkali throws it to the more liquid side. An acidified *Amœba* forms long slender pseudopodia because the peripheral back flow in the developing pseudopodium is quickly arrested by a setting of the protoplasm. The area of the base of the pseudopodium is, therefore, quickly limited and the extending pseudopodium conforms to this narrow base. In an alkalinized *Amœba*, on the other hand, the peripheral back flow of a developing pseudopodium tends to be arrested much more slowly. As a result of this the base of the pseudopodium spreads considerably before the protoplasm sets. The extending pseudopodium, having a larger base upon which to build, then becomes broadly lobate.

These observations harmonize with my experiments on injecting "acid" and "basic" organic dyes. The basic dyes, which contain a relatively strong acid radicle, jelly the protoplasm, whereas acid dyes, with a strong basic radicle, liquefy it.

It is interesting to note that these changes can be brought about in protoplasm while it is yet alive and that one can thereby change the character of the pseudopodia produced.

MICRODISSECTION STUDIES, III. SOME PROBLEMS
IN THE MATURATION AND FERTILIZATION
OF THE ECHINODERM EGG

MICRODISSECTION STUDIES, III. SOME PROBLEMS IN THE MATURATION AND FERTILIZATION OF THE ECHINODERM EGG.

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This paper is a record of operative work on the starfish, sea-urchin and sand-dollar eggs to ascertain the morphological nature of changes which take place in the egg during its maturation and fertilization. Results were obtained on the effect of nuclear material on cytoplasm, the nature of cortical changes in the maturing and fertilized egg and the difference between cortex and medulla of the egg with respect to fertilizability and to other life activities. The dissection and injection of the living eggs were carried out at first by means of Barber's ('14) apparatus and later with an improved micromanipulator of my own design ('21^b). A description of the technique as applied to microdissection has already been published (Chambers, '18^a). A detailed description of the new micromanipulator will appear both in the *Journal of Bacteriology* and in the *Anatomical Record*.

1. The Germinal Vesicle in the Maturing Starfish Egg.

Starfish eggs, on being shed naturally, have already begun maturing. In order, however, to secure large quantities of eggs, it has been the general custom to remove the ovaries bodily from a ripe female and to cut them up in a bowl of sea water. This procedure brings the eggs into the sea water in the immature condition with germinal vesicles intact. The germinal vesicle begins to disappear anywhere from thirty to fifty minutes after the eggs come into contact with the sea water and maturation usually proceeds in a normal manner (Wilson and Mathews, '95).

The undisturbed germinal vesicle or nucleus of a fully grown

immature egg is a hyaline sphere containing a sharply differentiated nucleolus and occupying about one fifth the volume of the egg. With the microdissection needle the vesicle may be moved about in the fluid cytoplasm without injury to the egg. With the needle one may considerably indent the surface of the vesicle. On removal of the needle the vesicle reverts again to the spherical shape (Fig. 1). The vesicle possesses a morphologically definite surface membrane inclosing an optically homogeneous liquid (cf. Chambers, '18[b]). Within this liquid lies a visible body, the nucleolus. By agitating the vesicle the nucleolus may be made to occupy any position within the nuclear fluid. The nuclear membrane is very easily injured. If, however, a microneedle be carefully inserted into the nucleus, the membrane about the puncture adheres to the body of the needle and the tip of the needle may push the nucleolus about with no apparent injury. The existence of considerable tension in the nuclear membrane is shown in the following experiment. An egg was cut into three fragments in such a way that the surface film forming over the cut surfaces of the middle fragment pressed upon the nucleus, deforming it considerably (Fig. 2). The attempt of the nucleus to return to a spherical shape bulged out one end of the egg fragment until it was constricted off from the remainder of the fragment (Fig. 2b–f).

Tearing the nuclear membrane in most cases results in a destruction of the nucleus. In a few cases it was possible to produce a slight rupture with no noticeable injurious effects. Such a case is recorded in Fig. 3. At 10:44 A.M. undue pressure on the germinal vesicle when cutting an immature egg in two resulted in its rupture followed by a lobular extrusion bounded by a very delicate film. During the following ten minutes the vesicle began slowly to revert to its original shape (Fig. 3b and c). Before that was attained the maturation process began and, at 10:55, the outline of the vesicle had disappeared. The nucleated egg fragment matured normally and five hours and a half after insemination it had segmented in two. At 8:40 P.M. it had developed into a swimming blastula.

The cytoplasm of the egg allows of considerable tearing without

apparent injury (Chambers, '17-a). If, however, the nuclear membrane be torn, a very striking phenomenon occurs. The cytoplasm immediately surrounding the nucleus disintegrates and
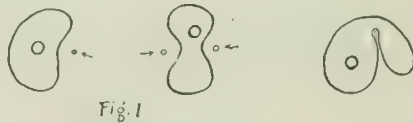
Fig. 1
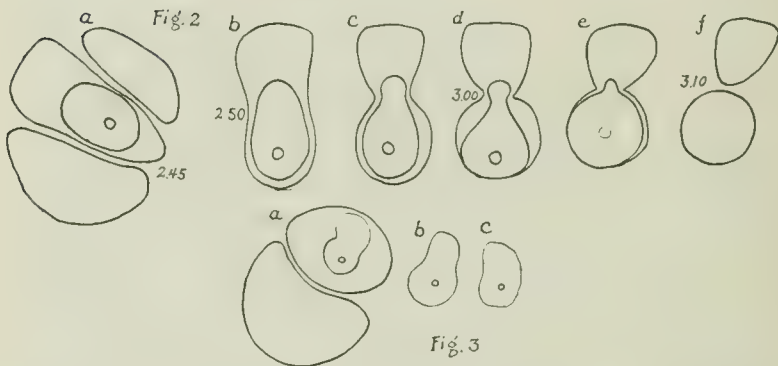
Fig. 2

Fig. 3

FIG. 1. Figures showing the extent to which the nucleus (germinal vesicle) of an immature starfish egg may be indented on one or both sides without rupture. On removing the needle the nucleus reverts to its original spherical shape.

FIG. 2. a, immature starfish egg cut at 2:45 P.M. into three parts; the nucleus has remained intact but is laterally compressed in the middle fragment. b, c, d, e and f, successive steps in attempt of nucleus to round up; b, 2:50 P.M.; d, 3:00 P.M.; f, 3:10 P.M.

FIG. 3. a, partial rupture of nucleus followed by a repair of its membrane. b and c, successive changes in the shape of the nucleus within the following ten minutes after which time it disappeared.

liquefies. If the rupture of the nucleus be violent, the disintegration of the cytoplasm spreads rapidly until the entire egg is involved. If the rupture be slight, the disintegrative process is quickly limited by a surface film which forms on the boundary between the disintegrating and the surrounding healthy cytoplasm (Fig. 4). This film tends to prevent any further spread of the destructive process. The destruction of the cytoplasm is evidently due to something which emanates from the injured nucleus. The injury to the cytoplasm does not start where the nuclear membrane is first torn, but from the entire surface of the injured nucleus.

This is analogous to results obtained by injuring red blood corpuscles with a needle upon which hemoglobin escapes immediately from the entire surface (Chambers, '15).
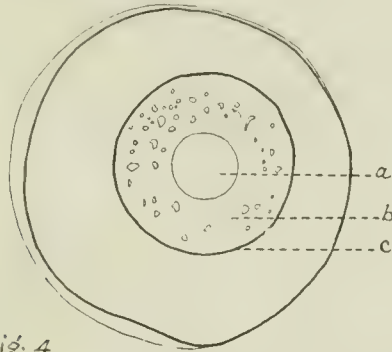
Fig. 4

FIG. 4. Disintegration of cytoplasm surrounding the nucleus on tearing the nucleus with a needle. (*a*) Faint hyaline sphere, a remnant of the destroyed nucleus. (*b*) Disintegrated cytoplasm. (*c*) Cytoplasmic surface film separating disintegrated from healthy cytoplasm.

Within the nucleus itself the immediate effect of the injury is a dissolution of the nucleolus. A nuclear remnant tends to persist after the injury as a hyaline sphere lying within the disintegration products of the cytoplasm. On being touched with the needle it fades from view.

In permanently immature eggs, such as eggs which have been standing in sea water for an hour or more without maturing, the disintegrative effect on the cytoplasm by injuring the nucleus tends to be much more restricted, and the nuclear sphere which persists after the injury can be shown to possess a morphologically definite membrane. Such a sphere is easily dissected out of the egg. Frequently, when the germinal vesicle lies close to the periphery of the egg, the disintegration of the cytoplasm quickly reaches the surface. With the formation of a surface film over the healthy cytoplasm the disintegrative area lies in a deep bay on one side of the egg. This hollow is slowly obliterated as the semi-fluid substance of the egg strives to assume a spherical shape. In this way the disintegrated material is forced out of the egg together with the persisting nuclear sphere. This nuclear sphere persists for some time in the sea water. It can be deformed by means of the needle and, on

tearing its surface, the fluid contents escape, leaving behind a col-
lapsed membrane which disappears within 10 to 15 seconds.

Fig. 5 shows the effect of cutting the mature egg nucleus of the
starfish egg. By pushing the nucleus against the inner surface of

Fig. 5

FIG. 5. Effect of cutting mature nucleus of a starfish (*Asterias*) or sea-
urchin (*Arbacia*) egg. *a*, intact egg nucleus; *b*, nucleus in process of being
cut in two. The nucleus was pushed against the periphery of the egg as it
was being cut by a vertical needle; *c*, the separated fragments of the nucleus;
d, reunion of the fragments; *e*, reconstituted nucleus.

the egg it is possible to pinch it into two pieces. Each piece
rounds up but, if the two are allowed to come into contact, they
will fuse into a single nucleus again. The same result obtains in
the sand-dollar and sea-urchin eggs. If, however, the nuclear
membrane be torn, a disintegration of the cytoplasm results
analogous to that produced on rupturing the germinal vesicle.
The extent of disintegration is much more limited, owing doubtless
to the much smaller amount of nuclear material present. Similar
results were obtained on tearing the nucleus of the *Arbacia* egg.

It was found possible to destroy the cytoplasm of one egg by
injecting into it nuclear material obtained from another egg. This
experiment has to be performed very rapidly, for if the nuclear
material be allowed to remain longer than ten seconds within the
pipette it has no effect whatever when injected into the cytoplasm
of an egg. If it be injected within that time the destructive effect
is very pronounced.

If an egg be allowed to undergo normal maturation, the ger-
minal vesicle disappears except for a small remnant which be-
comes the definite egg nucleus. This egg nucleus moves to the
surface of the egg, where it gives off the two polar bodies. It then
constitutes the female pronucleus, which remains quiescent until
fertilization occurs. The disappearance of the germinal vesicle is
a well-known phenomenon. In order, however, to locate definite
stages selected for my operations I introduce the following sum-

mary. The germinal vesicle with an intact membrane is shown in Fig. 6. Within thirty to forty-five minutes after standing in sea water the nuclear membrane exhibits wrinkles and its outline begins to fade from view. Within a few minutes no membrane is visible and cytoplasmic granules can be seen moving into the region hitherto occupied by the nucleus, while the nuclear sap appears to be diffusing out (Fig. 6-c). As the nuclear membrane disappears the nucleolus fades from view. The invasion of the nuclear area by cytoplasmic granules continues until all of the area except a small portion is rendered indistinguishable from the general cytoplasm of the egg. This small portion persists as the egg nucleus (Fig 6e and f). In Fig. 6-g two consecutive positions of the nucleus are shown. At 1:13 P.M. it lay deep in the substance of the egg. In twenty minutes it had moved to the periphery of the egg preparatory to the formation of the polar bodies.
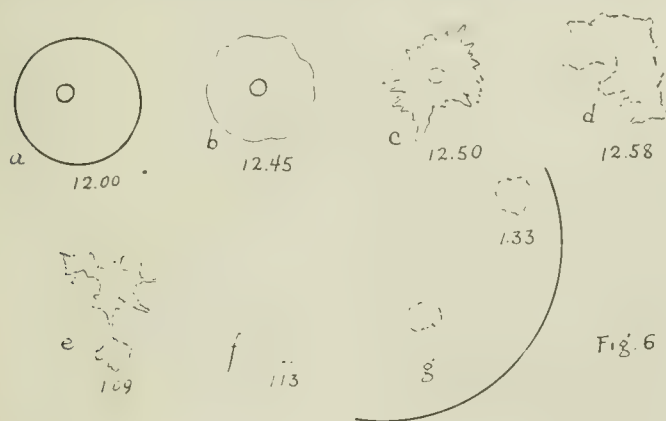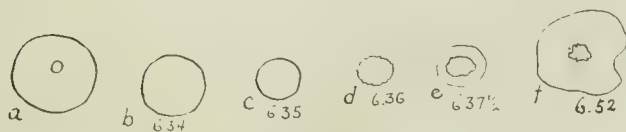
FIG. 6. Camera lucida drawings of the successive steps in the normal dissolution of the germinal vesicle in the maturing starfish egg. The process was somewhat slowed down owing possibly to the compressed condition of the egg necessary for detailed observation.

FIG. 7. a, intact germinal vesicle within the egg. b, nucleus after having been torn out of the egg and brought into sea water. c, d, e and f, successive changes undergone by the nucleus lying in sea water.

By means of the microdissection needle it is possible to show, at the stage shown in Fig. 6-*d*, that the membrane of the germinal vesicle no longer exists. By careful manipulation it was possible to push the cytoplasmic granules into the nuclear area. A slight rapid movement of the needle, however, was sufficient to give rise to disintegrative processes similar to those on tearing an intact germinal vesicle. In the normal maturation process the mingling of the nuclear sap with the cytoplasm is very gradual, being completed in the case recorded not under ten minutes. It is this gradual mixing which apparently prevents disintegration.

Morgan ('93) and Mathews (Wilson and Mathews, '95) found that maturation was accelerated by shaking starfish eggs shortly after they were placed in sea water. They concluded that the shaking ruptured the membrane of the germinal vesicle and so allowed the nuclear material to mix more quickly with the cytoplasm. I have repeatedly tried to intermix cytoplasm and nuclear material by rupturing the nuclear membrane of the starfish egg with the needle, but in every case I get an explosive disintegration of the cytoplasm. The ruptured nuclear membrane which Mathews (W. and M., '95) and Marcus ('07) describe in fixed and stained immature eggs which had been violently shaken is possibly the membrane of the sphere which I found to persist after injury to the germinal vesicle (see page 321). It is more likely that the shaking which accelerates processes within the egg leads to the normal gradual dissolution of the nuclear membrane and the subsequent diffusion of the nuclear material throughout the egg. I have been able to do this occasionally with the needle. An intact germinal vesicle which to all appearances should take fifteen to twenty minutes to go into dissolution will often immediately exhibit a wrinkled outline on being gently agitated with the needle. Then follows the gradual fading from view of its outline with the subsequent changes as shown in Fig. 6.

The intact germinal vesicle may be brought into the sea water by tearing away the surrounding cytoplasm. During the process the nucleolus fades from view. The slightest tearing of the nuclear surface then causes the entire liquid vesicle to disappear in the water. If, however, the nucleus be left alone, it shrinks for a

time and then swells. The changes appreciable to the eye are shown in Fig. 7. During the swelling of the nucleus a substance apparently separates out which collects into a small mass and persists as a gelatinous body. It is possible that this abnormal separating out is analogous to the formation of the definitive egg nucleus in the normal process of maturation. This separating out of a gelatinous material from a liquid nucleus upon injury may be similar to the method of precociously inducing chromosomes in spermatocytes of the grasshopper (Chambers, '14).

2. THE EXISTENCE OF AN EXTRANEOUS MEMBRANE ABOUT THE UNFERTILIZED EGG.

The existence of a membrane about the unfertilized egg rising off as the fertilization membrane upon insemination was first suggested by the earlier investigators (e.g., Hertwig, '76; Herbst, '93). Kite ('12) and Glaser ('13) agreed with them whereas McClendon ('14), Harvey ('14) and Elder ('13) claimed that the fertilization membrane is a new formation consequent to fertilization. Heilbrunn ('13) also identifies it with the actual protoplasmic surface of the egg, which he considers to be in a state of a gel and which lifts off as the fertilization membrane, a new surface film forming over the egg underneath it.

My experiments indicate that the unfertilized eggs of the starfish, sea-urchin and sand-dollar all possess a membrane extraneous to their true protoplasmic surface, and that it is this membrane which, upon insemination, is lifted off as the well-known fertilization membrane.

In the unfertilized egg the membrane is more or less tightly glued to the surface of the egg just as Kite ('12) described it. In the sea-urchin egg it is extremely delicate and can be demonstrated only as follows (Fig. 8) : The needle is inserted as nearly as possible through the periphery of the egg and left there. Within a few seconds the protoplasm, lying immediately under the egg membrane and distal to the needle, flow away from the needle until the needle lies in a small protuberance which is formed by a very slightly lifted portion of the egg membrane.

The existence of the egg membrane is easily demonstrated in the

starfish egg. In Fig. 9 the disintegration of the cytoplasm follow-
ing injury to the germinal vesicle has reached the surface of the
egg. The disintegrated area is quickly localized by a surface
film bounding a cup-shaped depression on the surface of the egg.
Roofing over the depression is the egg membrane. The egg
membrane can also be shown by cutting an egg in two by press-
ing the egg against the coverslip with the side of a needle. The
pressure of the needle cuts the egg in two without rupturing
the membrane, which, on releasing the egg, bridges the gap
between the pieces and holds them together (cf. Figs. 11 and
12, page 329).

The difference between the consistency of the egg membrane in
the starfish and the sea-urchin egg is strikingly shown in the fol-
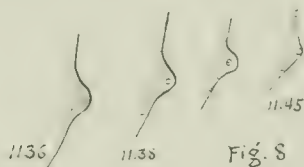
Fig. 8.
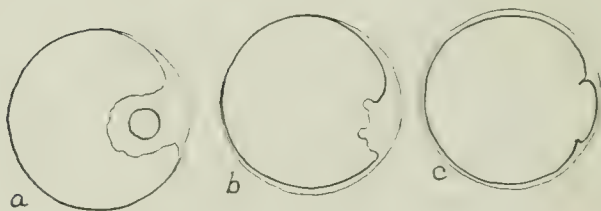
Fig. 9

FIG. 8. Needle inserted at 11:36 A.M. through periphery of a sea-urchin
egg and left there. At 11:38 the cytoplasmic granules have been flowing
away from the needle. A new surface film begins to appear with the needle
left outside. At 11:45 the original egg membrane appears as a delicate
membrane partially lifted off the surface of the egg by the needle.

FIG. 9. Lifting of a membrane from the surface of an immature starfish
egg following injury to the egg. a, local disintegration of cytoplasm following
destruction of the germinal vesicle (cf. Fig. 4). An egg membrane becomes
apparent as the cytoplasm retreats from it. b and c, gradual separation of
the membrane all over the surface of the egg.

lowing experiments. With the eggs in a hanging drop the egg is
pressed against the coverslip with the side of a glass needle until

the pressure divides the egg into two pieces. In the sea-urchin egg the two pieces immediately round up and roll away from one another. In the starfish egg the tougher membrane is not ruptured, but holds the two pieces together.

The membrane of the sea-urchin egg is so delicate that it is also possible to cut the egg in two in the following manner: In a hanging drop the horizontal end of the needle is brought *over* the egg (Fig. 10). The needle is now lowered. This brings the needle
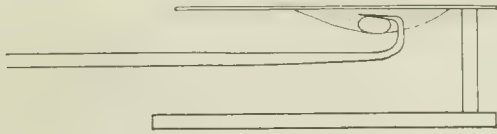
Fig. 10

FIG. 10. Side view of moist chamber to show one method of cutting an egg in two with the microdissection needle.

against the upper surface of the egg and presses the egg down against the surface film of the hanging drop. On lowering the needle still further it passes through the egg and out of the drop, cutting the egg cleanly in two. In the case of the starfish egg this procedure would drag the egg out of the drop along with the needle. The membrane of the sand-dollar egg is weaker than that of the starfish and stronger than that of the sea-urchin egg.

The consistency of the membrane varies with the age of the egg. The full-grown immature egg of the starfish has a relatively tough membrane. On the other hand, young ovarian eggs possess very delicate membranes and they can be cut in two with the same ease as mature sea-urchin eggs.

The strongest argument regarding the existence of a membrane about the unfertilized egg is that a membrane may be stripped off the egg whereupon the egg, which was previously non-adherent, now sticks to everything it touches. The fertilizability of such naked eggs is discussed under the next heading.

The existence of egg membranes is a fairly universal feature and it is, therefore, not surprising that we should find them in the

echinoderm eggs which have generally been considered as naked. The unfertilized *Cumingia* egg has an extremely tough membrane, so tough that it is difficult to rupture it without completely destroying the egg contents. The vitelline membranes in the frog and in the chick are undoubtedly analogous structures.

3. The Egg Membrane and the Fertilization Membrane are Identical.

Prior to fertilization no membrane enveloping the egg is visible. Upon fertilization a membrane lifts off which can easily be cut away from the egg. Figs. 11 and 12 indicate the identity of a preexisting membrane with the fertilization membrane. Fig. 11-*a* shows an egg cut in two with an investing membrane holding the pieces together. Upon fertilization the membrane lifts off, enclosing the two pieces in a single cavity (Fig. 11-*b*). One only of the pieces happened to segment, and the fact that the two pieces lie in one cavity is shown in Fig. 11-*c*, where the blastomeres of the segmented portion have encroached on the area around the nonsegmented piece. In Fig. 12 an egg was cut into three pieces, the egg nucleus lying in one of the pieces. Upon fertilization the membrane lifted off the pieces, each of which received sperm and developed into swimming larvæ. Fig. 12-*c* shows the empty fertilization membrane after the three larvæ had escaped. In Fig. 13 is shown an egg which, on being cut in two, was rolled about in an attempt to separate the pieces. The egg membrane between the two pieces was twisted into a thread joining the two. Upon fertilization each piece exhibited a complete fertilization membrane, but the fact that the two investing membranes are portions of one common membrane is shown by the connecting thread.

A conclusive test for the starfish and sand-dollar egg is the removal of the egg membrane prior to insemination. Occasionally, pricking the egg is sufficient to elevate the membrane. No subsequent development takes place. It is possible, however, to remove this membrane by tearing it and the egg then be made to slip out. This is more easily done on eggs which have been standing for some time in seawater. On catching at the sur-

face of such eggs with the needle, the membrane is often torn in such way that the egg slips out leaving the membrane stuck to the needle. Such an egg, when inseminated, is fertilized and subsequently segments with no investing membrane whatever.
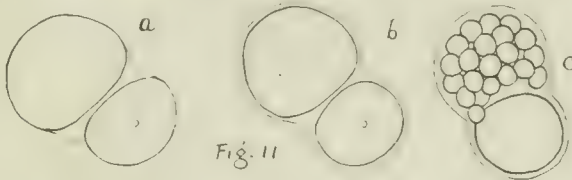
Fig. 11
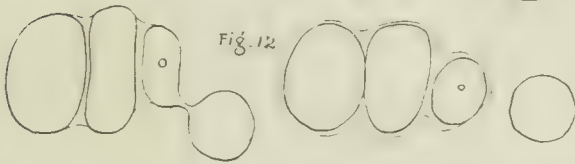
Fig. 12
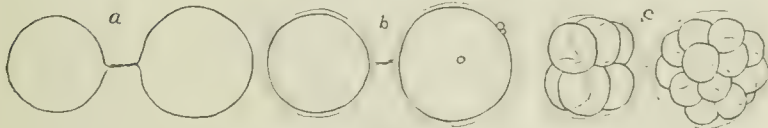
Fig. 13

FIG. 11. *a*, starfish egg cut in two without destroying the investing membrane. *b*, after insemination the investing membrane lifts off both fragments as the fertilization membrane. *c*, one of the fragments segmented, the other did not. That both fragments lie in a common cavity is shown by the encroaching of blastomeres of one fragment into the region of the unsegmented fragment.

FIG. 12. *a*, starfish egg cut into three pieces. One piece was squashed and produced an exovate. *b*, on being fertilized the exovate was pinched off as an endoplasmic sphere (cf. Fig. 25). The rest of the fragments produced a common fertilization membrane. Each of the three enclosed fragments developed into a swimming larva.

FIG. 13. *a*, sand-dollar egg rolled as it was cut in two. The egg membrane between the two pieces was twisted into a thread joining the two. *b*, egg shortly after fertilization showing fertilization membrane about each connected by a filament. *c*, the two pieces in an early segmentation stage.

The difference in reaction of sperm to an egg which has been denuded of its membrane as well as of its jelly, and to one which has not is very striking. An egg within its membrane is quickly surrounded by spermatozoa as they are trapped in the jelly surrounding the membrane. In a membraneless egg no crowding of spermatozoa is noticeable and heavy insemination is necessary

to bring about fertilization. When a cloud of sperm has been blown upon a naked egg, one may frequently observe a spermatozoön swim toward it, wander over its surface, and then swim away. On the other hand, the empty membrane with its investing jelly immediately becomes covered with a halo of spermatozoa. This observation accords with the interpretation of Buller ('o2), that the investing jelly determines the direction of the sperm which are captured by it, and that there is no apparent chemotactic substance excreted by the egg to attract the sperm.

The difference in position of the polar bodies in the starfish egg with respect to the fertilization membrane as shown by Gemmill ('12) (see also Chambers and Mossop, '18, and Garrey, '19) may be explained as follows: When the polar bodies form prior to fertilization they rise off the surface of the egg, carrying with them the closely adherent membrane. When they are pinched off the egg membrane remains continuous about the egg and subsequent insemination results in the formation of a fertilization membrane with the polar bodies lying outside. If, however, the eggs are inseminated before extrusion of the polar bodies, the egg membrane lifts off as the fertilization membrane and, when the polar bodies are formed, they lie within the membrane.

In the sea-urchin egg the identity of the egg membrane with the fertilization membrane is more difficult to demonstrate. In Fig. 14 is shown the effect of locally injuring the surface of the sea-urchin egg. In *a* is a disintegrated mass produced by tearing a spot on the surface with a needle. In *b* this area is shown as a bulge which may be explained as being produced by the interior pressure of the egg on a surface weakened by the loss of an investing membrane. In *c* the egg has been fertilized. The fertilization membrane is formed over all the surface except at the injured place. In *d* segmentation has occurred and a blastomere protrudes through the gap in the fertilization membrane.

A better demonstration is the case shown in Fig. 15. At 4:26 the tip of a needle was punched through the cortex. Within a few seconds the cytoplasm distal to the needle flowed away, leaving the needle lying under a delicate membrane (Fig. 15-*a*). At 4:27 the egg was inseminated with the needle still in place. At 4:29

the fertilization membrane was formed, showing its continuity with the delicate membrane previously noticeable (Fig. 15-*b*).
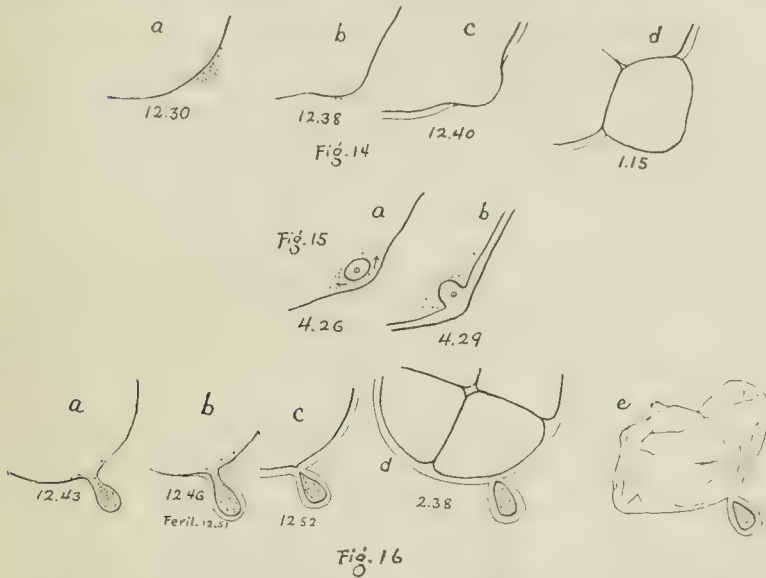
FIG. 14. Sea-urchin egg with surface torn producing local cytolysis. *a*, a new surface film has formed under the cytolyzed area which is being extruded. *b*, a bulge appears in the region of the new surface showing this region to be weaker than elsewhere on the egg surface. *c*, egg after fertilization exhibiting a fertilization membrane over the egg except at the place previously torn. *d*, the same egg 35 minutes later with a blastomere protruding through the tear.

FIG. 15. *a*, needle piercing sea-urchin egg near its periphery. The cytoplasmic granules are flowing in the direction of the arrows. One minute later the egg was inseminated. *c*, an intact fertilization membrane forms, inclosing both egg and needle tip.

FIG. 16. *a*, protrusion on surface of egg produced by pulling at cortex with needle. *b*, three minutes later the investing membrane lifted off surface of protrusion. *c*, one minute after fertilization. The protrusion has been pinched off from the egg and its investing membrane can be seen to be continuous with the fertilization membrane. *d*, empty and collapsed fertilization membrane.

In the sea-urchin egg the membrane often rises off a protrusion caused by pulling at the cortex with the needle. Such a case is shown in Fig. 16. The protrusion was formed at 12:43. At 12:46 a membrane had lifted off the protrusion. At 12:51 the egg was inseminated, and one minute later the membrane was

found continuous with the fertilization membrane. The protrusion subsequently pinched itself off and persisted in a sac-like protuberance of the fertilization membrane (Fig. 16-*d*–*e*).

In all of the various eggs studied a change in the consistency of the membrane takes place very soon after it has been elevated. The membrane, at first very soft and delicate, progressively toughens until it becomes almost parchment-like during the later segmentation stages. It is of interest to note that Harvey ('10) found a difference between the unfertilized and the fertilized sea-urchin egg when subjected to sulfuric acid. The acid dissolves the unfertilized egg completely, whereas it dissolves all of the fertilized egg except the fertilization membrane. Some chemical change apparently takes place as the membrane lifts off the egg.

Outside the membrane is a considerable zone of a structureless jelly. In the sand-dollar egg the jelly very loosely adheres to the membrane. On cutting into the jelly the egg with its membrane easily slips out. This is to a somewhat lesser degree true for the starfish egg. In the starfish egg one often sees the under surface of the jelly pushed away from the surface of the unfertilized egg by the protruding polar body.

The question as to whether the membrane lifts off the surface of the egg or whether the egg shrinks leaving the membrane behind has been raised by Glaser ('14) in spite of McClendon's ('10) statement to the contrary. Glaser, by making a large series of measurements, claims that the egg shrinks upon fertilization, and that the initial diameter of the completed fertilization membrane is equal to that of the unfertilized egg. Glaser's measurements were made on the assumption that the eggs always maintain a spherical shape. This is not true. The mature unfertilized egg is very soft and if allowed to lie on the bottom of a glass dish tends to flatten into the shape of a disc. Upon fertilization the egg rounds up as the fertilization membrane leaves its surface. One can readily see if the observations are taken of eggs in one plane only that erroneous conclusions may be arrived at.

I used two methods to ascertain the diameter of starfish eggs before and after fertilization. One method was to place a drop

containing a few eggs on a gelatin-coated slide. The eggs were rolled over by means of a micro-needle and only those which maintained their spherical shape were measured. With a micro-pipette sperm were introduced into the drop without disturbing the relative positions of the eggs. A second method was to place several eggs in a hanging drop in a Barber moist chamber. By piercing the surrounding jelly with a needle the egg to be measured could be held suspended in the middle of the drop. Numerous measurements of the starfish egg were made at different times through several summers and in every case the egg maintained its original size as the fertilization membrane rose off its surface. Not only does the egg not decrease in volume, but it slightly *increases* in size until segmentation occurs. The accompanying table is one sample of the measurements made:

	Un-fertil.	Minutes after Fertilization.					
		$1''$	$.2''$	$7''$	$10''$	$20''$	$70''$
Egg diameter.........	3.4	3.4	3.4	3.4	3.5 × 3.55	3.5 × 3.6	3.5 × 3.6
Fertilization membrane diameter..........		3.5	3.6	3.65 × 3.7	3.65 × 3.7	3.75 × 3.75	3.9 × 3.9

The conclusions from this table apply both to starfish and sea-urchin eggs. They may not necessarily be true for other species.

Fig. 17 shows successive steps in pulling a starfish egg out of its fertilization membrane. No second membrane is ever formed even with superimposed insemination. Occasionally the hyaline plasma layer in such an extruded egg swells up and simulates a second membrane, and it is probably this that has been described by certain investigators as a second fertilization membrane. The hyaline plasma layer will be discussed under heading 5.

An unfertilized mature sea-urchin egg may be rolled about and its contents churned to the extent of producing " fountain currents " within the egg (Chambers, '17-*b*). This is done by pushing an egg in a drop shallow enough to compress the egg. Currents are produced which flow backward immediately under the surface of the egg and forward along its central axis (Fig. 18). By careful manipulation it is possible to do this without rupturing

the investing membrane. Such an egg is capable of forming a normal fertilization membrane when inseminated. If the pushing process be carried too far, a distinctive quiver can be recognized, as of something giving way. On subsequent insemination such
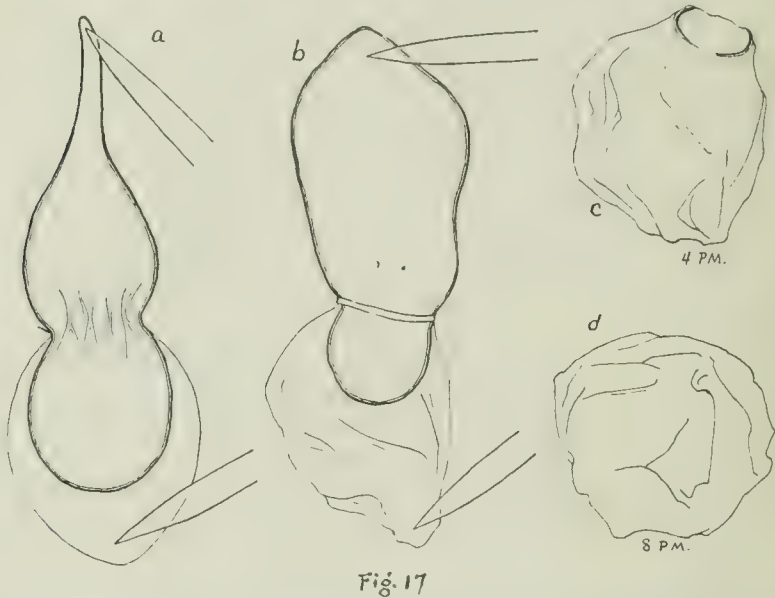
Fig. 17

FIG. 17. *a* and *b*, successive steps in pulling a starfish egg out of its fertilization membrane. *c*, empty membrane at 4:00 P.M. *d*, ditto four hours later at 8:00 P.M. The membrane persists as a collapsed remnant for a long time.

eggs produce a collapsed fertilization membrane. The quiver undoubtedly was due to a rupture of the egg membrane. On account of this rupture the fluid, which presumably collects under the membrane, leaks out and the membrane is not lifted uniformly.

4. THE CORTEX AND INTERIOR OF THE UNFERTILIZED EGG.

The cytoplasm of the immature starfish egg is uniformly semi-solid. A gash made in it with a needle is maintained for some minutes before closing up. When the germinal vesicle breaks down naturally, the egg protoplasm becomes more fluid so that a gash

through such an egg quickly closes up. The cortex—*i.e.*, the surface of the egg immediately beneath the egg membrane—tends always to remain more solid (Chambers, '17-*a*). Because of this difference in consistency the cortex and medulla of the egg can be separated from one another as follows ('21ᵃ) : If the surface of the mature starfish egg be torn with a needle and the egg then be caught at the opposite side and pulled to the edge of the
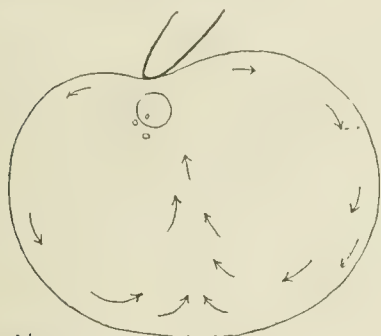
FIG. 18. Currents produced within a sea-urchin egg by pushing a sea-urchin egg held against a coverslip by a shallow film of water. The direction of the currents is shown by the arrows. The nucleus, after being carried about with the current, tends to come to rest in the location shown in the figure.

FIG. 19. Part of the cortex of a fertilized egg after the appearance of the hyaline plasma layer. The cortex was ruptured in one place and cytoplasmic granules can be seen issuing through the rupture in the hyaline plasma layer and the investing fertilization membrane.

hanging drop, the compression on the egg produced by the shallow water at the edge of the drop will cause the fluid interior to ooze out through the tear to form a spherical exovate (see Fig. 25, page 344). One may so manipulate the process as to cause the egg nucleus either to remain behind in the cortex (the cortical remnant) or to pass into the extruded sphere of endoplasmic material.

The cortical remnant is relatively solid and remains more or less inclosed within the egg membrane and its jelly. If left long enough it will eventually round up so as to present the appearance of a diminutive egg surrounded by a collapsed and wrinkled egg membrane.

The endoplasmic material which has escaped from the egg into the sea water is fluid and tends immediately to round up. On tearing with a needle its surface behaves like that of a highly viscous oil drop, adheres tenaciously to glass. As long as it possesses an intact surface it looks exactly like an egg fragment and will undergo disintegrative changes similar to those of entire eggs on being torn with the needle (cf. Chambers, '17-a).

The ability to produce endoplasmic spheres is possibly due to the relatively tough egg membrane in the starfish egg which helps to keep back the adherent cortex. In the sea-urchin egg, with an extremely delicate egg membrane, it has been impossible to cause the interior to flow out, as the cortex tends to flow with it.

The sand-dollar egg behaves very much like the starfish egg. The egg membrane is appreciable in the unfertilized egg and endoplasmic spheres are readily produced.

A difference in the functional activities of the cortex and interior of the starfish egg is discussed under the headings 6 and 7.

5. The Hyaline Plasma Layer.

Prior to fertilization the cytoplasmic granules in the sea-urchin and sand-dollar egg lie close to the surface. Within ten minutes after fertilization the granules have undergone a centripetal migration, leaving an appreciable peripheral zone of a hyaline appearance which has been called the hyaline plasma layer (Loeb's gelatinous film, '13, p. 19).

The microdissection needle indicates that this layer is relatively firm and gelatinous. The very fluid internal cytoplasm may be made to flow out through a rupture in this layer if the egg be torn. This is shown in Fig. 19. The cytoplasmic granules lie against the inner boundary of this layer and may be seen oozing out through the small tear in this layer and through a tear in the fertilization membrane to the exterior.

The hyaline plasma layer adheres very tenaciously to the needle and when an egg has been deprived of its fertilization membrane the egg sticks to everything it touches.

Loeb has called attention to the fact that the hyaline plasma

layer in a segmented egg bridges the segmentation furrow. When the furrow is first formed, however, the hyaline plasma layer does not bridge the furrow, but is carried in on the walls of the cleavage furrow (Fig. 20-*a*, *b*, *c*). The layer is thicker in the floor of the
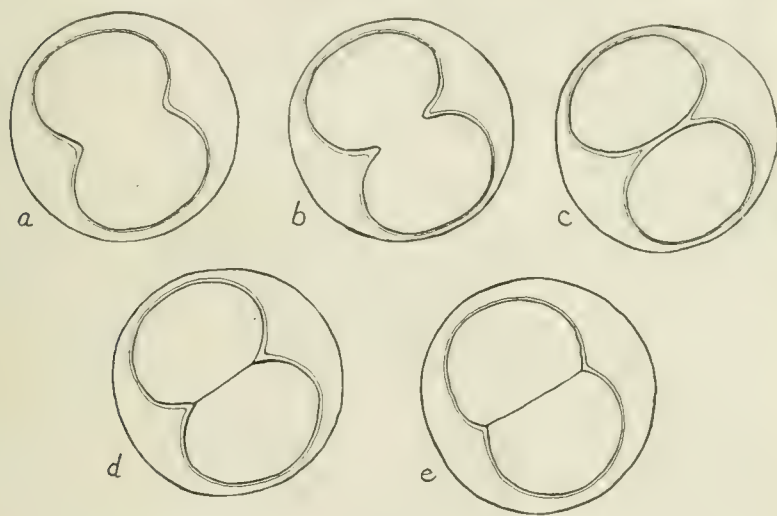
Fig. 20

FIG. 20. Contour of a sand-dollar egg at various stages of its cleavage into two blastomeres. In *a* and *b* the hyaline plasma layer is seen carried in on the walls of the deepening furrow. In *c* the egg has segmented in two with the hyaline plasma layer on opposite sides of the furrow tending to merge into each other. In *d* this process is carried further. In *e* the two blastomeres are tending to assume the shape of hemispheres with the hyaline plasma layer bridging the furrow.

furrow, but it is only later when the furrow has cut through the egg that the hyaline plasma layers on the opposite surfaces of the furrow run together. Each half of the segmenting egg tends to assume the shape of a sphere owing to the separation of the two asters of the amphiaster (Chambers, '17-*b*, '19). If there were no other forces at play, the two blastomeres, when formed, should be spheres. In the sea-urchin egg the adhesiveness of the hyaline plasma layer tends to draw the two blastomeres together; also the fertilization membrane, not rising to any great extent off the surface of the egg, must exert some pressure on the two blastomeres. In the sand-dollar the fertilization membrane is well

lifted, so that there is plenty of room within the membrane, per-
mitting the two blastomeres to assume almost spherical shapes
(Fig. 20-c). When the cleavage furrow is completed the two
blastomeres are contiguous only where the two spheres touch.
At this place the hyaline plasma layers of the two blastomeres
merge. We have here, apparently, two opposing forces; first,
the jellied aster holding each blastomere to a spherical shape,
and, second, the affinity of the plasma layer substance surround-
ing the two blastomeres. As soon as the asters disappear and
the cytoplasm of the blastomeres reverts to a more fluid state
the plasma layers of the two blastomeres merge more and more
and the blastomeres are pulled together till they assume shapes
approaching those of hemispheres (Fig. 20-c). The outlines in
Fig. 20 are camera lucida drawings taken during the successive
stages of one sand-dollar egg.[1] .

In the starfish, where there is no appreciable hyaline layer, and
where the fertilization membrane is lifted far beyond the surface

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[1] It has recently been intimated that the microdissection method is unre-
liable as a means of ascertaining changes in viscosity in the dividing egg
because of supposed discrepancies in the results obtained by Seifriz ('20)
and myself ('17[b] and '19). As a matter of fact the results of Seifriz har-
monize perfectly with mine. Seifriz states " there is a pronounced decrease
in viscosity of the central region of the cell with the first appearance of
the amphiasters." This statement has been interpreted as running counter
to mine. This is not true for although my results indicate that the astral
portion of the amphiaster is jellied, I definitely state (p. 494, '17) that the
central region and the zone between the two halves of the egg are fluid
where " a distinct flow of granules medianward can be observed."

Again, on completion of cleavage Seifriz notes that the two blastomeres
become liquid. This statement also fits in with my results. I state (p. 51,
'19) that, immediately after cleavage and while the two blastomeres *are
still spherical*, the firmness of the cytoplasm persists. Later, when the asters
disappear the cytoplasm liquefies and the two blastomeres crowd up against
one another. Seifriz noted this last liquid state of the two blastomeres
without considering the state prior to it.

I may mention here a possible criticism of the centrifuge method in ascer-
taining viscosity variations. There are critical stages in the developing asters
during which agitation causes their disappearance. This was noted long ago
by Wilson. On bringing the eggs to rest the asters reappear and develop-
ment proceeds normally. I have already discussed this matter fully ('19).
The centrifuge and microdissection methods of studying the physical state
of protoplasm should serve as valuable checks on one another, if only the
investigators in these fields would agree on cooperation.

of the egg, the blastomeres are practically non-adhesive, and they maintain more or less spherical shapes till well on into the later segmentation stages.

6. The Localization of a Material Which Affects the Life of the Unfertilized Starfish Egg.

It is well known that immature starfish eggs can be kept in sea water at room temperature for 36 hours or more without disintegrating. That the germinal vesicle or nucleus is responsible for this length of life can be demonstrated by cutting an immature egg in two. The nucleated fragment lasts fully as long as the entire egg. The non-nucleated portion, on the other hand, disintegrates within three to four hours. In mature unfertilized eggs the conditions are quite different. In the mature egg the germinal vesicle has broken down and the nuclear sap has diffused throughout the egg. Loeb ('02) and Mathews ('07) showed that such eggs have a higher rate of oxidation than immature eggs and if left unfertilized disintegrate within 8 to 10 hours whereas the immature eggs last for days.

The non-nucleated fragment of the mature egg lasts as long as the whole egg, evidently owing to the dispersed nuclear sap of the dissolved germinal vesicle. What is significant is that the nucleated fragment lives no longer than the non-nucleated fragment. Both contain the dispersed nuclear sap, while the nucleated fragment possesses also the definitive mature egg nucleus which is ultimately to become the female pronucleus. Apparently it is the dispersed nuclear sap and not the definitive mature egg nucleus which is chiefly concerned. In the formation of the nucleus of the mature egg we have possibly something analogous to the state of affairs in many Protozoa where the nuclear apparatus consists of a tropho- or macro-nucleus concerned chiefly in the metabolic activities of the cell, and the kineto- or micro-nucleus which has only to do with the reproductive activities. In the starfish egg we may consider the germinal vesicle as a combined tropho- and kineto-nucleus. On the approach of maturation the tropho-nuclear material (nuclear sap) diffuses throughout the egg, leaving behind the kineto-nuclear part, the mature egg nucleus, which gives off the polar bodies to become ultimately the female pronucleus.

The fluid interior of the mature unfertilized egg, if isolated by
being made to escape through a tear or the cortex, withstands dis-
integration for 24 to 36 hours. The presence of even a small part
of the original cortex in organic continuity with it causes it to
disintegrate in about the same time as an entire mature egg. This
would indicate that the reactions which make for disintegration
reside chiefly in the cortex. This, together with the fact that the
cortex of the egg is necessary for fertilization, would indicate that
the cortex is the seat of the initial activation processes of the egg.
The relatively inactive central material of the starfish and sand-
dollar egg somewhat resembles that of the *Linerges,* the Scy-
phomedusan, which Conklin ('08) has described. Conklin speaks
of "the large cavity in the line of the first cleavage furrow filled
with gelatinous or fluid substance, which forms the ground sub-
stance of the central area of the unsegmented egg." He found
that most of the ground substance escapes into the cleavage cav-
ity and suggested that it is the fluid yoke which is gradually used
up in the nourishment of the embryo. The central substance of
the *Linerges* egg is probably not strictly analogous with that of
the starfish or sand-dollar egg. In *Linerges* cleavage is of a type
peculiar to yolk-laden eggs and the central substance escapes
during the first cleavage. On the other hand, in the echinoderm
egg the nucleus lies well within the central substance of the egg
and, upon fertilization, all of the endoplasm is used up in the
formation of the cleavage asters and nothing apparently escapes
into the early cleavage cavity. We can not, therefore, con-
clude that the interior of the Echinoderm egg consists of entirely
inert material. It lacks certain essential features, but when co-
existent with the cortex it plays a full part in the cleavage of
the egg.

7. The Localization of a Substance Which Renders a Star-
fish Egg Fertilizable.

Wilson ('03[ab]) in *Cerebratulus* and *Renilla* and Yatsu ('04
and '08) in *Cerebratulus* have shown that non-nucleated frag-
ments of the egg are capable of fertilization only after the ger-
minal vesicle has broken down. With more delicate methods

rendered possible by the microdissection instrument it has been possible to work out this problem in detail and to ascertain to some extent the distribution of the material which renders fertilization possible.

A number of fully grown immature starfish eggs were enucleated by carefully dissecting out their germinal vesicles. None became fertilized when inseminated. In another lot of immature eggs the germinal vesicle was torn while in the egg (Fig. 21). Immediate
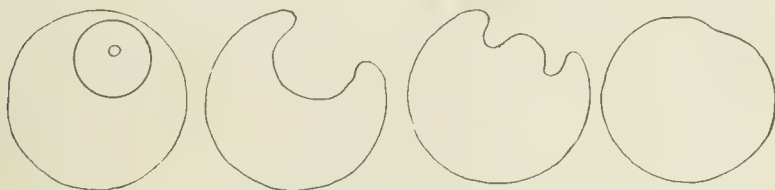

Fig. 21

FIG. 21. A starfish egg whose germinal vesicle is eliminated by puncturing it (cf. Fig. 9). The cytoplasm surrounding this nucleus was also destroyed. This enucleated remnant is nonfertilizable.

dissolution of the nuclear membrane took place with a disintegration of the cytoplasm around the nuclear area. Those eggs which succeeded in forming a protective surface film to prevent spread of the disintegration process subsequently rounded up. Upon insemination none of the eggs showed any sign of being fertilized.

Eggs were then taken with the germinal vesicle in various stages of normal dissolution and cut into nucleated and non-nucleated portions. The eggs may be grouped into stages b, c and d, according to the stage of dissolution of their germinal vesicles, as shown in Fig. 6 (page 323). Whenever the cut passed through the nuclear area during the nuclear stages b, c and d, disintegration always took place, involving all of the nucleated portion and a small part of the non-nucleated piece (Fig. 23 a, b and c). When the cut did not pass through the nuclear area all persisting nucleated portions matured normally and upon insemination formed fertilization membranes and segmented. Of the non-nucleated portions those from eggs in stage b are non-fertilizable (Fig. 22). Those from eggs in stage c form fertilization membranes upon insemination. Nuclear division also takes place, so that the egg

fragment becomes multi-nucleated but remains unsegmented (Fig. 23-c). Non-nucleated fragments of eggs in a later stage (stage d) proceed somewhat farther (Fig. 24). The multi-nucleated masses arising from them make several periodic attempts at segmentation (Fig. 24-c). Small furrows appear over the surface of the egg, cutting in between the peripherally arranged nuclei.
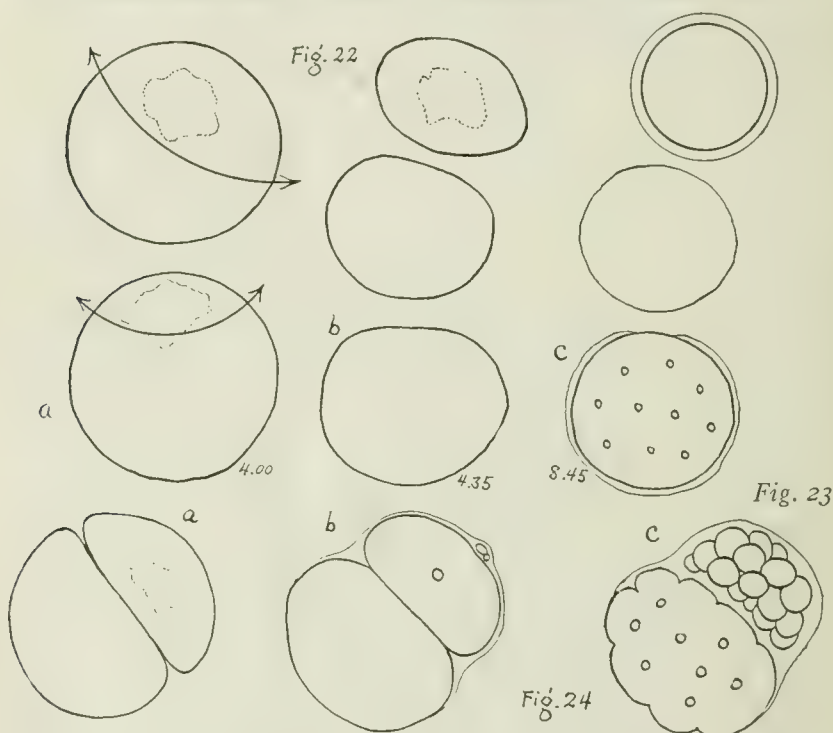
FIG. 22. Starfish egg in stage corresponding to b in Fig. 6 cut into two fragments. The non-nucleated fragment contains no material from the germinal vesicle and is nonfertilizable.

FIG. 23. Starfish egg in a later stage corresponding to c in Fig. 6 cut through the nuclear area. The cytoplasm in the injured nuclear area disintegrated leaving a non-nucleated fragment, b. That the fragment is fertilizable is shown in c by the formation of a fertilization membrane and the repeated division of the sperm nucleus. The fragment, however, is unable to segment.

FIG. 24. a, starfish egg in stage d of Fig. 7 cut into a nucleated and nonnucleated fragment. b, both fragments fertilized. The nucleated fragment segmented in the normal way with a number of blastomeres. The nonnucleated fragment became multinucleated and furrows appeared over its surface in an attempt at segmentation.

These furrows then disappear, to reappear again after a short interval. This may occur several times until the egg finally reverts to a spherical shape and remains so. In stage f the germinal vesicle has disappeared except for the definitive egg nucleus. Of such eggs any non-nucleated portion down to a certain size is capable of being fertilized and undergoing cleavage.

The above experiments lead one to infer the existence of a substance in the germinal vesicle which, on dissolution of the nuclear membrane, diffuses throughout the cytoplasm. The fertilizability of any egg fragment apparently depends upon the extent of diffusion of this substance. An egg fragment taken when a minimum amount of this substance has diffused into it will allow the sperm nucleus which has entered into it to divide. The presence of a little more of this substance will allow the fragment to undergo abortive segmentation. It is not until a sufficient amount is distributed throughout the egg that any fragment can develop properly.

Mature eggs were now studied, and it was found that any egg fragment in order to be capable of fertilization must contain a portion of the original cortex. The cortex and interior of mature unfertilized eggs were separated according to the method described under heading 4 (Fig. 25 a and b). The endoplasmic sphere and the cortical remnant were then inseminated. The fragment consisting of the cortical remnant is readily fertilizable and undergoes segmentation (Fig. 25 b and c). The endoplasmic sphere is nonfertilizable, no matter whether it contains the egg nucleus or not.

That the protoplasm of the endoplasmic spheres has not been irreparably injured in the process of flowing through a small tear in the cortex is shown in the following experiment. Eggs were squashed until the endoplasm protruded as lobate processes, whereupon the pressure on the eggs was lifted and the extrusion allowed to flow back into the egg. Such eggs are fertilizable and are capable of undergoing cleavage. One such case is illustrated in Fig. 26 where the cortex was torn in two places on squashing the egg and two exovates were formed. The nucleated exovate was allowed to pinch itself off. The other exovate flowed back into the remainder of the egg upon insemination (Fig. 26 b and c). A fairly com-

plete fertilization membrane formed around the egg except at
the two torn spots and cleavage followed.

Endoplasmic exovates were also produced which remain con-
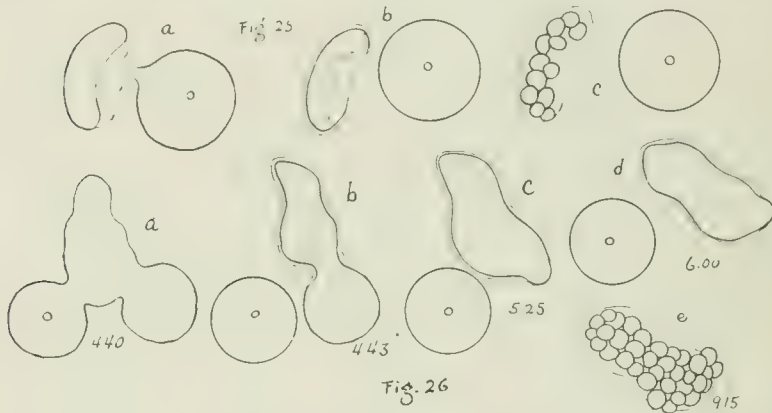nected by a bridge of protoplasm to the collapsed cortical portion

FIG. 25. *a*, nucleated exovate of internal cytoplasm produced by squashing
a starfish egg. *b*, fragments inseminated after the endoplasmic sphere was
pinched off. Only the ectoplasmic remnant forms a fertilization membrane.
c, the endoplasmic sphere remains inert and nonfertilizable (cf. Fig. 12).

FIG. 26. *a*, starfish egg squashed producing two endoplasmic exovates.
b, the nucleated exovate was pinched off. Upon insemination the other ex-
ovate drew back into the ectoplasmic remnant which formed a fertilization
membrane. *c*, *d* and *e*, the ectoplasmic remnant underwent segmentation
showing that the disturbance due to the squashing does not prevent segmen-
tation. The endoplasmic sphere remains inert (*d*).

of the egg. On being inseminated the exovate either is drawn back
into the cortical portion as the latter rounds up with the formation
of a fertilization membrane or is pinched off, after which it remains
as an inert body.

The possibility suggested itself that the substance which renders
an egg fertilizable has a tendency to collect in the surface film of
an egg and that, if an exovate remained in organic continuity with
the egg, this substance might spread to the surface film of the
exovate, thus rendering it fertilizable. Endoplasmic exovates were,
therefore, produced which remained connected for varying lengths
of time with the cortical portion of the egg. Some of the exovates
remained connected for as long as fifteen minutes. Before insemi-

nation they were pinched off from the cortical portion of the eggs. None developed of those which were separated in such a way that there was no question as to their lacking any of the original cortex of the egg.

An endoplasmic sphere, in order to develop at all, apparently must incorporate in its substance at least a part of the original cortex of the egg. This is shown in Fig. 27. An exovate was
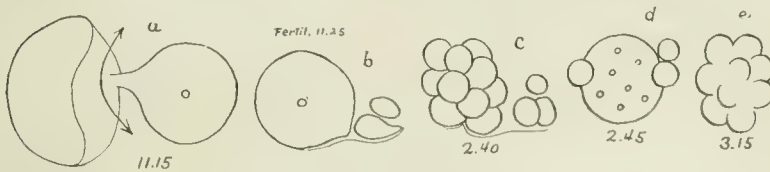
Fig. 27

FIG. 27. *a*, an exovate is produced by squashing and most of the ecto-plasmic part is cut away along line of arrow. *b*, the endoplasmic sphere formed itself incorporating a small part of the cortex. Upon fertilization the small cortical region formed a partial fertilization membrane. *c*, many furrows form simultaneously over the surface of the egg showing that it has been fertilized. (Note that the small cortical piece to one side of the egg has segmented in two.) *d*, the egg has reverted into a multinucleated nonseg-mented mass except for three blastomere-like bodies which were pinched off. *e*, the fragment is again attempting to segment.

produced by crushing an egg (Fig. 27-*a*). However, before the exovate was set free most of the cortical remnant was cut away, leaving a very small piece which was drawn into the circumference of the endoplasmic sphere. On being inseminated a small shred of the egg membrane lifted off from this remnant, and this was all that constituted the fertilization membrane (Fig. 27-*b*). A sperm on entering this sphere underwent nuclear division several times. This was followed by cleavage furrows which formed on the surface of the egg between the peripheral nuclei and gave to the egg the appearance of a mulberry (Fig. 27-*c*). Some of the furrows deepened sufficiently to pinch off nucleated bodies. A few minutes later the furrows became obliterated and the main body of the egg appeared again as a non-segmented but multi-nucleated mass (Fig. 27-*d*). This process may occur several times (Fig. 27-*e*). The ability of an exovate to approximate normal segmentation is a function of the amount of the original egg cortex which it incorporates.

The inability of the endoplasmic sphere to develop is not due to the lack of successful sperm entry. Sections show that the sperm enter with ease but they remain unchanged and no asters form about them. In this regard the sperm react exactly as they do when they have entered immature eggs.

There must be something localized in the cortex which is necessary for successful fertilization and development (cf. Lillie, '14, '18). On the evidence presented here we may assume that this substance, originally within the germinal vesicle, diffuses out upon its dissolution and accumulates in the cortex of the egg. It is held in the cortex of the egg and is not carried out in the endoplasmic spheres on crushing the egg. The spheres are, therefore, incapable of being fertilized. Finally, the variation in the ability to segment among exovates containing varying amounts of cortical material indicates that there must also be a definite minimum amount of this substance present in order that an egg fragment may develop.

Conclusions.

1. The nucleus possesses a morphologically definite membrane.

2. Tearing the nucleus results in an immediate change of the nuclear membrane, followed by a disintegration of the cytoplasm surrounding it. This is most striking in the relatively large nucleus (germinal vesicle) of the starfish egg.

3. Injection of the germinal vesicle sap of one egg into the cytoplasm of another egg starts up disintegration processes in the injected area.

4. The mature egg nucleus can be pinched into two fragments. The fragments behave like fluid droplets and will run together when contiguous. Eggs whose nuclei have been operated upon in this manner are capable of normal segmentation.

5. A membrane can be demonstrated adhering to the surface of the unfertilized starfish, sea-urchin and sand-dollar eggs. This egg membrane is most pronounced in the starfish and least of all in the sea-urchin. In the starfish and sand-dollar the membrane can be stripped off without injuring the egg. In the starfish a very delicate egg membrane can be demonstrated investing half-sized

immature eggs. This membrane becomes more pronounced as the eggs reach their full growth and still more so as the egg matures. In the sea-urchin the immature eggs exhibit no trace of a membrane until the eggs begin maturation. In the mature unfertilized sea-urchin egg the membrane has reached a development comparable to that of the half-grown immature egg of the starfish.

6. The egg membrane rises off the surface of the egg upon fertilization and constitutes the fertilization membrane. No appreciable diminution in volume of the egg occurs during this process.

7. An egg, whose membrane has been removed, is fertilizable and segments without a fertilization membrane.

8. The hyaline plasma layer, which forms on the surface of the sea-urchin and sand-dollar egg within ten minutes after fertilization, binds the blastomeres together. In the starfish egg no such layer is formed, and, if the fertilization membrane be removed, the blastomeres tend to fall apart.

9. The fertilizability and approach to normal development of an egg fragment is directly proportional to the amount of a substance which emanates from the germinal vesicle during maturation.

10. The unfertilized mature egg possesses a more solid cortex of appreciable thickness inclosing a highly fluid interior. The fluid interior of the starfish and sand-dollar eggs can be made to ooze out through a tear in the cortex, whereupon it forms a surface film on coming into contact with sea water. In this way the internal and cortical material of the egg can be isolated from one another. Both round up, the internal material immediately and the cortical after some time.

11. Endoplasmic material, possessing a small part of the original cortex, is fertilizable and the approach to normal development is in direct proportion to the amount of cortical material present. The presence of even a small amount of cortical material causes disintegrative changes to set in at about the same time as in a whole egg.

12. The following table gives, for the various kinds of fragments of immature and mature starfish eggs, the length of time that they withstand disintegration when left standing in seawater and also whether they are or are not capable of being fertilized:

	Immature		Mature			
	Nucl. fragm. or entire egg	Non-nucl. fragm.	Nucl. fragm. or entire egg	Non-nucl. fragm.	Nucl. or Non-nucl.	
					Ectoplasmic remnant	Endoplasmic sphere
Longevity in hours...	24–36	2–3	8–10	8–10	8–10	24–36
Fertilizability...	+ (when mature)	—	+	+	+	—

As regards longevity it will be seen that the immature egg depends upon its nucleus (germinal vesicle) to prevent disintegration, for a fragment lacking the nucleus disintegrates very quickly. On the other hand, the mature egg, which has become permeated with the nuclear sap of the germinal vesicle, behaves quite differently. The non-nucleated fragment of a mature egg lasts longer than that of an immature egg and it is significant that the presence of the nucleus of the mature egg, which consists of not much more than the chromosomal constituents, has no effect in preventing disintegration.

The long period that the endoplasmic sphere withstands disintegration indicates that the factors which make for disintegration reside chiefly in the original cortex of the mature egg.

In regard to fertilizability it is evident that the substance which renders cytoplasm fertilizable emanates from the germinal vesicle and finally becomes localized in the cortex of the mature egg.

We can, therefore, distinguish three factors in the starfish egg; one affecting longevity, the second affecting disintegration and the third affecting fertilizability. The first and third have been traced to the germinal vesicle of the immature egg. The second is a function of the egg cortex.

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147 (1894)

Merogony experiments on sea-urchin eggs.

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By merogony in the broader sense is meant the fertilization and development of egg fragments whether nucleated or not.

By means of the more accurate method of using a mechanical apparatus for microdissection an attempt was made to repeat the work of earlier investigators (O. and R. Hertwig, Boveri, Driesch, Morgan, Loeb, Wilson and others) especially for the purpose of cross-fertilizing egg fragments of the sea-urchin and sand dollar. Owing probably to the lateness of the season the cross-fertilization experiments were unsuccessful.

However, the following results were obtained in the self-fertilization of sea-urchin egg fragments which indicate that the size of the nucleus in the swimming larvæ depends directly upon the initial size of the nucleus in the fertilized egg fragment whereas the size of the larva bears no direct relation either to the size of the nucleus or to the initial amount of cytoplasm in the fertilized egg. Mature eggs were deprived of their nuclei by cutting them out together with a minimum amount of cytoplasm. The non-nucleated fragments were about 4/5 the size of the entire eggs. These, when fertilized, developed into dwarf larvæ of about half the size of the control and with abnormally small nuclei. Other eggs were deprived of more than half of their cytoplasm. These, upon fertilization, developed into dwarf larvæ of about half the size of the control but possessed nuclei equal in size to that of the control.

45 (1792)
Apparatus for micro-manipulation and micro-injection.

By ROBERT CHAMBERS.

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This apparatus is designed for the purpose of dissecting living cells or injecting substances into them, and for isolating microorganisms. Its advantage over that which Barber described in the *Philippine Journal of Science* in 1914 is its simplicity of construction, and the accuracy with which it can be manipulated.

The apparatus consists of two instruments, the micro-manipulator for producing movements in the microscopic field in any of three dimensions and, second, the micro-injection instrument for securing the necessary pressure to drive or suck substances through a micro-pipette. The method of making glass micro needles and pipettes is given in full in Barber's paper and in mine in the *Biological Bulletin* of 1918.

The micro-manipulator is small and compact and can be attached to the stage of any microscope. It consists of a system of rigid metal bars connected together with spring hinges. By turning certain screws the bars are forced apart. On reversing the screws the springs return the bars to their original positions. The instrument moves the tip of a needle or a pipette in three arcs at right angles to one another. The arcs are small enough so that, in the microscopic field, the needle moves practically in straight lines. The movements are fine and steady enough to be under perfect control when viewed under the highest power of the microscope. The instrument can be used singly for one needle only or with a companion when two needles, or a pipette and a needle, are to be used simultaneously.

In the micro-injection instrument mercury or an inert oil[1] (Nujol) is used to procure the necessary pressure. The instrument consists of a thin-walled steel tube about six inches long and half

an inch in diameter, one end of which is provided with a stopcock. The other end leads into a small steel tube fine enough to be flexible and long enough and so bent that, while the large tube lies on the table beside the microscope, the tip of the fine tube can be held in the pipette carrier of the micro-manipulator. Into this tip a glass Barber pipette is sealed. Mercury or oil is introduced through the stopcock of the large tube and is forced on into the micro-pipette. The stopcock is then shut off. By means of leverage clamps on the thin-walled tube the mercury or oil can be driven through a pipette having an aperture of only one micron in diameter. By turning the screws of the micro-manipulator the tip of the pipette can be brought into a hanging drop in a Barber's moist chamber. Release of pressure on the steel tube draws substances into the pipette. Injection and suction in microscopic quantities is accurately controllable as the meniscus of the mercury or oil in the pipette responds instantly to the pressure of the leverage clamps.

NEW APPARATUS AND METHODS FOR THE DISSECTION AND INJECTION OF LIVING CELLS

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FIVE FIGURES

INTRODUCTION

Operative work on the living cell has long been the aim of investigators in cytology and in experimental embryology. It was, however, not till Barber developed his method that any serious attempt could be made to dissect cells under magnifications high enough to enable one to observe in detail the various steps of the operation. The big feature of his method, aside from the making of needles and pipettes stiff and yet fine enough to puncture red blood corpuscles, consists in his moist chamber, which allows the needle tips to be operated in a drop hanging from a cover-slip in the chamber. This method eliminates all obstacles between the objective and the cover-slip, thereby permitting the use of the highest-powered objectives. Unfortunately, his instrument for manipulating the needles, unless very skillfully made, has too much lost motion, and wear and tear soon render the movements jerky and undependable.

Barber uses his apparatus principally for the isolation of bacteria. In 1912 Kite (Kite and Chambers, '12) applied Barber's method to cytological investigation. The difficulty of handling Barber's apparatus limited the number of investigators in this field and as the work in microdissection progressed the need of a more accurate and simple instrument became imperative.

The instrument described in this paper, a preliminary account of which has been published ('21), has the following advantages over any instrument hitherto made: a) simplicity of construction,

1

b) no lost motion through wear and tear, c) accurate and con-
tinuous control of the movements of the needle or pipette tip in
any direction under the highest magnifications of the microscope,
d) maintenance of the needle tip in one plane while it is being
moved back and forth in any of the three directions, and e) exist-
ence of preliminary adjusting devices which facilitate placing the
needle or pipette quickly into position.

The basic principle of the instrument consists in rigid bars
which are screwed apart against springs. The movements im-
parted are in arcs of a circle having a radius of about two and a
half inches. As the extreme range of movement of the fine ad-
justments is only 2 mm. (of which only one is necessary) the
curvature of the arc is unnoticeable.

The movements performed by the instrument are so accurately
controlled that one can readily carry out such delicate operations
as puncturing mammalian blood corpuscles, tearing off the
sarcolemma of a muscle fiber, drawing out nuclear chromatin
strands and even cutting up the chromosomes of insect germ
cells. The glass needles used for these operations taper rapidly
to a point invisible under the oil immersion objective. With the
micropipette, the bore of which need be no larger than one micron
in diameter, one can either inject substances into or withdraw
material from a cell.

For the isolation of bacteria the instrument is not only steadier
than Barber's apparatus but has new features which facilitate
greatly the method of procedure. Its application to bacterio-
logical purposes is more specifically dealt with in the Journal of
Infectious Diseases.

I take this opportunity of expressing my deep obligation to
Mr. W. H. Farnham, mechanician in the department of Chemical
Engineering in Columbia University, to whose skill and faithful
workmanship the practical evolution of the instrument is due.
I wish especially to acknowledge assistance from Dr. Milton
J. Greenman of The Wistar Institute and Dr. C. V. Taylor of
the University of California. I wish also to express my apprecia-
tion to many friends for valuable suggestions. The principle
involved in the construction of the micromanipulation instru-
ment is patented.

A MECHANICAL MICROMANIPULATOR FOR CONTROLLING THE
MOVEMENTS OF A MICRONEEDLE OR MICROPIPETTE IN
THE FIELD OF A COMPOUND MICROSCOPE

The principle of this device is demonstrated on considering the
mechanism for the movements in one plane only (fig. 1, b). This
consists of three bars of rigid metal connected at their ends to
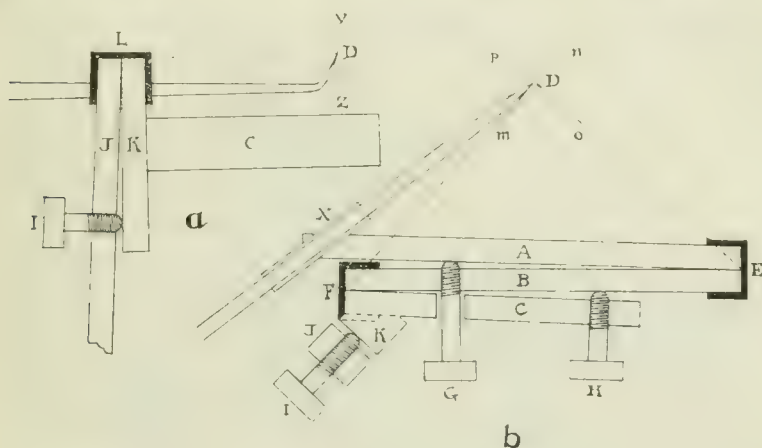form a Z-like figure by resilient metal acting as a spring hinge.

Fig. 1 Diagram showing the working principle of the micromanipulator. In
1,a, where the instrument is viewed from the side, screw, *I*, moves needle tip
through vertical arc, *y–z*. In 1,b, where the instrument is viewed from above
screws, *G* and *H*, move the needle tip through the horizontal arcs *m–n* and *o–p*.

By the action of certain screws the bars can be forced apart;
on reversing the screws the bars return to their original position
owing to the spring action at the end of the bars. By these
means arc movements may be imparted to the tip of a needle
when placed in the proper position.

The needle or any instrument, the tip of which is to be manip-
ulated, is held in a carrier fastened to the free end of a bar,
A at *X*. The needle is made to extend so that its tip is at the
apex of an imaginary triangle at *D*. In order to obtain two
movements at right angles to one another and in the horizontal
plane the tip of the needle must be at the apex, *D*, of a right-

angled isosceles triangle the base of which is a straight line joining the centers, E and F, of the two springs holding the three bars, A, B, and C, together. The shank of screw, G, passes through a large hole in bar, C, and is screw-threaded in bar, B. Turning it spreads apart bars, A and B, and imparts an arc movement to the needle tip at D at right angles to that procured by turning screw, H.

The movement in the vertical plane at right angles to the aforementioned movements is produced by screw, I (fig. 1, a), which is screw-threaded in a rigid vertical bar, J, and abuts against a vertical extension, K, of bar, C. The extension, K, is parallel to the bar, J, and is connected to it at its top by means of a solid spring hinge. Turning screw, I, spreads apart bars, J and K, and lifts the whole combination (A, B, and C) and imparts an arc movement in the vertical plane to the tip of the needle at D. To procure a vertical movement, the tip of the needle at D must lie in the same horizontal plane, L-D, with the spring fastening K and J together. When screw, I, is turned, the needle tip will then move in an arc, Y to Z, more nearly vertical than any other arc on the same circumference of which the point, D, is the center.

There are two models of the micromanipulator. One is fitted with a clamping device with which it can be fastened directly to the front of the microscope stage (fig. 2; cf. fig. 3, e).[1] The other is fastened to a rigid pillar rising from a large metal base on which the microscope is clamped (fig. 3, a). The horizontal bars of the instrument extend diagonally across the corner below the level of the stage. They do not interfere with the substage accessories of the microscope nor with any of the known types of mechanical stages.

The necessity of having one or two instruments is, of course, conditioned by the type of work to be done. For picking up bacteria one is sufficient. For microdissection in experimental embryology a great deal can be done with one instrument, but for cell injection in general and for tissue cell dissection two

[1] Steadiness may be assured by a brace, one end being screwed to the rigid vertical part of the instrument and the other end to the foot of the microscope.

instruments are indispensable so that two needles or a needle
and a pipette may be manipulated simultaneously.   When two
instruments are to be used both must be placed at the front of
the microscope so that the needles may extend, side by side, into
the moist chamber from the front.   As the horizontal bars of
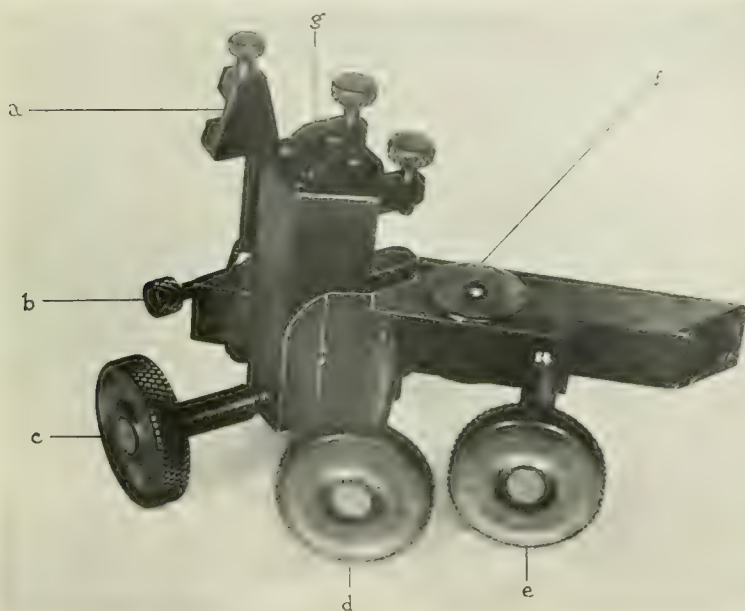each instrument extend diagonally under the microscope stage

Fig. 2   Left-handed micromanipulator to be clamped to microscope stage.
a, needle carrier with clamping screw; *b*, screw to clamp post of needle carrier; *c*,
screw for up-and-down movement; *d* and *e*, screws for lateral movements; *f*,
disc guide for the horizontal bars; *g*, stationary or rigid part of instrument with
lugs by means of which instrument is clamped to microscope stage.   Screw, *b*,
clamps the coarse adjustments.

one must be a mirror image of the other.   According to their
position with respect to the microscope these two models have
been designated as left-handed and right-handed.   For bacterio-
logical work, where it is more convenient to work from the left,
the right-handed model is to be preferred as it can be swung
around and fastened to the left side so that the pipette may

extend into the moist chamber from the left. For cytological
work, if one desires to have only one instrument, it is advisable
to secure the left-handed form and to use it as shown in figure 3, c.
The mechanical stage may then be operated with the right hand
and the instrument with the left. Eventually this instrument
may be supplemented with a right-handed form to be clamped
to the stage or attached to a pillar. When a pair of instruments
is to be used the best combination is a left-handed one clamped to
the microscope and a right-handed one attached to a pillar (fig.
3). This allows one to hold the tissue on which one is operating
with one instrument while the microscope is being temporarily
removed for renewing the pipette of the other (see page 14).

THE SETTING UP AND THE WORKING OF THE INSTRUMENT

Figure 3 shows two instruments in place ready for work.
They should be as close together as possible so that the open
end of the moist chamber need not be too wide to accommodate
the needles. This leaves ample room on either side for the attach-
ment of a mechanical stage on the microscope.[2]

The instrument is provided with means for a preliminary
adjustment of the needle in any direction. By these means the
needle tip can be quickly centered in the field of a low-powered
objective and raised close to the hanging drop in which it is to
operate. Before centering the tip the bars which control the
fine adjustments must be put into a state of tension by giving
a few turns to the milled heads of each of the three screws.
The instrument is now ready for action.

The milled heads of the screws which control the lateral
movements are provided with holes for rods to be used as levers.
A most useful accessory is a wire-wound flexible shaft about 2
feet 6 inches long (fig. 3, c) with a milled head at one end (fig. 3, d)
and the other end attached to the screw controlling the up-and-
down movement. Curving the shaft around one side of the micro-
scope brings the control of this screw, which is the one most

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[2] In the case of the Bausch & Lomb and Spencer stages, it may be necessary
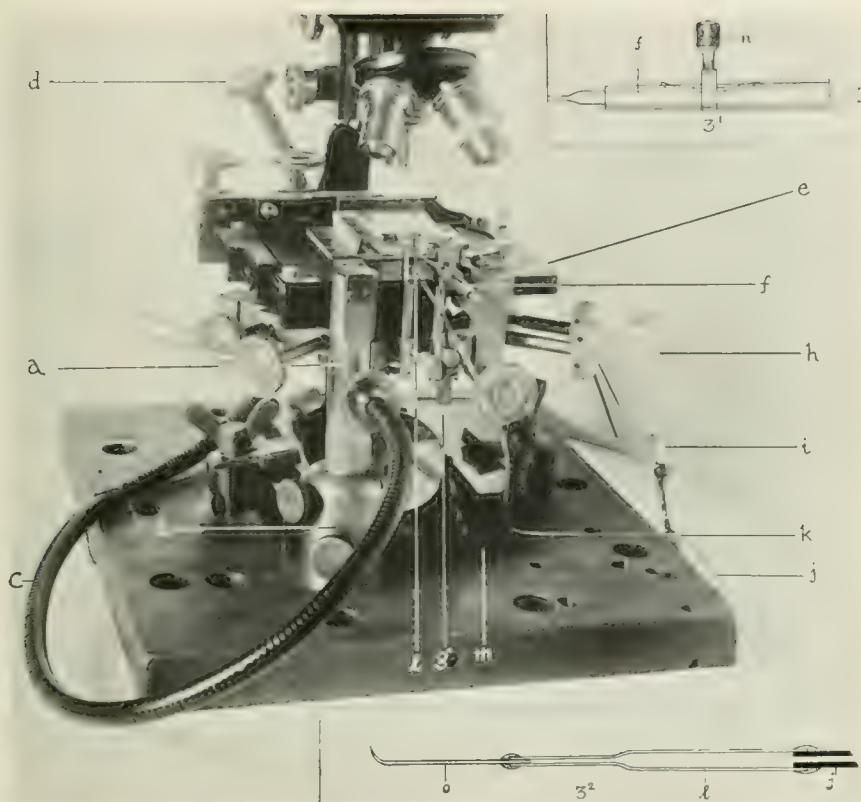to replace the screw clamping the front end of the stage by one with a smaller
head.

Fig. 3 Microscope with two micromanipulators and the microinjection apparatus in place. *a*, right-handed manipulator on pillar set in collar, *b*, fastened to base on which microscope is clamped; *c*, flexible shaft attached to screw for up-and-down movement with its milled head at *d*. (Note that screws for lateral movements are controlled by levers.) *e*, left-handed manipulator clamped to left front of microscope stage. In its needle carrier is clamped brass collar, *f*, within which shaft of needle slides. (See detail in fig. 3¹.) The coarse adjustment for raising and lowering needle carrier is done by screw, *g;* that for the lateral movements is done by turning the post on its axis.

Injection apparatus. *h*, Luer syringe set in its butt, *i*, cemented to curved brass tube, *j*. This is clamped to base at *k*. Its other end is cemented into glass tube, *l* (see detail in fig. 3²), clamped in needle carrier of the right-handed manipulator, *a*.

Most of the holes in the base are unnecessary. Foot of microscope is held by two screw clamps. The adjustable guide, *m* keeps microscope in proper alignment.

Fig. 3¹ Detail of brass collar (*f* in fig. 3) which facilitates in-and-out movement of needle or pipette; *n*, screw which presses on a spring to clamp the needle in the collar.

Fig. 3² Detail of glass tube of injection apparatus (*l* in fig. 3) cemented on brass tube, *j; o*, shank of micropipette cemented into end of glass tube. The pipette is readily changed by softening the sealing wax which holds it.

7

frequently used, close to that of the fine adjustment of the micro-
scope. The shaft also facilitates the use of both hands for the
various movements of the one instrument.

Another useful accessory is a brass collar $1\frac{1}{2}$ inch long (fig. 3)
with a spring which projects into its lumen through a slot. The
shaft of the needle is slipped through the collar and the screw,
clamping the spring, tightened sufficiently to enable one to slide
the shaft evenly. The collar is then clamped into the needle
carrier of the instrument. This arrangement facilitates sliding
the needle into or out of the moist chamber without danger to
the tip of the needle.

The micromanipulator is intended to be used with the mechani-
cal stage of the microscope. The mechanical stage moves the
moist chamber (fig. 3). As the cell or tissue to be dissected lies
in a drop hanging from the roof of the chamber, the motion im-
parted by the mechanical stage moves the cells against the micro-
needle. Indeed, most of the dissection, where a single needle is
used, is done by first bringing the needle tip into the cell and then
dragging the cell away by means of the mechanical stage.

The horizontal movements of the micromanipulator are used
mostly for the purpose of bringing the tip of the needle accurately
into a desired spot in the field of the microscope preparatory to
the actual operative work. In order to insure the greatest
possible steadiness to the vertical movement, the part of the
instrument which imparts this movement adjoins and is mani-
pulated from the stationary and rigid part of the instrument.
To make this possible the present design incorporates a theoreti-
cal error which can be understood from figure 1, a. Turning screw,
I, to produce the vertical movement throws the combination of
bars A, B, and C, out of the horizontal, and it is these bars upon
which the lateral movements of the needle depend. However,
the angle at which these bars are placed minimizes the error so
that it is unnoticeable.

Guides exist in the instrument to insure a true travel of the
bars as they spread apart or come together. The guide for the
bar which produces the vertical movement consists of a depres-
sion in the stationary part of the instrument into which the verti-

cal bar fits. The guides of the lateral movements are two metal discs which can be tightened or loosened by screws. The upper one is seen in figure 2, f. They correct two possible errors which may occur on reversing the direction of movement, viz., a dropping of the needle or pipette out of focus and a shifting to one side.

The first error can be corrected by tightening one or both of the guides; the second, by loosening them. The guides, therefore, must be neither too tight nor too loose. The first error is the more serious of the two. It is due to an unequal tension in the springs which throws the tip of the moving screw to a different spot on the bar against which it abuts. If this be not corrected, the screw will in time wear a depression in the brass bar that is out of center thus accentuating the error. The second error is due to the guides being too tight so that they bind and prevent the bars from making a true return. If not corrected, this error will be gradually eliminated with the wear of the frictional surfaces.

By an accidental knock the horizontal bars of the instrument may be jarred out of place or the fine adjustment screws injured. If the upper and lower surfaces of the horizontal bars are not flush loosen the guide discs (fig. 2, f) also the screws of the springs on the ends of the bars and, with a wooden mallet, gently hammer the bars till they are flush. Then tighten the guide discs to keep the bars flush and carefully tighten the screws of the spring. If the screws have been bent by the accident they must be changed otherwise tightening them will again pull the bars out of place. If the guide discs are bent they also must be changed. A more serious accident is when the fine adjustment screws are injured. The steel shafts of the screws may be bent or they may have cut into the brass so as to loosen the threads. This tends to throw the shaft of the screw out of center. In such a case somewhat larger screws must be made and accurately centered opposite the bar against which it abuts.

THE SUBSTAGE CONDENSER AND THE METHOD OF MAKING BARBER'S MOIST CHAMBER AND GLASS NEEDLES

For critical illumination the height of the moist chamber must be equal to the working focal distance of the substage condenser.

The Abbe condenser can be used by removing the top lens. The focal distance of the remaining lens is almost one inch. In the Bausch and Lomb microscope the substage can easily be arranged to raise this lens sufficiently to have at least half its focal distance above the surface of the stage. This is ample, for one seldom requires a moist chamber as high as half an inch. The focal distance of this lens can be reduced and its illuminating power correspondingly increased by placing the lens of a 10X dissecting lens on top of it. This combination has a focal distance of about $\frac{3}{8}$ of an inch and, if the substage can be raised to bring the top lens flush with the upper surface of the stage, all of this distance may be used for the height of the moist chamber. Better results are secured with a triple lens condenser with its top lens removed. Such a condenser from Leitz which I am using has a working focal distance of $\frac{3}{8}$ of an inch. One may also use condensers which are made with a specially long working distance for projection apparatus, in which a cooling trough is placed between the condenser and the slide.

If the working focal distance of the condenser be less than $\frac{3}{8}$ of an inch, it is well to have two moist chambers, one for critical work and the other, from $\frac{3}{8}$ to $\frac{1}{2}$ an inch high, for ordinary work. This is advisable, because it is easier to make needles for the higher chamber.

The moist chamber is of glass (fig. 4). The base is a thin glass slide about $2\frac{3}{8}$ x 2 inches in size. The sides consist of strips of plate glass about $1\frac{7}{8}$ inches long and $\frac{1}{4}$ inch wide, and of a height determined upon by the available condenser. One end of the chamber is closed with a strip of glass of the same height as the sides and backed by another strip a fraction higher, in order to prevent a cover-slip from sliding beyond it. The trough of the chamber should be from $\frac{3}{4}$ to $\frac{7}{8}$ of an inch wide. The strips are cemented with any ordinary glass cement. Heated Canada balsam serves well. Near the closed end of the trough a small strip of glass should be cemented across the trough to provide a well for water. When cementing the long strips to the base, care must be taken to have the top surface of the strips horizontal. This may be done while the cement is still soft by

focusing on the upper surface of the strips and by manipulating the strips until all parts of their surfaces lie in one focal plane.

The well in the chamber is to be filled with water and, in order to distribute the moisture throughout the chamber, strips of blotting-paper should be placed along the sides of the trough with the inner end in the water well. One may substitute for the well strips of blotting-paper laid across the trough. This moist chamber is designed for cover-slips of a size 24 x 40 mm. The cover-slip is sealed on the chamber with vaseline. Square
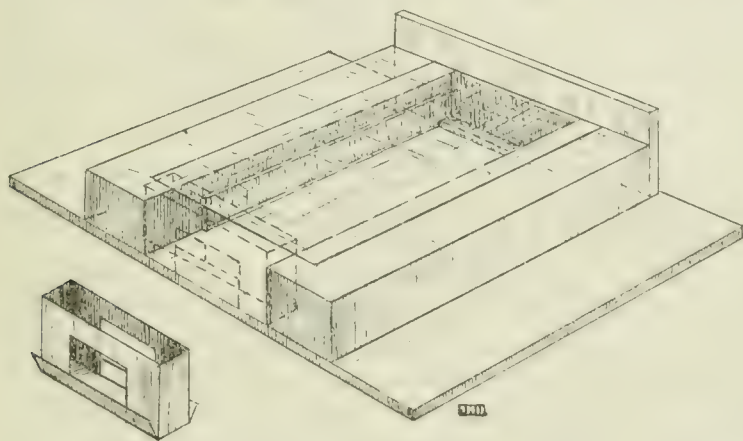
Fig. 4 Moist chamber and cardboard trough for closing open end of chamber. When the needles are in place (cf. fig. 3), the trough is placed over shanks of needles (dotted lines at open end of chamber) and filled with vaseline.

cover-slips may also be used, if the rest of the chamber be roofed with other strips of cover-glass.

The moist chamber is open at one end to permit the entrance of the microneedles or pipettes. To prevent undue evaporation, especially when a preparation is to be left over night, the open end may be temporarily closed by means of a paraffined thin cardboard trough of a shape shown in figure 4. The trough is placed over the shafts of the needles and filled with soft vaseline containing a few threads of cotton to give substance to the vaseline. The vaseline closes around the shafts of the needle and seals the opening of the chamber without interfering with the movement

of the needles. To prevent the vaseline from spreading on the floor of the moist chamber, it is well to have a shallow pan of cardboard set under the shafts of the needles for the trough to rest upon.

The hanging-drop containing the cells or tissue to be operated upon is placed on the cover-slip which is then inverted over the moist chamber.[3] To prevent the vaseline from spreading on the cover-glass and from contaminating the hanging-drop, a thin film of melted paraffin may be spread and cooled on the cover-glass bounding the area to be occupied by the hanging-drop.

The needles are made from either soft or hard glass tubing. If a brass collar is used to serve as a guide for the in-and-out movement (fig. 3), the glass tubing should be selected to fit the collar. What I use is a fraction less than $\frac{1}{8}$ inch in outside diameter. The thicker the wall of the tubing the firmer tends to be the tip of the needle made from it. The method of making the needle is given in a paper of Barber's ('14) and in one of mine ('18). A brief account will suffice here. Acetylene or ordinary illuminating gas may be used. For a microburner use a piece of hard glass tubing bent at right angles and with the burner end closed except for the smallest aperture that will retain a flame. This may be done by heating the end and pinching it with forceps. The size of the flame may be regulated by a screw pinch-cock on the rubber tube, figure 5, h.

To make the needles, proceed as follows: 1) In an ordinary burner draw out one end of a glass tube with a capillary of about 0.3 to 0.5 mm. in diameter (fig. 5, a). 2) Lower the flame of the microburner to the smallest flame possible. Now hold the shank of the tube in the left hand and grasp the capillary at its end either with the thumb and finger of the right hand or with forceps having flat tips coated with Canada balsam. Bring the capillary over the flame and pull gently till the capillary parts. The hands should remain on the table during the

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[3] For placing a hanging-drop after the moist chamber has been covered, a convenient pipette is one with its end drawn out into a curved capillary and the tip bent at an angle so that, on insertion into the moist chamber, the tip will touch the undersurface of the cover-slip. With a rubber tube to reach one's mouth, a small drop is readily deposited.

process and, as the capillary parts, lift the glass away from the
flame by turning the hands slightly outward. The capillary will
separate with a slight tug. The tip should be like that in
figure 5, c. If too little heat be used and the pull made too sud-
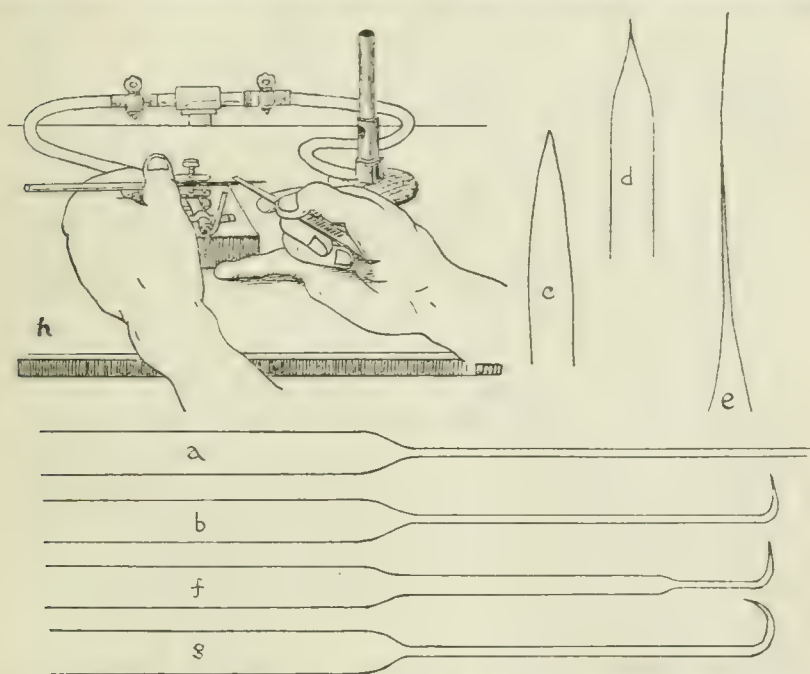denly, the capillary may part with a snap with a broken tip.

Fig. 5 Method of making the needles. *h*, position of hands when making
needles over microburner; *a*, glass tube with capillary; *b*, needle with tip bent
up; *c*, a good needle tip; *d*, needle tip serviceable for converting into a pipette;
e, unserviceable tip drawn out into a hair; *f*, needle with stout shank; *g*, needle
with tip bent back for cutting purposes.

If too much heat be used, the tip is drawn out into a long hair,
figure 5, e. 3) Bend the capillary at right angles by heating it
just back of the point and pushing up with a dissecting needle, 5, b.
The length of the needle beyond the bend is conditioned by the
height of the moist chamber to be used. The type of needle
shown in 5, g, is used for cutting by bringing the upper limb of
the needle below and up into the cell.

APPARATUS FOR INJECTION AND FOR THE WITHDRAWAL
OF MATERIAL FROM A LIVING CELL

Barber's mercury pipette method, which depends upon the expansion and contraction of mercury by heat and cold, although excellent, is troublesome to make and easily broken. Taylor ('20) devised an instrument which depends upon a plunger to exert pressure on an enclosed mercury column. With mercury, however, it is difficult to maintain a plunger for any length of time without leakage. I described an apparatus ('21) in which mercury or Nujol oil is enclosed in a thin-walled steel cylinder. Pressure on the wall of the cylinder exerts the driving force necessary for injection. This works very well, but it requires special apparatus and the difficulty of securing a cylinder the walls of which are sufficiently resilient renders the apparatus somewhat unserviceable.

The apparatus shown in figure 3, does all the work of any device hitherto described and has the advantage of being extremely simple to make. All that is required is a carefully selected glass Luer syringe of about 2 cc. capacity, a piece of fine brass tubing of about 2 mm. outside diameter and two feet long (small, extra soft brass tubing used for lighting purposes is also serviceable), a metal rod 1 inch long with a hole through it large enough to receive the brass tubing, a piece of $\frac{1}{8}$-inch glass tubing, some de Khotinsky cement or ordinary sealing wax and an ordinary small horseshoe clamp.

First seal the metal butt of the Luer syringe to one end of the brass tubing. Slip the metal rod over the tubing and cement it an inch or two away from the syringe attachment. At the other end of the brass tubing seal a short piece of $\frac{1}{8}$-inch glass tubing, the free end of which has previously been drawn out into a capillary an inch or so long and about 1 mm. in inside diameter (fig. 3²).

When cementing the brass tube to the syringe attachment and to the glass tube, have a wire inserted far into the brass tube before applying the cement. The tip of the brass tube, from which the wire projects, is then coated with cement and the part to be cemented pulled over it. While the tube is still warm,

withdraw the wire with a gentle twirling motion. This draws the cement out around the ends of the brass tube on the inner surface of the projecting glass tube and prevents the formation of pockets in which air may be trapped. In the make-up of the entire system one must exercise care to prevent air from being trapped, for the presence of the air-bubbles vitiates the accurate control of pressure in the apparatus.

The brass tube where the metal rod encloses it is to be clamped to the foot of the microscope or to a base which is rigidly attached to the microscope, figure 3. The short end of the tube, projecting from the rod, is bent so that the syringe, when set into its butt stands more or less upright. The long end of the tube is carefully curved and bent, so that the glass tube which is sealed on the end will rest in the needle carrier of the micromanipulator and its capillary project over the stage of the microscope with its end about $1\frac{1}{2}$ inches from the field of the microscope objective.

The Luer syringe must now be charged with distilled water which has been boiled and the apparatus filled to within $\frac{1}{8}$ of an inch from the tip of the glass capillary. Before stopping, however, it is well to run water through the apparatus for some time to drive out all the air. Before charging the syringe for the last time the plunger should be coated with heavy stop-cock grease. This much of the apparatus can be kept permanently ready for use.

The micropipettes are made from microneedles drawn out of thin-walled capillary glass tubing. When finished, the shaft of the needle should be at least $1\frac{1}{2}$ inches long and large enough to fit snugly into the glass capillary of the apparatus. This can be readily done by drawing out a supply of thin-walled glass capillaries and preserving those which fit a sample the size of the capillary of the apparatus. The needle end of the shaft should be bent at an angle, the length from the knee of the bend to the tip depending upon the height of the moist chamber. The shaft of the needle near its end is now thinly coated with de Khotinsky cement or sealing wax and, while the cement is still soft, inserted into the glass tube of the apparatus. An extra coat of cement should be added over the joint to insure the seal. The apparatus is now ready for use. The tip of the needle is brought into a

hanging-drop of water or a solution to be injected and converted into a pipette by jamming the tip against the under surface of the cover-slip until it breaks off. During the process continual pressure should be exerted on the plunger of the syringe in order to prevent pieces of glass from being sucked into the pipette. Occasionally, while attempting to make the needle in the flame, a serviceable pipette results instead. When the pipette is finally in place, all or most of the air in it should be driven out.

One can readily see that the sealing of the micropipette into the apparatus must be done away from the microscope. It is in this operation that the type of micromanipulator fastened on a pillar is of advantage. The pipette has to be frequently changed, and it is very convenient to be able to release the microscope from its base by loosening its clamps and to slip it out of the way. As soon as a fresh needle has been inserted, the microscope is readily slid back into place. For this purpose the base on which the microscope rests is provided with guides to insure its true return. When exchanging a pipette, care must be taken not to clog the lumen. This can be done by using a minimum amount of cement and by having the lumen of the tube into which the shaft of the pipette is to be inserted as clean as possible.

The use of thin-walled tubing for making the micropipette is to insure having the largest bore possible at the tip of the pipette. The thickness of the wall and the size of the lumen of the glass tube tend to maintain their original proportions when drawn out in a flame. Often, however, it is more convenient to have pipettes with stouter walls. Such pipettes are less readily broken but, owing to the smaller-sized lumen, run the risk of quickly clogging. The best pipettes are made from hollow needles with a rapidly tapering tip (fig. 5, d), for needles with a long taper are apt to break anywhere.

A necessary precaution is to have the capillary from which the needle is to be made perfectly dry. The presence of the least moisture may result in alternating columns of water and air in the pipette tip which no amount of pressure will expel.

Water seems to be the best medium for transmitting pressure in the apparatus. Mercury is apt to break and allow air or

water to leak past it when it reaches the tip of the pipette. When this occurs, the separated droplet of mercury clogs the aperture. Mercury also tends to leak past the best plunger made.[4] The disadvantage of using water is the risk of its diffusion into the solution to be injected. If a considerable amount of the solution be drawn into the pipette, this risk is minimized. A good method is to color the water (e.g., with Nile-blue chlorhydrate or with neutral red). The solution drawn into the pipette from a hanging-drop is then visible by contrast. For ordinary purposes a cushion of air between the water and the injection fluid serves well.

Oil is unsuitable because, in spite of all precautions, it occasionally comes into contact with the hanging-drop containing the tissue to be operated upon; it then spreads over the surface of the drop and injures the preparation. It also dissolves de Khotinsky cement and sealing wax which are so convenient for cementing the pipette to the apparatus.

Manipulation of the syringe is facilitated by fastening it in a frame and by using a milled screw to press the plunger. I use a microscope for this purpose with the objective, substage and mirror removed. The syringe is passed through the center of the microscope stage where it is held firmly with a tight-fitting collar of cork. The lower end of the microscope tube rests on the top of the plunger so that pressure can be brought to bear on it by either the coarse or fine adjustments. There is no need of fastening the plunger to the microscope tube, because the resiliency of the water in the apparatus is sufficient to cause suction in the micropipette when the plunger is released from pressure.

APPENDIX

Barber's instrument is based on the principle of a carrier pushed along a groove by a screw at one end. By having a series of three carriers built up on one another, each traveling in a different direction, movements in any one of three dimensions may

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[4] Leakage in the syringe can be avoided by placing a cushion of oil between the plunger and the mercury. This may also be done when water is used.

be imparted to a needle clamped to the top carrier. Hecker ('16)
improved Barber's instrument, but added materially to the in-
tricacy of its make up.

Other investigators that I know of who have devised instru-
ments for micro-operative work are Schmidt ('69, '70), Birge
('82), Chabry ('87), Schouten ('05, '11), Tchahotine ('12, '21),
McClendon ('07), Malone ('18), Bishop and Tharaldsen ('21).

Schmidt's instrument is one of historic interest only. I have
already described it ('18). Chabry used a delicate spring device
with which he could shoot the tip of a glass needle into an ovum
to any desired depth. Schouten uses his for the isolation of
bacteria. It consists of a pillar carrying a needle which may be
mechanically raised and lowered. For the horizontal movements
Schouten depends upon pushing the microscope on a base.
McClendon attached an up-and-down movement to a Spencer
mechanical stage. Tchahotine uses a mechanism attached to the
tube of his microscope from which extends a glass needle curved
in such a way as to bring its tip into the field of a low-power
objective where it is brought into focus. Dissection of cells is
carried out by moving the microscope tube and by pushing the
cells against the needle tip by means of the mechanical stage of the
microscope. Malone uses Schouten's method, but, instead of
having a special pillar with a raising device, he mounts his pipette
carrier on the tube of a second microscope whose adjustments
serve as a means for raising and lowering the pipette. Bishop
and Tharaldsen have a simple instrument based on a principle
somewhat resembling mine but lacking in proper control for one
of the two lateral movements. Recently I have heard that
Zeiss is manufacturing a micro-dissection instrument which
is a modification of Barber's apparatus with both coarse and
fine adjustments.

Tchahotine and Bovie have recently devised a method for
producing localized injury in a cell by means of ultra violet rays.
The method is very ingenious but, of course, is rather limited in
its application to micro-dissection.

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A MICROMANIPULATOR FOR THE ISOLATION OF BACTERIA AND THE DISSECTION OF CELLS

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I have recently described (Chambers, 1922, b) an apparatus for the manipulation of micro needles and micro pipettes under the highest magnifications of the microscope. This apparatus is an improvement on Barber's Pipette Holder (Barber, 1914) because of its simpler construction and the greater accuracy with which one can control its movements. An additional advantage consists in the existence of certain devices for bringing the pipette or needle, quickly into position before starting actual operation.

The working principle of the apparatus (which is being patented) is illustrated in figure 1. It consists in the use of bars of rigid metal connected at their ends to form a Z like figure by resilient metal acting as spring hinges. The bars are forced apart by screws and return when the screws are reversed. By these means arc movements are imparted to the tip of a pipette which is attached to one of the bars. As the radius of each arc is about two and a half inches, the fine movements imparted to the tip of the pipette are practically in straight lines because the excursion never exceeds one millimeter.

The instrument can be used by itself for one needle or pipette, or with a companion apparatus when two needles, or a needle and a pipette are to be used simultaneously. When a pair is used, one is a left handed and the other a right handed apparatus, both being clamped to the front of the microscope stage. For the isolation of bacteria, one instrument is sufficient. It may be clamped on the left side of the microscope stage, figure 2, so that the pipette projects into the moist chamber from the

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left. The tip of the needle or pipette is bent up so as to project from below into a drop suspended from the coverslip which roofs the chamber. The cells to be operated upon lie in the hanging drop. When a cell is to be dissected or injected it tends to retain its position on account of the shallowness of the drop and the inertia of the cell. However, it is more satisfactory to use two instruments, one with a needle for holding the cell or tissue, and the other with a needle or pipette for the actual operation.
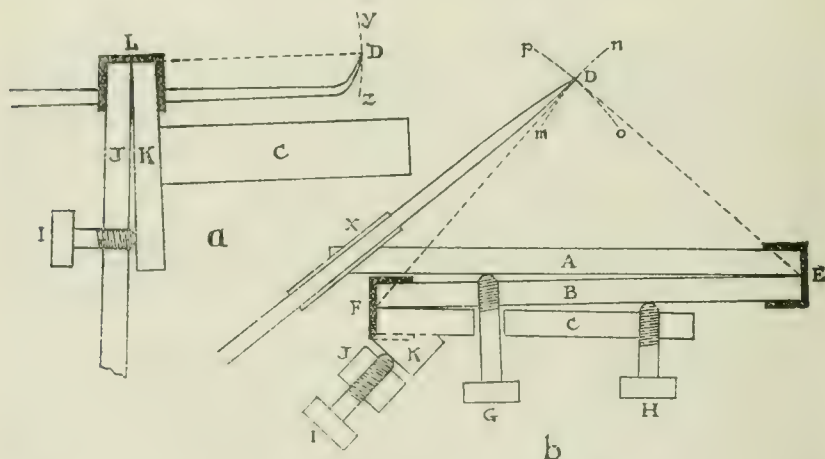
Fig. 1. Diagram Showing Working Principle of Micromanipulator

(a) Side view. Screw I in stationary pillar J pushes against K, and causes needle tip D to move through vertical arc y-z.

(b) Surface view. Screws G and H move the needle tip through horizontal arcs m-n and o-p.

For dissecting purposes, the glass needles may be curved or straight and with obtusely or gently tapering tips. They can be made fine enough to puncture red blood corpuscles and to tear up leucocytes.

For injecting and for withdrawing materials from a living cell, the micro pipettes are made with apertures varying from two to less than half a micron in diameter. I have recently described (Chambers, 1922, a) an effective and easily made apparatus for exerting the necessary pressure to drive materials through

such small pipettes, and at the same time to control, with considerable accuracy, the amount to be injected or withdrawn.

For isolating bacteria, much coarser pipettes are used, which can be blown into by the mouth through a length of rubber tubing, figure 2. At my suggestion, Dr. Kahn has kindly pub-
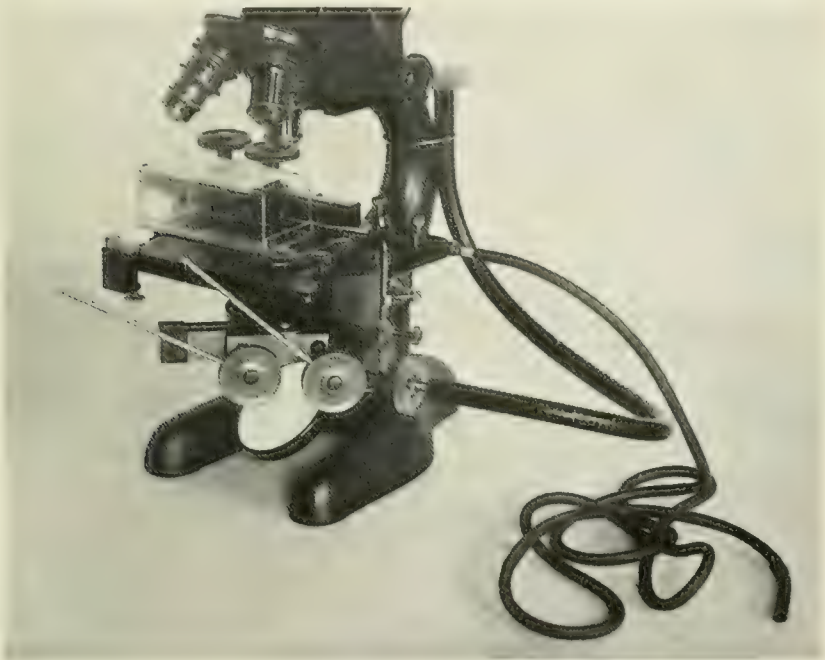

FIG. 2. MICROMANIPULATOR MOUNTED ON LEFT SIDE OF MICROSCOPE FOR ISOLATING BACTERIA

Note Barber's moist chamber with the coverslip marked with cross lines to aid in locating areas. The chamber shown here is higher than necessary. The screw producing the vertical movement is connected with a flexible shaft, which allows its control to be brought into close proximity with the fine adjustment of the microscope.

lished an account (Kahn, 1922) of the procedure, together with a discussion of the application of the micromanipulator to the isolation of bacteria. In brief, the procedure is as follows: A sterile, hollow glass needle is first made. The bent up tip is then inserted into a test tube of a liquid culture of bacteria,

and converted into a pipette by breaking the tip against the wall
of the test tube. A small amount of the culture is sucked up,
and the filled pipette placed in the micromanipulator attached
to the microscope. The tip of the pipette is then brought into
the microscopic field and brought close to the coverslip of the
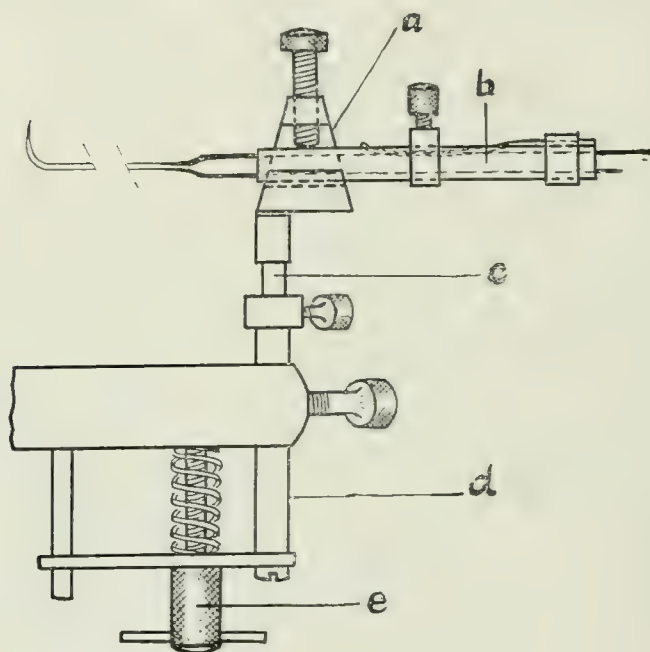
Fig. 3. Detail Showing Devices for Preliminary Adjustments of the
Pipette

Carrier, *a*, for clamping brass collar, *b*, in which needle or pipette has been in-
serted. The needle or pipette slides evenly within the collar for the in and out
movement. Telescoping pillar, *c*, for lengthening vertical post of carrier. Post,
d; rotates and serves to move needle tip laterally, Screw, *e*, raises and lowers
post, *d*, to move needle tip vertically.

moist chamber by means of the preliminary adjusting devices
shown in detail in figure 3. The tip is now further raised by
means of the fine adjustment screw until it reaches the under-
surface of the coverslip. By alternately raising and lowering
the pipette, and by moving the moist chamber with the mechani-

cal stage, a series of hanging droplets[1] are placed on the coverslip. The pipette is then removed from the instrument and discarded. A search is now made for droplets containing only a single organism. Each such droplet is drawn up into a fresh sterile pipette, which is then removed from the instrument and inserted into a tube containing a suitable sterile medium. The contents of the pipette are now expelled by blowing. In this way, one can quickly obtain cultures known to have originated from a single organism.

The micromanipulation technic is not very difficult. The making of the glass needles and pipettes, and the working of the instrument can be quickly mastered.

For the bacteriologist, the isolation method as introduced by Barber, has long proved most successful. With the apparatus described here, it should soon be more widely used.

For the cytologist and cell physiologist, the problem is to find the proper material with which to work. Through micro-operations on certain tissue cells and on such material as Protozoa and marine ova, considerable light has already been thrown upon the nature of living protoplasm.

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[1] Barber uses coverslips smeared with petrolatum to aid in the maintenance of the droplets. The excess, having been washed off with soap and water, the slips are dried with a cloth, and then heated and wiped a second time while still warm. They are sterilized by flaming.

A MICRO INJECTION STUDY ON THE PERMEABILITY OF THE STARFISH EGG.

BY ROBERT CHAMBERS.

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It is well known that selective permeability, or semipermeability, is one of the essential characteristics of the living cell. So far, however, there is no evidence as to whether the semipermeability of protoplasm is a property of its entire mass or of its surface only.

Apparently the only means by which the action of substances on the interior alone of protoplasm may be studied is by injection. Animal cells can be injected by using the very fine glass pipettes and the mercury injection method which Barber devised for bacteriological work. I have used this method. The pipettes, both as regards their size and the ease of making, leave nothing to be desired. The method, however, is not only very difficult, but is unsatisfactory, owing to the fact that the pressure required for injection depends upon the expansion of mercury by heat, and this cannot be instantly controlled. Kite[1] tried it, but substituted for most purposes the far cruder method of blowing into his pipettes through a rubber tube. This operation necessitates larger pipettes than can be properly used for cell injection. The erroneous conclusions arrived at by Kite were due not only to the difficulty of the procedure, but mainly to the extraordinary ability of protoplasm to form films over torn surfaces. Pushing a pipette, especially a comparatively large one, into an egg cell frequently causes the surface of the cell to become invaginated and thus forms a deep pocket. The tip of the pipette, even if it should finally break through the surface, is apt to be separated from the protoplasm of the interior by the formation of a new surface film continuous with the original surface of the cell. Kite apparently did not guard against this contingency, and his experi-

[1] Kite, G. L., *Biol. Bull.*, 1913, xxv, 1.

mental results indicate that his solutions never actually entered the
protoplasm of the cell. The injected fluid simply seems to have filled
the bottom of a pocket and then flowed down the side of the pipette
to the exterior. When Kite says that he found no difference in
permeability, whether a substance be applied to the surface of a cell
or to any spot in its interior, he was perfectly correct. The spot in
the interior was an infolding from the surface of the cell. At best,
he was only comparing the permeability of the original surface of the
cell with that of a newly formed surface film which surrounded the
tip of his pipette.

I have recently succeeded[2] in devising a simple but efficient piece
of apparatus with which one can accurately and easily control the
injection of fluids through a micro pipette having an aperture of
less than 1 micron in diameter. The pipette, when properly made,
tapers rapidly to a tip with a sharp cutting edge. The apparatus is
so constructed that the pipette can be quickly changed. By keeping
in mind the ease with which protoplasmic surface films are formed,
one can, with this method, readily and accurately inject fluids directly
into the interior of the protoplasm of a cell.

This summer Jacobs kindly set at my disposal a manuscript which
the reader will find in this number of this Journal, in which are de-
scribed the interesting results that were obtained by immersing neu-
tral red vitally stained starfish eggs in ammonium chloride, and in
sodium bicarbonate solutions. Jacobs found that a $1/2$ M NH_4Cl
solution, which is sufficiently acid to redden neutral red, will cause
the neutral red within the eggs to turn yellow, indicating the entrance
into the eggs of NH_3 and not of HCl. He also found that neutral
red stained eggs will turn a deeper red when immersed in an alkaline
solution of $1/2$ M $NaHCO_3$ charged with CO_2, indicating the entrance
into the eggs of CO_2 and not of NaOH.

Jacobs' results confirm those of Loeb,[3] Bethe,[4] Warburg,[5] Harvey,[6]

[2] Chambers, R., *Anat. Rec.*, 1922, xxiv, 1.

[3] Loeb, J., *Biochem. Z.*, 1909, xv, 254.

[4] Bethe, A., *Arch., ges. Physiol.*, 1909, cxxvii, 261.

[5] Warburg, O., *Z. Physiol. Chem.*, 1910, lxvi, 305.

[6] Harvey, E. N., *J. Exp. Zool.*, 1911, x, 507; *Internat. Z. physik. Chem. u. Biol.*,
1914, i, 463.

and Crozier[7] that weak acids and bases freely penetrate living cells, whereas strong acids and bases do not. This is presumably because of their solubility in the organic solvents (lipoids) of the protoplasmic surface layer.

I have injected the solutions which Jacobs used into starfish eggs vitally stained with neutral red, and obtained decided and consistent results, which show that HCl and NaOH will permeate protoplasm freely as long as there is no protoplasmic film to serve as a barrier. The semipermeability of protoplasm, in all probability, depends upon the surface film having properties different from those of the continuous internal protoplasm.

EXPERIMENTS.

Mature starfish (*Asterias forbesii*) eggs were vitally stained with neutral red. They were then placed in a hanging drop in Barber's moist chamber, and those eggs selected which showed a neutral tint of an orange-red hue. All the experimental work was done under a Leitz $\frac{1}{7a}$ oil immersion objective and ocular 15. This objective gives a remarkably long working distance together with a sharp definition that allows of the use of high powered oculars.

Treatment with 1/2 M NH₄Cl.

Eggs were placed in a hanging drop of $1/2$ M NH_4Cl in which, as Jacobs has shown, their vitality remains unimpaired for a period of 10 to 15 minutes. With a microdissection needle deep cuts were made in the eggs. The cut surfaces were immediately bounded by surface films continuous with the surface of the egg, and no injurious effect of the surrounding medium was noticeable. During this time, the neutral red within the egg, gradually turned yellow.

This experiment indicates that the NH_4Cl does not prevent the formation of films over the cut surfaces of the egg, and also that the solution will not, within the time limits of the experiment, penetrate those films.

The interior of stained eggs was made to flow out in a drop of $1/2$ M NH_4Cl by the following means. The egg was torn at one spot on its surface and then caught on the other side and pulled to the edge of the drop. In every case the rapidly outflowing interior turned rose-red upon coming into contact with the surrounding solution and cytolyzed into a frothy semisolid mass. The change from an orange color to a red, with an accompanying cytolysis, extended from the out-

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[7] Crozier, W. J., *J. Biol. Chem.*, 1916, xxiv, 255.

flowing area into the egg itself, and spread to the original cortex until the entire egg was cytolyzed.

This experiment shows that if the egg be torn in such a way as to cause its interior to flow out rapidly, no surface film forms. The NH_4Cl at once penetrates the protoplasm which undergoes the characteristic color change and cytolysis.

Stained eggs were placed in a hanging drop of sea water and $1/2$ M NH_4Cl injected under the egg membrane. This is fairly easy to do in the unfertilized egg but more so in the fertilized egg in which the membrane has already lifted off as the so called fertilization membrane. In the unfertilized egg, the injected solution at first bulges the membrane giving rise to a localized blister, and then usually spreads quickly over the egg, lifting the membrane from its entire surface. The permeability of the egg to the NH_4Cl is not affected by this treatment. This demonstrates that it is not the egg membrane which protects the egg from the NH_4Cl, but the actual surface film of the protoplasm lying under the membrane.

Stained eggs in a hanging drop of sea water were punctured with a glass pipette having an aperture of 1 micron in diameter, and a minute quantity of $1/2$ M NH_4Cl injected directly into the interior of the egg. The injected area immediately changed from an orange to a rose-red color, and then underwent cytolysis. The color change and accompanying cytolysis spread from the injected area. In some cases this spread was arrested by the formation of a surface film which converted the injected and disintegrated area into a vacuole. In other cases the cytolysis spread till it reached the cortex which disintegrated from within outward.

This experiment demonstrates that $1/2$ M NH_4Cl, which causes an alkaline color change within eggs immersed in it, will, when injected into the interior of the eggs, produce the acid color change and accompanying cytolysis which characterizes the presence of HCl.

Treatment with $1/2$ M $NaHCO_3$ + CO_2.

Stained eggs were cut and torn in a hanging drop of the $NaHCO_3$ solution. In contrast to the reaction in the presence of NH_4Cl there was no tendency for the formation of surface films over their cut surfaces. The protoplasm simply flowed out and was dispersed in the solution, the color changing meanwhile from red to yellow.

Injection of $NaHCO_3$ beneath the egg membrane of eggs in sea water had no other effect than that produced upon eggs by immersing them in the solution; *viz.*, deepening of the red color in the egg owing to the selective penetration of CO_2.

Stained eggs were injected with the $NaHCO_3$ solution. The injected area immediately turned yellow, and cytolysis with liquefaction took place. No surface film formed about the cytolyzing area, and the yellow color spread throughout.

CONCLUSION.

The experiments with the NH_4Cl are similar to, and corroborate micro injection experiments performed in connection with some work on mustard gas in which the writer[8] collaborated. Eggs immersed in sea water containing decomposed mustard gas, at a certain low concentration are not affected. If, however, the solution be injected, the egg quickly cytolyzes owing to the free HCl present.

A similar impermeability of the protoplasmic surface film to certain substances was also encountered in injection work on *Amœba*.[9] *Amœbæ* immersed in an aqueous solution of eosin will not take the stain till after death. On the other hand, the eosin, when injected into the *Amœba*, quickly permeates the protoplasm, to be arrested only at the surface.

The semipermeability of a living cell appears primarily to be a function of its surface film. It is immaterial whether this film be that of the original cortex of the cell, a film newly formed over a cut surface, or a film that surrounds an artificially induced vacuole within the cell. As long as such a surface film exists neither the acid group of the NH_4Cl nor the alkaline group of the $NaHCO_3$ can, within certain concentration limits, penetrate the protoplasm. These solutions, if injected beneath the surface film, however, will produce their characteristic effects upon the protoplasm.

[8] Lillie, R. S., Clowes, G. H. A., and Chambers, R., *J. Pharmacol. and Exp. Therap.*, 1919–20, xiv, 75.

[9] Chambers, R., *Proc. Soc. Exp. Biol. and Med.*, 1920–21, xviii, 66.

67 (2027)

A note on the entrance of the spermatozoon into the starfish egg.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

In 1876 Fol made the classic discovery that the spermatozoon actually enters the egg in fertilization. This fact he observed in the starfish egg. Fol's treatise was apparently so exhaustive and so carefully worked out that no one has questioned the details of his observations and his interpretation of the process is generally accepted to this day. Conical elevations were seen to form on the surface of the egg and the spermatozoa travelled in a straight line toward them. When a spermatozoon reached a cone its head penetrated it. Fol called the conical elevation the "attraction cone" and believed that it attracted the spermatozoon from a distance.

The starfish egg is surrounded by a zone of glutinous jelly the thickness of which is about one fifth the diameter of the egg. When the eggs are placed in a sperm suspension all the spermatozoa that accidentally come into contact with the surface of the jelly stick and are unable to penetrate it to any extent.

My observations confirm those of Fol regarding the formation of the cones on the egg's surface. The number of cones depends upon the age of the egg and upon the density of the sperm suspension surrounding it. An overripe egg forms these cones

quickly and in considerable numbers. A fresh mature egg forms only a few cones unless the sperm suspension is very dense.

Fol, however, failed to observe the following: From the tip of each cone a slender filament grows outward piercing the jelly until it reaches the periphery where the trapped spermatozoa are lying. If there be no spermatozoa in the immediate vicinity nothing more happens. If, however, the tip of the filament comes into contact with a spermatozoon the cytoplasm of the tip and that of the sperm head immediately flow together so that the sperm nucleus now lies within the cytoplasm of the egg filament. An extraordinary reaction then takes place. The filament begins to draw back into the egg dragging the spermatozoon along with it. Not only this but all the other filaments projecting from the egg are similarly withdrawn. Apparently, a wave of response is started when a filament fuses with a spermatozoon. This wave must travel down the filament and over the egg.

As the filament with a spermatozoon on its tip shortens, the spermatozoon is pulled deeper and deeper into the jelly and the lashing of its tail becomes more and more restricted. The spermatozoon behaves like an unwilling victim and occasionally, frees itself, especially when other filaments have been slightly ahead in activity and have also secured spermatozoa which they are now pulling in. With the microdissection needle one may free a spermatozoon by breaking the filament to which it is attached. Such a spermatozoon is generally unable to extricate itself from the jelly in which it lies embedded. After a few vibrations of its tail it becomes permanently quiescent.

By the time the filament has dragged the spermatozoon half way through the jelly the base of the cone changes in shape. The convexly rounded border, which gives it the appearance of a rounded nipple, draws in so as to become concave. In doing so it leaves the egg membrane behind and this now becomes plainly visible owing to the space intervening between it and the surface of the cone. By the time the filament is withdrawn so as to bring the sperm head to the summit of the cone, the lifting of the egg membrane has spread from the base of the cone over the egg and is recognized as the fertilization membrane.

When the filament is completely withdrawn into the base of the cone the head of the spermatozoon is taken in with it. The

tail of the spermatozoon remains for a time outside the fertilization membrane. As long as the tail maintains organic continuity with its head it keeps up a feeble oscillatory movement. As the cone recedes into the egg, the strand extending from it to the tail outside the fertilization membrane breaks and the tail then lies motionless. The tail can be seen for several minutes marking the site where the sperm head had gone in.

DISTURBANCES IN MAMMALIAN DEVELOPMENT PRODUCED BY RADIUM EMANATION

BY

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The effect of radium on animal development has been the subject of several researches since the early work of Bohn (1), in 1903, upon the ova and larvæ of the sea-urchin. Experiments on developing nematodes, molluscs, amphibians, fishes, and birds are associated with the names of Perthes (2), P .Hertwig (3), Schaper (4), O. Hertwig (5), and G. Hertwig (6). These investigators report developmental retardations following radiation of the ova and developing embryos. They found a particular susceptibility of the nuclei of the cells and a general slowing up in the developmental processes, especially in the case of the central nervous system. The total disturbances, depending upon the period of development when the radiation was applied, resulted in the formation of monstrosities conforming more or less to a general type.†

Similar experiments concerning the effects of x-rays on development have been conducted by many investigators. After exposure to x-radiation, Perthes (7) noted abnormal cell division and a retardation in the development of the ova of Ascaris megalocephala. Gilman and Baetjer (8), after radiating the ova of Amblystoma, and Baldwin (9), the fertilized ova of frogs, were able to produce a fairly constant type of development defect. Injurious results have followed in all cases where mammals have been exposed to x-radiation. It has been shown that when any particular part of a young animal is exposed to a sufficient amount of radiation, that part fails to reach its normal size and is unable to exercise a full degree of function.

Arrests in development and the production of abnormal types may be induced not only by radio-activity, but by many physical or chemical agents. Abnormal temperature changes, treatment by many chemicals, lack of oxygen supply, or the overabundance of carbon dioxide, etc., have produced marked changes in the developing embryo.

The present experiments are mainly concerned with disturbances in mammalian development, before and after birth, as a result of exposing the embryos of rats, at various times during the prenatal period, to irradiation from radium emanation. The effect on the embryos following radiation of the mother at varying intervals before mating was also determined. These experiments were designed not only to study the factors underlying the production of abnormal types, but through an

†In connection with the above statement, and applying to x-ray treatments as well, the question of dosage is an important one. A survey of the literature shows that there was a very wide range in the severity of the dose employed, and in several cases the experimental settings were inadequately described (Bohn used 'some centigrams' of pure radium bromide for from twenty minutes to two hours). The amount of radium metal used in the investigations that have been mentioned varied from 2 mg. to 35.1 mg., and the time from a few seconds to several hours. The deleterious changes in the animal tissues varied with the amount of radium and the time of exposure.

3

examination of the abnormal to gain a clearer insight into the nature of normal development and differentiation.§

I acknowledge with pleasure my indebtedness to Dr. James Ewing for his aid in the interpretation of the pathological results.

METHODS AND APPARATUS

Two methods were used for applying the radium emanation. In the first method an 'active deposit' was obtained by exposing a definite quantity of common salt to a comparatively large amount of radium emanation, about 500 millicuries were used, or the amount of radium emanation initially equivalent to one-half a gram of radium metal. To the radio-active salt thus produced sufficient water was added to make a physiological solution. The pregnant rats were injected subcutaneously in the shoulder region and intravenously through the caudal vein; 3 to 4 minims constituted the usual dose. Because of the rapid loss of radio-activity of these solutions, the injections were made immediately after the preparation. The details involved in preparing and measuring the doses, as well as the methods for protecting the experimenter, are described elsewhere (10 and 11). The activated solution exhibited all the known phenomena of radium metal itself; alpha, beta, and gamma rays were present, but the greatest physiological effects were probably due to alpha-ray activity. After long experimentation, a dose of 5 millicuries was found to be the maximum applicable to the aims of this experiment. In the second method gamma-ray radiation was applied through the ventral body wall of pregnant rats at nearly full term. A large amount of radium emanation was used, an amount equivalent to $1\frac{1}{2}$ grams of radium metal, filtered by 2 mm. of lead and $\frac{1}{2}$ mm. of silver. The source of emanation was 1 cm. away from the animal. The applicator, called a 'lead tray' in clinical usage, was 6 cm. in diameter and 1.5 cm. high. This was placed in the bottom of a small wire cage, 10 by 13 cm. in diameter and 10 cm. high, and was covered by a thin sheet of cardboard. The animal was placed on this paper immediately above the applicator.

Preliminary tests showed that a dose of about 1300 millicurie hours was sufficient to produce developmental arrests in the embryos without killing the pregnant animals. Doses as high as 2900 mc. hrs., however, were successfully used in some cases. The embryos were killed by ether, and histological material procured at various periods after the treatment. The tissues were fixed in Bouin's solution, cut in serial section, and stained with haematoxylin and eosin.

EXPERIMENTAL RESULTS

Series A. Injections of Radio-Active Solutions.

1. *Subcutaneous Injections After Mating.* Sixty-five full-grown, normal, pregnant rats were treated in this series. They were divided into four groups, each treated at different periods after mating. Ten pregnant females were injected 7 days after mating; twenty-four, 10 to 14 days after; twenty-one, 15 to 17 days after; and ten, 18 to 21 days after mating. Many of the animals were killed at weekly intervals after treatment, although some were allowed to reach full term.

Various degrees of developmental disturbances were noted, as shown in the following groups:

§Dr. J. F. Gudernatsch was a co-investigator with the writer during the year 1919. A preliminary report of the work done with him at that time is given in the Proceedings of the Society for Experimental Biology and Medicine, 1920, vol. 17, p. 183.

1. There was a large number of cases where no embryos developed, in others many began development, but were absorbed or aborted at an early time. The females in which no embryos were found, although they were definitely considered pregnant before treatment, occurred among cases treated soon after mating and in those instances where females were autopsied a considerable time after treatment. Figure 1 shows the remnants of maternal and embryonic structures; from the size of the placentae one can see that the foetuses had reached a fair degree of development before the radiation retarded the normal physiological processes. In one case (fig. 2) a small ovoid sac was found attached to the uterine wall by a thin stalk. This apparently represented the remnants of a former embryo and placenta. Extravasated blood and cell detritus were found in this sac and a great many large cells of an epithelioid nature that probably belonged to the former embryonic syncytium. The wall of this cyst was formed by fibrous connective tissue.

2. Embryos were killed by the treatment, but were removed from the mother and preserved before they were absorbed. These showed various extravasations from the vessels of the subcutaneous connective tissue, within the meningeal sinuses, and mainly along the dorsal midline of the body. Figure 3 shows a typical example of such a lesion which was situated in the mid-dorsal line. The mother of this embryo, No. 1167, was mated on April 22, 1919, injected with 4.9 millicuries on May 7th, and was killed two days later. When the embryo was cut in serial section, it showed that the haematoma in the dorsal subcutaneous tissues had exerted sufficient pressure upon the spinal column to produce at one place a complete dislocation. Microscopical examination of the viscera showed no pathological changes. Not all the foetuses of a litter were affected in the same degree. In one case seven foetuses were found, three showing haemorrhagic lesions, two beginning to macerate, and two in the progess of absorption. This variation in resistance was due either to the higher or lower vitality of the embryos themselves or to the amount of radioactivity which passed the placentae. In another case the foetuses, although injured, were carried to full term, and among a litter of six young, two were apparently normal and four showed haemorrhagic spots on the head, face, and along the dorsal midline of the body.

3. Several young of a single litter showed areas of extravasation and were born alive. Their mother died, however, and foster mothers refused to nurse them.

4. Eight litters gave normal living young. This number is low, because, as previously stated, many pregnant rats were killed by the experimenter at various intervals after treatment. The average number of young per litter was 4.8, as compared with 6.5 per litter for the control rats, but the probable errors indicate that this difference is, very likely, not significant. Only one litter, containing four young, survived a treatment given seven days after mating. Several of the rats of this group, which had apparently escaped the full radium exposure during the uterine period or perhaps they were more resistant to it, when mated inter se produced litters of apparently normal young of normal fertility. The offspring of these animals, about twenty in number, were observed for two generations, but no abnormalities were noted.

II. *Subcutaneous Injections Before Mating.* Seventy-seven females were treated in this group, eleven died as a result of the injec-

Fig. 1. Two well-developed placentae are shown at the left attached to a uterus which has been partly opened. The remnants of embryonic tissue are superimposed on the placentae. At the right is a placenta which has been dissected from the uterus, and shows more clearly the remains of embryonic material, here represented as a lighter area in the upper portion of the drawing. Female mated April 22, 1919, injected May 7th, killed May 6th. Dose = 4.6 mc. (subcutaneous).

Fig. 2. A stalked sac partly dissected from the uterus, showing the remnants of a former embryo and placenta. Female mated April 22nd, injected May 7th, killed May 16th. Dose = 4.8 mc. (subcutaneous).

Fig. 3. This is a dorsal view of a rat embryo, showing a characteristic area of extravasation due to the treatment of the mother during pregnancy. Female mated April 22nd, injected May 7th, killed May 9th, at which time

tion before they were mated, while several were killed at weekly intervals after mating, and some were allowed to continue to full term. Thirty-four animals were injected between 5 and 7 days before mating; seventeen, 10 to 14 days before, and fifteen, 20 days before mating.

Only three litters in this group showed abnormal young. The most interesting was a litter of seven, in which case the female was treated with 4.2 mc., 22 days previous to fertilization, and the foetuses, approximately 16 days old, showed very pronounced areas of extravasation, which in one case (fig. 4) covered a large area on one side of the head and a few small scattered areas on the other side. These areas were not only along the dorsal midline, but also on the lateral surfaces of the body as well (fig. 5). The lesions were much more widely distributed and more variable in size than in the cases recorded under Section I. Although the conditions that produced these results were repeated many times, the above is the only case where positive data were obtained. Usually the female had either been rendered sterile or the young were killed and absorbed during early stages. There were two other cases, however, where young were found with haemorrhagic areas, and these occurred in a group of females that were treated seven days before mating. Female 85 was given a dose of 6.6 mc. on November 7, 1919. It was mated on November 14th, and as three young were born December 11th, fertilization took place about fourteen days after the treatment. Two of the young were apparently normal, but one showed a large haemorrhagic area, which involved most of the right side of the snout, the right eye, and a portion of the lower jaw on that side. This area disappeared after three days. Female 99, injected and mated at the same time with female No. 85, received a dose of 5.6 mc. Five young, three males and two females, were born on December 13th, making the date of fertilization about sixteen days after treatment. One male and one female showed definite haemorrhagic areas on the face. Consideration of these cases will be deferred until later.

Seventeen females following treatment were killed at varying intervals after mating and showed markedly haemorrhagic or cystic ovaries and congested uteri. In these cases radium emanation apparently had either so altered the maternal tissues as to prevent fertilization or development when started was soon followed by the death of the embryo and its absorption. Many nodules were found in the uteri in which it was impossible to differentiate between embryonic and maternal structures.

The remaining females (as previously stated, eleven died between the period of treatment and mating) produced either full-term normal young or young apparently normal at autopsy. Several of these living

seven foetuses were found about fifteen days in development. Two of the litter were macerated and two absorbed. Dose = 4.9 mc. (subcutaneous).

Fig. 4. Areas of extravasation are shown in the two views of this embryo, similar to the condition shown in figure 3, but in this case resulting from treating the mother twenty-two days before fertilization. There are a few small scattered areas over the right side of the head and a large area of extravasation on the left side. Female injected April 22nd, mated May 12th, killed May 30th. Seven foetuses were found, fifteen to sixteen days old. Dose = 4.2 mc. (subcutaneous).

Fig. 5. These are three views of another foetus, a litter mate of the one shown in figure 4, showing the wide distribution of the extravasated areas over both sides and back of the animal. The experimental conditions are the same as for figure 4.

young grew normally and were mated inter se, but produced no abnormal offspring, although observed for two generations.

III. *Intravenous Injections After Mating.* The intravenous injections were primarily planned to act as a check on the series of subcutaneous treatments. The object was to determine the immediate reactions that might occur in the embryo as a result of injecting a comparatively large dose of radio-active solution into the circulation of the pregnant female, and whether these reactions would be similar to those already recorded for the subcutaneous series. The toxic reactions were so prompt and fatal that it was not necessary to treat many animals to settle this point. A typical case in that of female No. 123. This animal, of about nineteen days' pregnancy, was treated with 30 mc. injected directly into the blood stream through the caudal vein. This was six times greater than the usual dose in the first two series. Three young were born dead twenty-four hours later. They showed very definite radium changes, typical of those already recorded for the subcutaneous series. Figure 6 shows a foetus still attached to an apparently normal placenta, but a characteristic area of extravasation was found over a considerable portion of the left side of the head. In figure 7A a dorsal view shows another embryo with two comparatively small haemorrhagic areas along the dorsal midline, and the placenta in this case is also normal. The third foetus in this litter was apparently normal, but the placenta (fig. 7B) had acted in the nature of a 'shock absorber' in protecting the foetus from exposure to the radio-activity, and it was so swollen and completely filled with blood as a result of its injury, that it had the appearance of a large haemorrhagic sac.

Series B. Results from Radiating Nearly Full-Term Pregnant Rats With Gamma-Ray Radiation.

Ten rats were treated at the end of about nineteen days of pregnancy. It was found that exposure to about 1350 mc. hrs. of radium emanation was sufficient to produce very decided changes in the embryo and yet leave the pregnant females sufficiently uninjured to be able to nurse their young and care for them until after the weaning period. When the dose was increased to 3378 mc. hrs., the young were severely injured, and were either killed outright or died two or three days after birth.

The following are the conditions that resulted in the first generation of animals treated in utero with a dose of about 1350 mc. hrs.:

1. The young of each litter were born two or three days after the treatment, alive and apparently normal.

2. About ten days after treatment, about half of each litter became markedly anemic, showed symptoms of diffuse edema, and promptly died. There was an easily recognizable slow development of meningeal and spinal-cord haemorrhages, similar to those already described as a result of treatment by radio-active solutions. A series of these lesions is shown in figures 8, 9 and 10. Figure 8 shows a young rat with the dorsal integument partly dissected away, exposing a typical haemorrhagic area in the region of the frontal lobes. The slow development of this lesion could be easily noted through the thin, transparent scalp. This young was one of several treated in utero with 1350 mc. hrs. of gamma-ray radiation on February 21, 1920. It was born two days later, and died on March 3rd. The young rat shown in figure 9 was a litter mate of the previous animal. It shows the presence of three distinct haemor-

rhagic areas, a small frontal lesion, a fairly extensive one in the occipital region, and a small lesion in the subcutaneous tissues in the thoracic region, near the middorsal line on the left side of the body. This animal also died on March 3rd. A third animal belonging to the same litter is shown in figure 10. Here is seen a still more acute reaction, as shown by the fact that the animal died a day sooner than in the two cases above. There is an extensive area of meningeal haemorrhage which covers most of the dorsal portion of the brain, involving the frontal and occipital regions and the medial area between, as well as a considerable portion of the right temporal area. In addition, a distinct, rounded haemorrhagic lesion may be noted on the reflected skin on the left side of the body. This lesion occurred in the midshoulder region of the back.

The heads of several of the young rats showed marked lateral compression. In one case a haemorrhage so affected the spinal cord as to produce complete paraplegia. The tissues of these animals were studied histologically. Save for the mechanical disturbances produced by the presence of the extravasated areas, the most marked pathological conditions were seen in the liver and intestines. In the first case there was a pronounced fatty degeneration of the hepatic cells, and in the second, a desquamation of the lining cells of the intestinal mucosa.

3. It is interesting to note that the other half of each litter survived the treatment, grew to a normal size, and some animals have lived for over eighteen months. They showed the effects of the late uterine treatment by the following arrests in development:

a. The first pathological condition noted was that the eyes became smaller, the pupils opaque, and there finally was a complete, or nearly complete, closing of the lids and total blindness. This condition was first observed a short time after the eyes had opened. The photographs in figure 11 show three views of a female rat about one year old with typical eye deformities. The upper view shows the entire animal, which had grown to normal size and weight for its age. The left eye was nearly completely closed, as is shown more clearly in the lower right-hand view of the head at a higher magnification. Both pupils were opaque, but, as shown in the illustration, the right eyelids were slightly more opened than those of the other side. The animal was one of a litter treated in utero on March 8, 1920, was born six days later, and the photograph was taken on March 1, 1921. The dose in this case was 2920 mc. hrs. of gamma-ray radiation, which was a dose higher than that usually tolerated.

b. Mating tests showed that both the males and females were completely sterile in the first lots, but subsequently a first-generation female, that had been treated with 1350 mc. hrs., mated with a male similarly treated, gave birth to nine apparently normal young.

c. Before these adult offspring of treated animals were killed for histological examination, their neurological reactions were very carefully studied. The animals, being blind, when startled assumed various defensive attitudes, but save for these reactions their behavior was remarkably normal. There was no ataxia in locomotion or in any of the feeding reactions, auditory acuity was normal, and there was no cutaneous hypoesthesia or other sensory disturbances. Except for blindness, there was nothing to suggest abnormal sensory function.

d. When these animals were autopsied, marked developmental disturbances were noted in the condition of the central nervous system.

9

Fig. 6. There is a large and a small area of extravasation on the head of this foetus. The mother was injected intravenously with 30 mc. of radioactive solution and three young, about full term, were born twenty-four hours later. All were dead. In this case the attached placenta is apparently normal.

Fig. 7. The lower figure (A) shows an embryo with two dorsal head lesions and an apparently normal placenta. This is a litter mate of the animal shown in figure 6. At B is indicated a large haemorrhagic placenta from the third young of this litter, which itself was apparently normal.

Fig. 8. Dorsal view of a young rat with the skin dissected to either side. There is a prominent area of meningeal extravasation in the frontal region. This animal was treated in utero with 1350 mc. hrs. of gamma-ray radiation on February 21st and was born apparently normal on February 23rd. It died on March 3rd.

Fig. 9. Dorsal view of a young rat showing areas of frontal and occipital extravasations which were within the meningeal sinuses. There is a smaller lesion in the left dorsal thoracic region. This is a litter mate of the animal shown in figure 8, and the experimental conditions were identical. Death occurred on March 3rd.

Fig. 10. There is an extensive meningeal extravasation over a considerable portion of the hemispheres, and a haemorrhagic lesion is shown on the reflected skin from the dorsal interscapular region. This is a litter mate of the animals shown in the two preceding figures. Death occurred on March 2nd.

The cerebral hemispheres were greatly reduced in size, and in several cases very little cortical material remained. Those portions of the brain that were ontogenetically older (the archiostriatum and the cerebellum) were apparently normal. The optic tracts were markedly atrophic. Correlated with this disturbance in brain development, the skull was found to be asymmetrical, narrow, thicker than normal, and concave in the frontal region.

Figure 12 shows a dorsal view of a normal, untreated brain of an adult rat, magnified five diameters. In figures 13 and 14 are dorsal and lateral views of a brain of one of the rats which belonged to the same litter as those of section 2 of this series. This animal was treated with 1350 mc. hrs. on February 21, 1920, was born on February 23rd, and was killed December 31, 1920. This was one of the animals which (except for blindness) showed no abnormal neurological reactions. The magnification in figures 13 and 14 is the same as that for the control brain in figure 12. The dorsal view in figure 13 shows an apparently normal cerebellum and normal olfactory lobes, but the part of the brain which represents the rudiments of the hemispheres shows a great lack of development of cortical substance. In a side view of the brain in figure 14, the cortex may be seen to be very thin; indeed, not completely covering what should normally be the frontal, occipital, and lateral aspects of the brain. The remains of the hemispheres do not sufficiently approach each other in the median line to cover the colliculi beneath. In figure 13 the meninges on the left side of the brain have been removed, but on the other side they have been left in place. It was possible in this specimen to see the lateral ventricles through the transparent membranes. Several other brains have been studied which showed various degrees of developmental arrests resulting from radium treatment. In some cases the hemispheres were markedly reduced in size, were widely divergent in the median line, and yet the pallium was complete over the entire surface. In all these cases there was marked optic atrophy. These brains are now being sectioned, and a study of them in greater detail will be the subject of a separate communication.

e. A histological study of the eye showed that the eyeball was reduced to one-fourth the normal diameter. The retina was missing, but traces of the choroid remained as a few scattered pigment cells. The cornea was three times as thick as normal and covered with four or five layers of opaque squamous epithelium. The optic nerve was extremely small, not more than one-third the normal dimensions.

f. The testes of the radiated animals were decidedly atrophic, and a comparison with the normal is shown in the photograph in figure 15. The diameters of the testicle alone (minus the epididymis) of the experimental animal was 14 mm. for the length and 7 mm. for the width, while the control measurements from normal animals of the same age and weight and with the same method of fixation were 21 mm. for the length and 11.5 mm. for the width. The epididymis of the radiated testis was practically missing. A small portion of the tail remained, but the head and body of the epididymis had failed to develop. Histological examination shows that there is little evidence of spermatogenesis. Some tubules seem to contain imperfect spermatoblasts and forming spermatozoa, but the great majority of tubules show complete degeneration and loss of epithelial cells, and contain loose granular material, which in places is calcified. Some spermatic tubules are greatly dilated and filled with granular material. Very few interstitial cells are visible.

The ovary of the radiated animals was reduced to one-fourth or one-fifth the normal size. The graffian follicles were entirely missing. Groups of lutein cells persisted in small numbers, but showed marked hydropic degeneration. Some of the large vessels about the ovary were sclerosed.

g. The liver, kidney, lungs, spleen, and the other organs were examined, but showed no pathological disturbance.

CONTROL GROUP

Pregnant rats of the same stock, the same age and weight, were injected subcutaneously and intravenously with equal amounts of solutions that previously had been strongly radioactive, but were allowed to 'decay,' until they had lost their radio-activity. These experiments gave absolutely negative results. As a control to the gamma-ray experiments, pregnant rats, sisters of the treated animals, were allowed to breed under exactly the same experimental conditions. No abnormal young were observed.

DISCUSSION AND SUMMARY OF RESULTS

It has been shown that when doses of radio-active solutions are injected into an animal marked physiological reactions take place. Large doses produce severe toxemia, resulting in pronounced pathological changes in the various viscera of the white rat (10). A study of metabolic changes in dogs, as determined by urine analysis, showed that, following intravenous injections of such solutions, there were very decided increases in the total nitrogen content of the urine, the urea, creatinine, uric acid, and the total phosphates (12). A prompt reduction occurred in the number of white blood cells of the dog after intravenous injections of these solutions, associated with a marked decrease in the relative percentage of circulating lymphocytes (13). In order to reduce as much as possible the severity of the reaction, very small doses of radio-activity were used in the experiments recorded in this article. But even with comparatively small doses, certain rats treated in utero showed very acute reactions. Many were killed by the treatment and and were absorbed or aborted. Others were found showing pronounced areas of subcutaneous extravasations, mainly situated along the mid-dorsal line of the body and within the meningeal sinuses. This condition was probably due to the destructive action of radium on the endothelium of the blood vessels, as well as a possible increase in blood pressure, as was shown to occur in the dog by Burton-Opitz and Meyer (14) after intravenous injections of very small quantities of radium bromide. A similar reaction of the blood vessels to radiation was previously reported by Halkin (15) for the skin of pigs, and by Danysz (16) for radiated mice. This destructive action of radium on the blood vessels is in line with clinical observations on the usual prompt regression of very vascular tumors (the angiomata, in particular) after exposure to irradiation.

The changes in the rat embryos of this experiment are interesting in so far as they show that a sufficient amount of radio-activity was able to pass the placenta and subsequently affect the developing embryo. This occurred after subcutaneous as well as intravenous injections of the mother. By far the most interesting observation concerned the presence of lesions similar to those described above in rat embryos whose mother was treated with radio-active solutions a considerable time before mating.

Fig. 11. The upper photograph shows an adult rat with eye deformities due to gamma-ray irradiation during the prenatal period. The size and weight are normal for its age. The lower figures show the lateral views of the head at a higher magnification. Both pupils are opaque and the left eyelids are nearly completely closed. Mother treated on March 8, 1920, young were born on March 14th, and photograph was taken on March 1, 1921. Dose = 2920 mc. hrs.
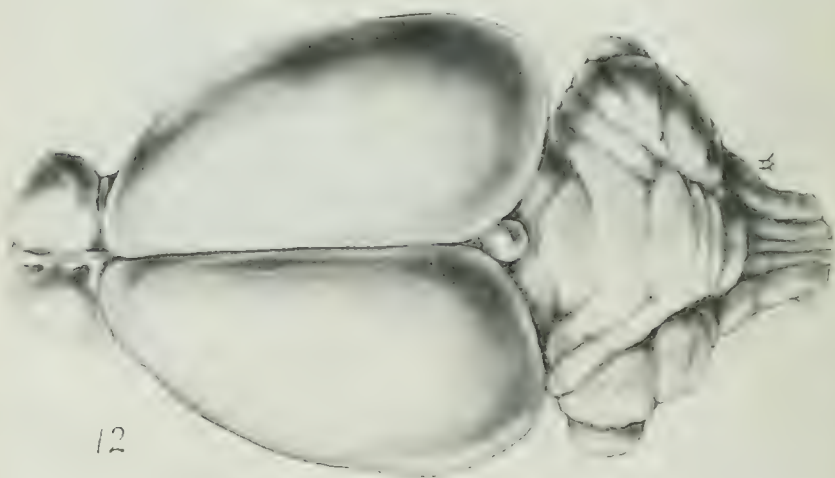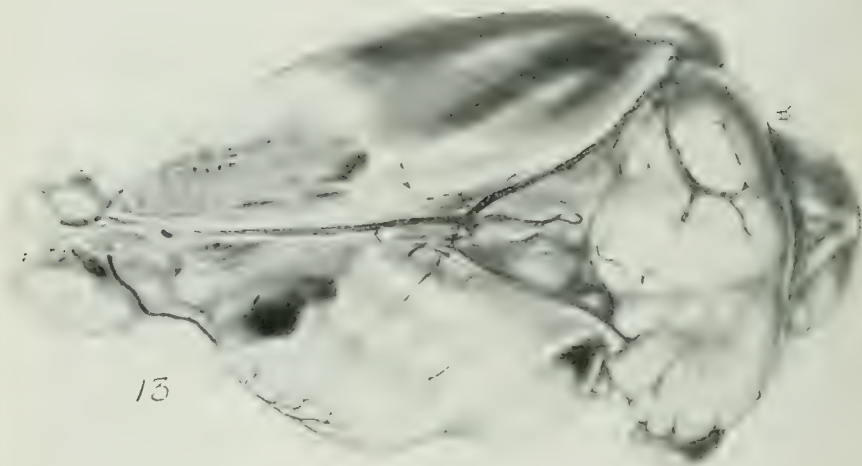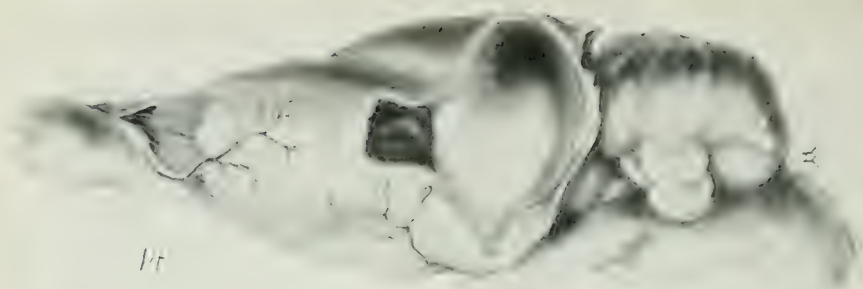
Fig. 12. Dorsal view of a normal untreated brain of an adult rat.
Fig. 13. Dorsal view of the brain of an adult rat showing the marked devel-

14

mental arrest in the formation of the neopallium. The meninges on the left side of the brain are removed. Mother treated with gamma-ray irradiation on February 21, 1920, young were born on February 23rd. Brain from radiated young removed on December 31, 1920. Dose = 1350 mc. hrs. (For further reference see text). The magnification is the same as for the drawing of the normal brain.

Fig. 14. A lateral view of the brain shown in Fig. 13. This shows the thinness of the remains of the cerebral cortex and its total absence in the frontal and occipital regions. (For further reference see text).
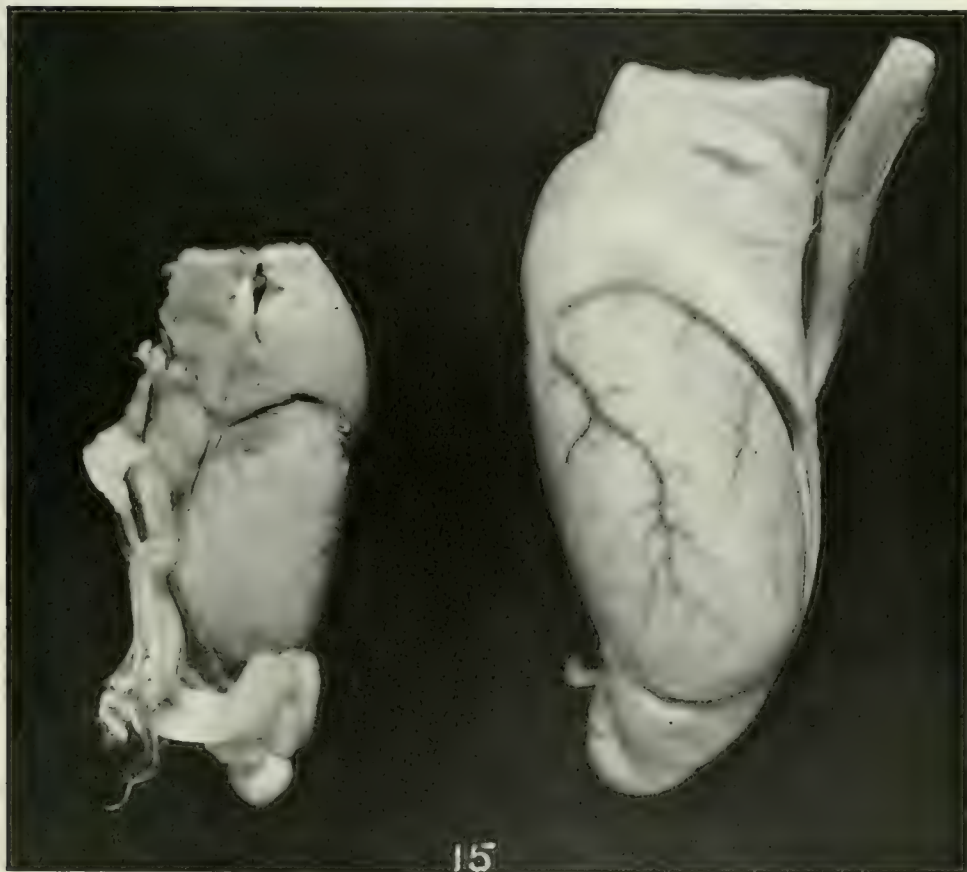
Fig. 15. At the right is shown a normal untreated testicle of a white rat surmounted by a well-developed epididymis, which is partly obscured by fat. At the left is a radiated testis showing considerable atrophy. The single small lobe at the very bottom of the photograph represents the remains of the tail of the epididymis, the head and body of that part being completely missing. The animal was treated in utero on February 21, 1920, was born February 23rd, and was killed December 31, 1920. Dose = 1350 mc. hrs. of gamma-ray irradiation.

The writer has no explanation to account for this phenomenon. It would appear that the treatment of the mother several days previous to conception has lessened the faculty of the later-developing embryo to form proper endothelium of the blood vessels, and the wide distribution of these lesions over the body of the embryo (peculiar to this group of animals) would tend to substantiate this view. One female was injected twenty-two days before fertilization, and since the solutions lose their radio-activity very rapidly (there is about a 50 per cent reduction in the first hour after the preparation) the likelihood of any radio-activity remaining over during this period and affecting the egg at a later critical moment is remote. The amount of radio-activity remaining after twenty-two days, if present at all, should, as determined from physical computation, be infinitesimally small.

The series of intravenous injections again emphasize the specific action of radium emanation in the production of typical areas of subcutaneous extravasations in the developing young, and in addition shows that the placenta may act in the nature of a 'shock absorber' and prevent the embryo from receiving the full effect of the radiation.

We now come to a consideration of the cases wherein pregnant females were treated with external applications of comparatively large doses of gamma-ray radiation. At this time a report is given only for embryos treated towards the end of pregnancy. The writer plans to continue this line of investigation and treat at earlier prenatal periods.

The results emphasize the well-known delayed reaction associated with gamma-ray radiation. There was approximately a ten-day interval following treatment during which no changes were noted in the embryo, and during this period the young animals were born in an apparently normal condition. Acute reactions promptly occurred at the completion of this time, killing half of each litter. The young rats died showing typical radium changes, such as anemia, diffuse edema, and meningeal, spinal-cord, and subcutaneous extravasations. These extravasations were markedly similar to those already described for the series of solution treatments. The liver in these animals showed a fatty degeneration of the hepatic cells similar to the condition reported by Mills (17) after exposing a series of mice to gamma-ray radiation. The only other pathological change noted in these embryos was a desquamation of the lining cells of the intestinal mucosa. This observation is in line with the results emphasized by Hall and Whipple (18) in their experiments on Roentgen-ray intoxication in dogs.

While the animals described above died after showing acute reactions certain of their litter mates (half of the litter) continued to develop apparently normally. This difference in reaction may possibly be due to individual variability or tolerance for the radiation, but it probably can be explained by the fact that certain embryos were slightly farther away from the source of radiation than others, and as the intensity of radiation varies inversely as the square of the distance, even such slight differences in distances that did exist would be sufficient to subject the embryos to a considerable range in intensity of radiation. This is especially important in this case because the source of radiation was only 1 cm. from the body wall of the mother. The quality of radiation, however, remained the same for all the embryos.

It was soon apparent that the animals that lived over the ten-day period had not completely escaped the effect of the radiation, as was shown by a suppression of the full development of the eyes. Eye defects

were noted, such as opaqueness of the pupil, atrophy of the lens, and closing of the lids, which resulted in complete blindness. These animals grew to a normal size, successfully competed with their cage mates for food, and showed absolutely no abnormal neurological condition, except those clearly incident to blindness. At autopsy, in some cases over a year after birth, very decided developmental arrests were noted in the structure of the brain. All grades of such maldevelopment were noted in the condition of the neopallium, from merely a decrease in the size of the hemispheres, which permitted the corpora quadrigemina to be clearly visible from above, to a more marked absence of the cerebral cortex until only a very thin lamina of tissue remained to represent that structure, and there were large areas in the frontal, occipital, and temporal region where no cortex existed at all, so that when the meninges were removed the basal ganglia were clearly seen from without.

This correlation between defects in the development of the eye and the brain has been emphasized by Stockard (19) in his recent paper on developmental rate and structural expression. He states as follows: "The periods of arrest necessary to induce the eye and the brain modifications are so close together or so nearly the same, that one generally finds combinations and mixtures of the defects among the same experimental group of embryos." Again in the same article Stockard has shown that the type of deformity that results from experimental disturbance depends upon the developmental moment at which the interruption occurs. It is significant that the animals of this experiment showed arrests in the development of the neopallial portions of the brain and not in those regions which are ontogenetically older. Apparently the radium emanation, acting towards the end of pregnancy, had affected the development of the brain after the basal ganglia, the cerebellum, and medulla had become fairly well differentiated, and therefore those portions showed no gross changes. But the radium had slowed the developmental rate of the neopallium (which we know is one of the last portions of the brain to differentiate) during its period of active cell proliferation, and that portion of the brain was never able to re-establish its proper rate of development in relation to the other parts of the brain. If the period of treatment had occurred earlier in prenatal existence, other portions of the brain would probably have shown disturbances as well. The writer does not believe that the deformities in the brains of these animals were due to the early production of vascular disturbances later recovered from, but to an actual inhibitory effect of the radiation upon the developing nerve cells. If extravasations had occurred in this group of animals, and were so situated as to affect the development of the cerebral cortex in particular, they probably would have been detected as were even the comparatively small lesions which were associated with the acute reactions. Also, if the effect was largely due to vascular disturbances, from the nature of the radiation employed, one would expect more generalized changes throughout the entire brain. However, on the other hand, Craigie (20), in his recent paper on the relative vascularity of various parts of the central nervous system of the albino rat, suggests that "the vascularization of the more recently evolved centers (of the cortex cerebri) is more susceptible than the more ancient regions to sexual, hereditary or environmental influences."

From a neurological point of view, it is interesting to consider that the animals with practically no cerebral cortex reacted so normally in their ordinary behavior. Except for blindness, there was no other

apparent sensory disturbance, and motor co-ordination appeared perfect. The physiological functions localized in the cerebral cortex of the rat were in these animals apparently transferred to the basal ganglia and other paleokinetic portions of the brain, showing the remarkable degree of compensation possible in the mammalian brain when the disturbing element acts at an early period in its development. In this connection it is worth mentioning that the radium emanation did not produce a sudden traumatic effect, as is normally the case with the experimental production of brain lesions, and in fact, the radium changes were probably prolonged over a considerable period. This condition favored the establishment of compensatory reactions, and exists (as shown by the writer in a recent article (21) even in the case of radium lesions experimentally produced in adult mammalian brains.

The reproductive system completes its development a considerable time after birth, and so it is not at all surprising that these structures should have shown a considerable amount of atrophy due to the developmental arrest during the prenatal period. The ovaries and testes appeared to suffer with equal severity.

An interesting correlative relation was shown by the fact that the other viscera (digestive, excretory, etc.) of the animals that showed marked developmental arrests of the nervous and reproductive systems were apparently normal and the animals grew to an average size. The lungs, liver, kidneys, etc., had differentiated before the physical agent was employed. Further studies with earlier prenatal treatments should throw some light on establishing the critical growth periods of the various embryonic structures.

As a final point, the results of this investigation may be of interest to the clinicians and the laboratory workers who handle large quantities of radium and utilize x-rays. Although the results of this paper deal only with irradiation of the female, there is no reason to believe that the germ cells of the male are more resistant to these destructive agents than those of the female, and, in fact, there is very good experimental evidence to show that spermatozoa of some animals are especially likely to produce abnormal young after exposure to comparatively small quantities of irradiation. Physicians should guard against the possibility of producing developmental arrests such as shown in this article when treating pregnant women, as well as the possibility of altering the human germ cells by irradiation previous to conception.‖

<center>CONCLUSIONS</center>

1. The marked selective action of radium emanation on fast-growing embryonic structures was noted in these experiments.

2. Very decided developmental arrests occurred in the differentiation of the nervous and reproductive systems of mammalian embryos exposed to irradiation towards the end of pregnancy.

3. Experimental animals with greatly reduced, or practically no

‖The writer does not mean to be understood as stating that present-day clinical irradiation treatments produce such effects in the developing young, but it is his personal opinion that such changes are biologically possible. It is not possible to obtain desired information by comparing the amount of exposure that a small mammal can stand with the corresponding dose that a man should tolerate, judging by comparative weights. The small mammal can tolerate very much more radiation in proportion to its weight than a man can.

neopallium, gave apparently normal neurological behavior, except for blindness.

4. Radium emanation, used either in the form of a radio-active solution injected into the adult female, or employed as an external gamma ray radiation, produced marked areas of extravasation in the subcutaneous connective tissue of the developing young. This suggests that the action of radium emanation might be selective upon the endothelium of blood vessels.

5. Extravasations occurred in the developing young of females treated with radio-active solutions a considerable time before fertilization, and suggest that in some way the faculty of the later developing embryos to form proper blood vascular endothelium had been interfered with.

6. The results so far obtained indicate that gamma-ray radiation is a physical agent admirably adapted to the study of experimentally produced developmental arrests in mammalian embryos.

7. When women are subjected to therapeutic irradiation, especially during the early stages of pregnancy, the clinician should be forewarned concerning the possibility of producing very grave disturbances in the developing child.

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A SIMPLE APPARATUS FOR DELICATE INJECTIONS.

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ONE FIGURE

Methods and apparatus for dissecting and injecting cells have been worked upon for a number of years. With the micromanipulator apparatus recently described by Chambers[1] is an injection device consisting of a syringe, a brass tube, and a glass cannula. This device, which is a very simple one, may be easily adapted to injecting the blood vessels of chick embryos or any delicate accurate free-hand injection work under a binocular dissecting microscope. The accompanying illustration shows this device held in a system of ball-and-socket joints which takes the place of the micromanipulator. In addition is a scheme providing for a warm injection mass.

For descriptive purposes this apparatus is divided into three parts:

First, the actual injecting part consists of a syringe[2] (*A*); a hypodermic needle with the tip sealed into one end of a coiled piece of fine extra soft brass tubing[3] (*C*); and into the other end of which (*n*) is sealed a piece of $\frac{1}{8}$ inch glass tubing (*D*). The glass tube (*D*) is drawn out at one end (*p*) to about 1 mm. inside

[1] Chambers, R. New apparatus and methods for the dissection and injection of living cells. Anat. Rec., vol. 24, 1922.

[2] A 2-c.c. Luer syringe is shown in the figure, but any convenient syringe may be used.

[3] The soft brass tubing used here is one with 2 mm. outside diameter. This or others of somewhat larger sizes may be obtained from any wholesale brass dealer or automobile supply house. If the tubing is too stiff, it may be softened by annealing.

diameter, into which the cannula (*E*) is sealed.[4]   The cannula
is made by drawing out a glass capillary which will fit into the
drawn out end of *D* at *p*.   The tip of the cannula is made by
momentarily heating the capillary over a microburner and pulling
it out just as it softens.   This procedure results in a tapering
tip which may be broken off to form a pipette of the size which
suits the operator.   The cannula may be bent at any desired spot
to facilitate insertion into the tissue to be injected.

Second, the support for the injecting device in the figure is
built out of two Leitz dissecting stands.   One complete stand,
and the ball-bearing arm, *d'*, of a second, connected together
with wooden plugs, as shown in the figure, gives ample freedom
for moving the glass tube (*D*) which bears the injecting cannula
(*E*).   The coils of the brass wire allows the cannula to be moved
about while the syringe is held rigid by the clamp, *c'*.

Third, if a warm injection be desired, this is accomplished by
arranging a flow of warm water to heat the injection fluid in the
glass tube (*D*).   This is done by means of a 500-cc. flask (*I*)
fastened on the support (*b'*) and a $1\frac{1}{4}$ inch rubber tube connected
with it at *j*.   The glass tube (*D*) is thrust through the wall of
the tubing in two places as indicated in the figure.   The lower
end of the rubber tube is shunted off to one side of the apparatus
and the outflow of the warm water is regulated by the screw clamp
(*III*).   The water in the flask (*I*) can be heated by an alcohol
lamp or a small Bunsen burner and its temperature regulated
with the aid of a thermometer.   Through the cork (*m*) a small
funnel may be inserted for the addition of more water.   If a
flask with an opening as shown in the figure is not available, a
second glass tube must be thrust through the cork of the flask (*I*)
to allow the entrance of air.   A third tube may be added for the
addition of water.

[4] The sealing may be done with ordinary sealing-wax or de Knotinsky cement,
which can be obtained from any dealer in chemical and physical apparatus.
As the micropipette has to be frequently changed, it must be sealed with a
cement which can be readily softened.   The de Knotinsky cement is admirable
for this purpose.
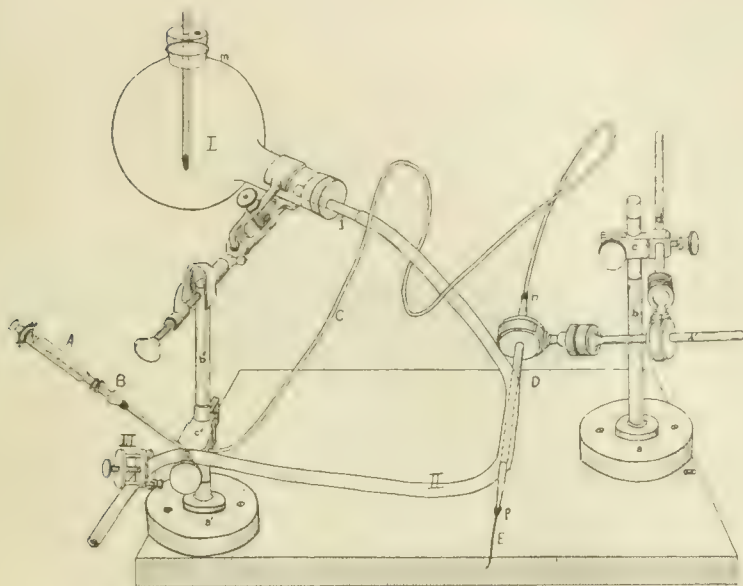
Once this apparatus is set up, the bases a and a' may be screwed down to a board of convenient size, thus making a permanent apparatus of which only the cannula has to be renewed.

To operate the apparatus the brass tube (C) must be filled with water through the syringe before the cannula (E) is sealed at p. The injection mass is drawn up through the cannula, an air space being left between the water and injection mass to prevent diffusion. The tissue or embryo to be injected is placed in position under a binocular dissecting microscope, and the injecting device brought into position with the cannula immediately over the tissue. The operator's fingers control the cannula by gripping D where the rubber tubing covers it.[5]

[5] For other types of apparatus for similar purposes, see Knower, H. McE., A new and sensitive method of injecting the vessels of small embryos, etc., under the microscope. Anat. Rec. vol. 2, no. 5.

Chambers' Micromanipulator for the Isolation of a Single Bacterium

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CHAMBERS' MICROMANIPULATOR FOR THE ISOLA-TION OF A SINGLE BACTERIUM

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As I have been working for several years on the isolation of bacteria with Barber's apparatus and have recently been using Chambers' instrument for the same purpose, Dr. Chambers suggested that I make some comments on the relative merits of the two instruments together with a short review of Barber's isolation method.

The mechanical principles involved in the construction of this instrument are entirely different from those of Barber's pipet holder, while the basic methods of manipulation are essentially the same. There are several noteworthy features in the Chambers' instrument which modify and improve the original Barber technic. These features may be best described under two headings: (1) advantages in construction, and (2) advantages from the use of various accessories to aid in manipulation.

ADVANTAGES IN CONSTRUCTION

The mechanical principles on which the instrument is based are fully described in the preceding article by Chambers.[1] The absence of parts which may loosen by wear and tear renders possible great precision in the manipulations. While excellent work may be done with the Barber pipet holder, the parts wear somewhat after several months' use, giving rise to a certain amount of false motion. For instance, when one desires to move the pipet laterally one may encounter an unexpected vertical motion. The Chambers apparatus used by me had been in use for two years, and in spite of this I was unable to detect any false motion.

A second advantage is that the instrument clamps directly on the stage of the microscope giving much greater rigidity than is possible with the metal flange which has to be attached to the stage of the microscope when Barber's instrument is used. Third, the smaller size of the instrument brings all manipulations closer to the microscope and

eliminates accidental jostling of the pipet holder which may shift the needle out of focus. This compactness also makes it feasible to use a short pipet which is easier to focus and is in less danger of contamination as less of the pipet is exposed.

Barber's instrument possesses no accessories so that all the adjustments, both preliminary and operative, have to be made by means of the same finely threaded screws with a consequent waste of considerable time.

In Chambers' instrument the several accessories are as follows:

1. There is a brass collar (fig. 2') through which the shank of the pipet is inserted before clamping it in the pipet carrier of the instrument. Besides insuring rigidity to the pipet and thus greater accuracy for manipulation, the collar facilitates bringing the pipet into the field of the microscope. It also steadies the pipet as it is being withdrawn from the moist chamber, thus minimizing contamination or injury to the delicate tip.

2. For the vertical manipulation of the pipet there are three different adjusting devices: first, the telescoping pillar for roughly adjusting the pipet to the height of the moist chamber, after which it may be tightly clamped; second, another coarse adjustment operated by a spring screw with which one may bring the pipet into focus, and, third, the fine adjustment of the knurl headed screw (fig. 1), which is used in the actual operation of isolation. The first two devices are for the coarse adjustment and enable one to use moist chambers of practically any height. They aid greatly in the technic of the vertical adjustment, which is the most important one from the bacteriologic point of view.

3. The use of levers on the screws controlling the lateral movements insures great delicacy to the touch and is a decided aid in bringing the pipet directly under the droplet containing the organism, especially when working with the higher power or oil immersion lens where the slightest movement is greatly magnified.

4. The flexible shaft attached to the vertical control screw brings the movement of that screw behind the microscope away from all working parts and close to the fine adjustment of the microscope.

For bacteriologic work it is more convenient to have the moist chamber as Barber originally devised it, viz., with its open end so placed that the pipet may project into it from the left side. This

facilitates the frequent interchange of pipets so necessary in isolating bacteria. For this purpose it is necessary to have the pipet holder attached to the left side of the microscope. This can be done with the form which Chambers designates as right-handed by fastening it on the left side of the microscope stage near the outer corner (fig. 1).

In the preceding article it has been recommended to have the height of the moist chamber equal to the working focal distance of the sub-
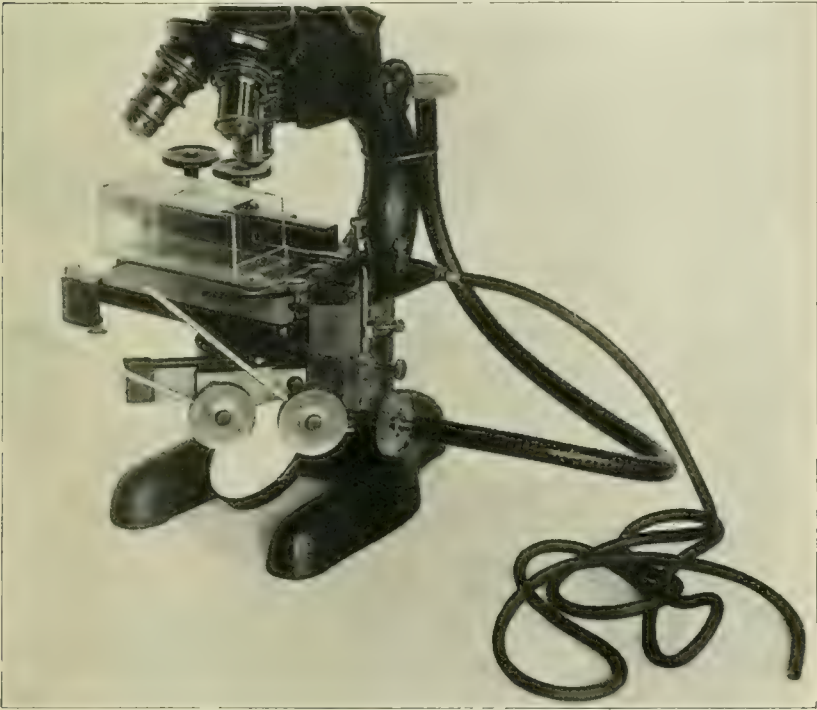
Micromanipulator mounted on the left side of the microscope for isolating bacteria. Note Barber's moist chamber with the coverslip marked with cross lines to aid in locating areas The chamber shown here is higher than necessary.

stage condensor. This limits the height to one which may make the chamber too shallow to be convenient for frequent interchanges of the pipet. I, therefore, use a chamber $\frac{3}{4}$ of an inch high, 1 inch wide and $2\frac{1}{2}$ inches long. It will be noted that this moist chamber is not only deeper but also wider than the one Chambers uses for cytological work.

THE ISOLATION METHOD

For the isolation of a single bacterium one must have the under surface of the coverslip so treated as to hold minute droplets without fear of their spreading or possibly running together. The droplets placed on the coverslip must be slightly hemispheroidal in order that their outlines may be distinct and all parts of it clearly visible. Also, in order to maintain these droplets throughout the operation, the moisture conditions within the chamber must be sufficient to prevent their evaporation and at the same time must not be too great for fear of flooding them.

It is necessary, therefore, to have the surface of the coverslip specially prepared. Barber, after smearing the cleaned coverslips with petrolatum, washes them with soap and water to get rid of the excess of petrolatum. The coverslips are then carefully cleaned with a dry cloth, heated enough to soften the petrolatum and rubbed again while still warm. The aim is to remove as much petrolatum as possible without the use of excessive heat or any fat dissolving reagent other than soap. If an excess of petrolatum is left on the cover, small particles will appear in the droplets and may be mistaken for bacteria. If all petrolatum is removed, the droplets run together and make successful isolation impossible. Instead of soap and water one may use 95% alcohol with equally good results. One must realize that success or failure in isolation work depends on a proper treatment of the coverglass.

The method of procedure for the isolation of a bacterium may be summarized as follows: [2]

1. Prepare a young liquid culture from a subculture not more than 18 hours old.

2. Insert the tip of a needle into a tube of the liquid culture and convert the needle into a pipet by gently rubbing it against the wall of the tube. Then with a rubber tube on its shank suck up a small amount of the culture.
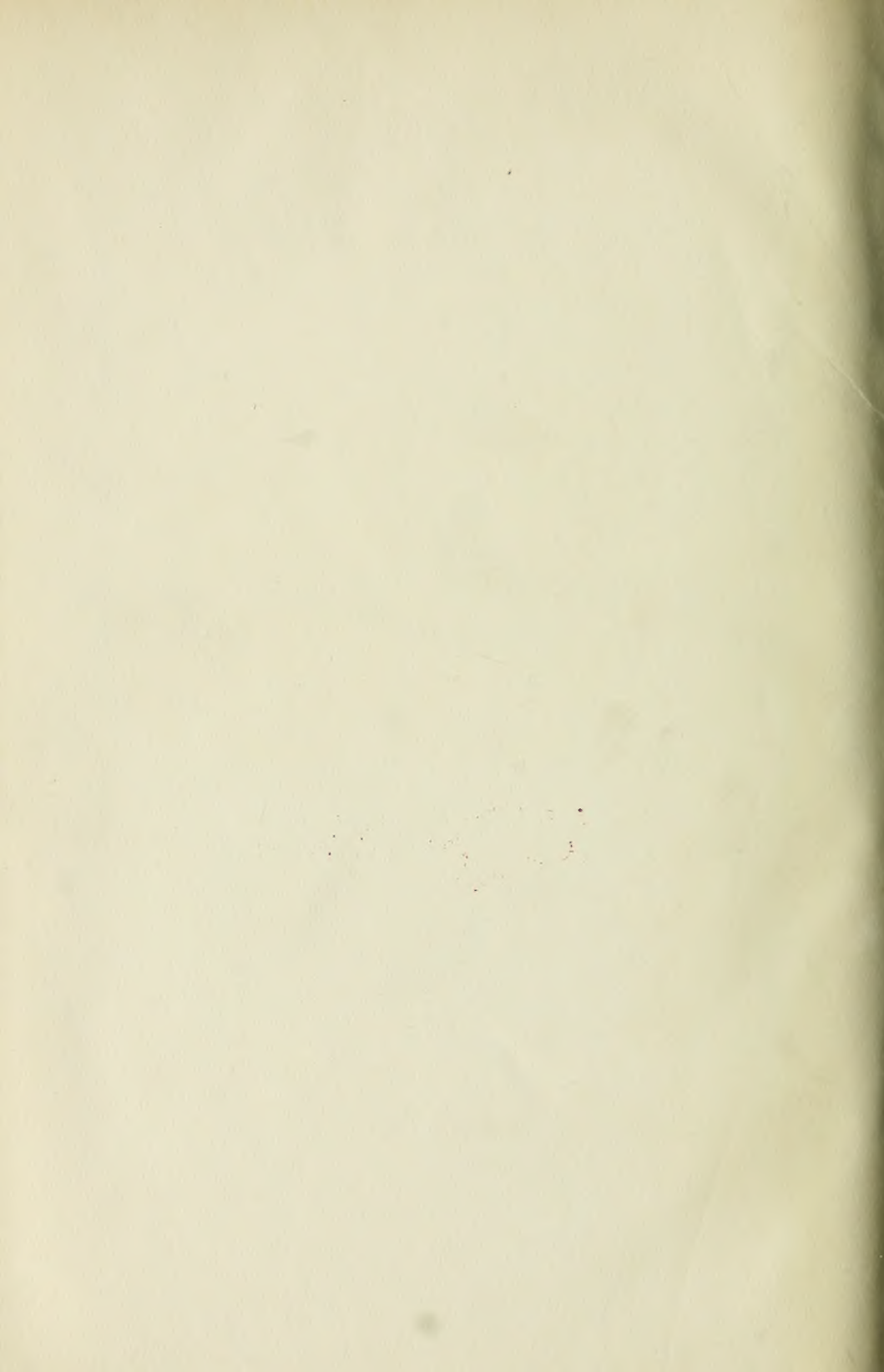
3. Insert the pipet in the brass collar, then clamp it in the pipet holder of the instrument and bring the tip into focus in the center of the microscopic field (see figure). Raise the pipet until its tip touches the undersurface of the coverslip and expel an appreciable droplet. This may have to be diluted with sterile fluid if the culture is too dense.

4. After securing a moderately dilute preparation fill the same or a new pipet to a little below its bend. Lower the pipet, and with the aid of the mechanical stage, bring another portion of the coverslip into view. By alternately raising and lowering the pipet a series of minute droplets will be produced on the coverslip wherever the pipet touches it. The fluid runs out by capillary attraction and needs no blowing. Some of these droplets will be found to contain a single micro-organism.

[2] The method of making the pipets is described in the preceding article by Chambers.

5. Replace this pipet with a new sterile one containing a small amount of sterile liquid medium which must not run below the elbow. This new pipet is now brought directly under a droplet containing a single micro-organism. The pipet is then slowly raised and as soon as it touches the surface the droplet with the contained organism will flow into it. This occurs by capillary attraction and no suction is required.

6. This pipet, which is known to contain only one micro-organism, is carefully removed from the apparatus and its tip inserted into a tube containing a suitable sterile medium. The entire contents of the pipet are now to be expelled by blowing. As an added precaution it is well to break off the tip of the pipet in the culture medium. The blowing may be done by mouth or by a rubber bulb operated either by the hand or the foot.