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La marcha del desarrollo y la expresión estructural. Un estudio experimental de los gemelos, "monstruos dobles" y deformidades aisladas, y la interacción entre los órganos embrionarios durante su origen y desarrollo.

En una larga serie de experimentos el autor ha interrumpido de diversos modos el desarrollo normalmente continuo de un pez, para determinar los efectos de tales modificaciones sobre el desarrollo de las diversas estructuras. Los momentos críticos y pasivos del desarrollo han podido localizarse. La interrupción del desarrollo en un momento crítico frecuentemente es causa de marcadas desviaciones estructurales, cuyo tipo varía según el momento afectado. El autor ha localizado varios momentos definidos durante los cuales las interrupciones provocan un tipo particular de anomalía. Existe un momento temprano durante el cual la interrupción tiende a producir embriones dobles y gemelos; una interferencia un poco mas tardía induce la aparición de varios defectos oftálmicos, y existen otros momentos en los cuales la interrupción origina deformidades en la boca y branquias, anomalías óticas, la supresión de las vesículas cerebrales primarias etc. Todas las reacciones estructurales, incluso los gemelos y embriones dobles son "cesaciones de desarrollo" típicas. Un estudio de los componentes de monstruos dobles ha suministrado ayuda valiosa en la comprensión de la competencia del crecimiento que existe entre las partes que están creciendo y los órganos del embrión. El autor discute la importancia de estos experimentos sobre la poliembrionia en las formas inferiores y la formación de gemelos en el hombre. También analiza el factor tiempo en el desarrollo del individuo y sus diversos órganos.

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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<sup>1</sup>

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THIRTY-TWO TEXT FIGURES AND SIX PLATES

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## 1. INTRODUCTION

In the present contribution an endeavor will be made to analyze the causes and conditions which determine the usual type of structural expression or form. The ordinary progress of embryonic development gives rise to individuals of rather uniform structure, yet there may be numerous slight variations and defects in the structural composition of various organs and parts. Minor defects in structure are found in almost every individual of a group, but rarely do two individuals present exactly the same kind or degree of defects. These facts are readily recognized in a group of human beings where small differences are easily appreciated,

but no doubt the same conditions obtain among vertebrate animals in general, although in lower forms such differences in developmental results are more difficult to detect.

The end products of development differ from one another to varying degrees, slight differences are of little concern and are classed as ordinary variations, but when the same deviations become exaggerated they may be ranked as serious deformities or anomalies. This fact renders the analysis of normal developmental processes and the experimental study of monstrous development one and the same problem. It should be understood that the present study is not intended solely as a contribution to so-called 'teratology,' but is an experimental analysis and consideration of the processes involved in all normal embryonic development and growth. The experimental treatments have in many of the cases caused the formation of well-known monstrous structures, but the point of importance is not the production of the monster, but the simple alterations in the usual course of events which have induced the modified structural expression.

For the past ten years I have claimed that all types of monsters not of hereditary origin are to be interpreted simply as developmental arrests. Such a position has been taken by others (Daresté, '91). However, I propose at this time to present evidence which clearly demonstrates the truth of the claim. By arresting development in very simple ways all types of monsters may be obtained. The experiments have now reached such a degree of exactness that the following propositions may be stated as true, the evidence for which is recorded in the body of this paper.

First, all types of monster, double as well as single, may be caused by one and the same experimental treatment; second, any one type of monster, such as cyclopia, may be produced by a great number of different experimental treatments; third, all effective treatments tend primarily to lower the rate of development, and, fourth, the type of monster induced depends upon the particular developmental moment or moments during which the developmental rate was reduced. Slowing the rate at one

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 developmental 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 consideration 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 inhibiting 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, usually 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 oxygen 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 continuous. That is, development begins with fertilization which is soon followed by cleavage, and then continues without interruption 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 considered 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  $\text{CO}_2$  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 modification 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 seawater 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  $\text{CO}_2$  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<sub>1</sub> was placed in the refrigerator at 5°C., C<sub>2</sub> at 6°C., C<sub>3</sub> at 8°C., C<sub>4</sub> at 9°C., and C<sub>5</sub> 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<sub>1</sub> at 5°C., D<sub>2</sub> at 6°C., and D<sub>3</sub> 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 temperatures 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<sub>1</sub>, 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<sub>2</sub> showed much the same condition as C<sub>1</sub> and were also removed from the refrigerator.

Lot C<sub>3</sub> seemed as completely stopped as the other two and was returned to room temperature.

The members of the C<sub>4</sub> 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<sub>5</sub> 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<sub>1</sub> 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<sub>2</sub> was in a closely similar condition to D<sub>1</sub>, but remained at the reduced temperature.

Lot D<sub>3</sub> had also failed to make noticeable progress during the 45 hours at 8°C., but was allowed to remain at this temperature.

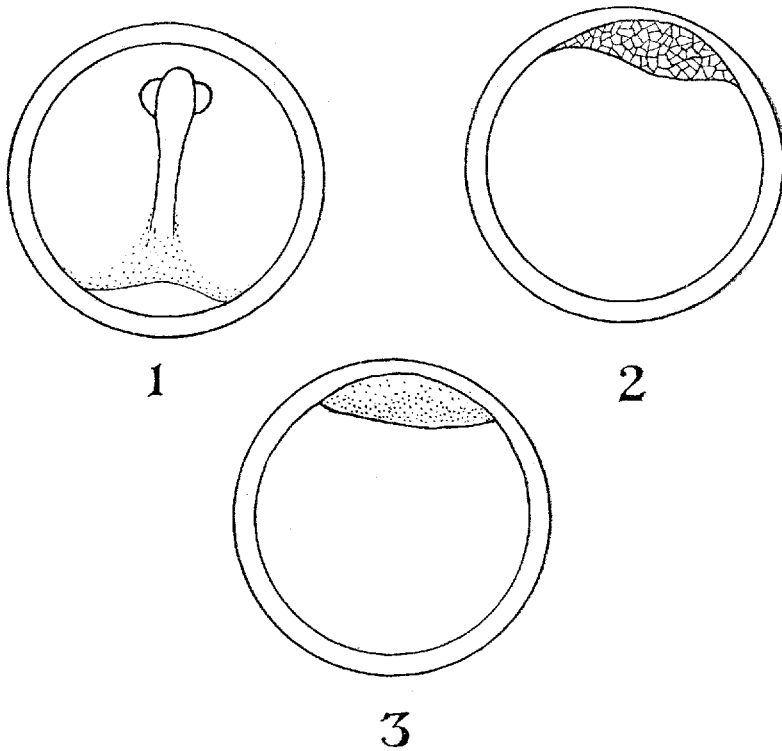


Fig. 1 A control embryo 72 hours old, the body is well outlined and the germ-ring almost completely over the yolk.

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<sub>1</sub>, 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<sub>1</sub> eggs had now actually developed at room temperature for about 47 hours, the first 23 hours after fertilization and the fourth day.

Lot C<sub>2</sub> 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<sub>3</sub> the germ-rings had all descended about half way over the yolk-sphere.

The D series showed somewhat the same response. Lot D<sub>1</sub>, 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<sub>1</sub>, 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<sub>2</sub> were about in the same condition as C<sub>1</sub>.

Lot C<sub>3</sub> seemed on the average a little further along, though closely similar to the two foregoing lots.

Lot C<sub>4</sub>, 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<sub>5</sub> at about 10°C. for four days, were possibly a little further along than C<sub>4</sub>, though in general they showed a similar condition. These were also returned to room temperature.

Lot D<sub>1</sub> 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<sub>2</sub>, 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<sub>3</sub>, 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<sub>3</sub> group a considerable advantage in progress over the D<sub>2</sub> 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<sub>1</sub>, 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<sub>2</sub>, embryos were also in a stage just prior to the heart beat, and the C<sub>3</sub> group was about the same.

Lot C<sub>4</sub> 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<sub>5</sub> group are still further advanced with the optic outpushings prominently shown.

Lot D<sub>1</sub> 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<sub>2</sub>, 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<sub>3</sub> contained long embryos with the optic outpushings just beginning, so these were still ahead of D<sub>2</sub>.

At seven days the control embryos were actively moving and the yolk vessels were now clearly mapped out by the pigmented arrangement.

Lot C<sub>1</sub>, 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<sub>2</sub> showed a good circulation in almost all.

Lot C<sub>3</sub> 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<sub>1</sub> showed practically every specimen normal and strong, apparently just as good as the control, though somewhat behind.

Lot C<sub>2</sub> were in equally as good a condition.

Lot C<sub>3</sub> was much the same as the other two groups.

Lot C<sub>4</sub> also seemed to contain all normal embryos.

Lot C<sub>5</sub> were further advanced than C<sub>4</sub>, 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<sub>1</sub> 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<sub>2</sub> contained specimens further behind in development than the D<sub>1</sub> group, since they remained in the cold longer, but all appeared perfectly normal at this time.

Lot D<sub>3</sub> 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<sub>1</sub> three specimens had not developed and sixty were normal. This is as good a record as is usually found under ordinary conditions. Lot C<sub>2</sub> contained about 100 specimens, which were all living and normal. Lot C<sub>3</sub> had about the same number in similar conditions. Lot C<sub>4</sub> 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<sub>5</sub> 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<sub>1</sub>, 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<sub>2</sub> lot were all normal, and so was the D<sub>3</sub> 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<sub>1</sub> contained a majority hatched and all seemed normal.

In lot C<sub>2</sub> there were not quite as many hatched, but all were in good condition.

Lot C<sub>3</sub> 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<sub>4</sub> had remained longer in the refrigerator, 4 days, and at this time none had hatched, though they seemed fully ready. In lot C<sub>5</sub> also none had hatched.

Lot D<sub>1</sub> contained a majority hatched, almost as large a proportion as the control. These had remained in the cold only two days. Lots D<sub>2</sub> and D<sub>3</sub> 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<sub>4</sub> had hatched. In lots D<sub>2</sub> and D<sub>3</sub> many had now hatched, so these are not very much later than the control in spite of their 4 days' arrest.

In lot C<sub>5</sub> 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<sub>1</sub> had 12 unhatched and 50 hatched. Lot C<sub>2</sub> contained 18 unhatched and about 80 hatched. Lot C<sub>3</sub> had 29 unhatched, 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<sub>4</sub> lot had hatched, and all seemed normal, though they remained in the refrigerator twice as long as C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub> had.

Lot C<sub>5</sub> also showed about half of the specimens hatched.

Lot D<sub>1</sub> had 7 unhatched and about 60 hatched, all of them seemed normal.

Lot D<sub>2</sub> contained 29 unhatched and about 40 hatched, all of which were normal.

Lot D<sub>3</sub> showed 20 unhatched and about 30 hatched.

When 25 days old, every egg in the control had hatched.

Lot C<sub>1</sub>, 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<sub>2</sub>, every specimen hatched. In lot C<sub>3</sub> 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<sub>4</sub> 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<sub>5</sub> contained 10 unhatched, one of which was abnormal, the others were all normal.

Lot D<sub>1</sub>, only one unhatched, all seem fine.

Lot D<sub>2</sub> contained 2 unhatched, and lot D<sub>3</sub> 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<sub>4.1</sub>. 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<sub>4.2</sub> 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 unhatched. 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  $F_2$  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  $F_2$  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  $F_2$  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  $F_2$  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  $F_2$  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 deformities 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 indicate. 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 necessarily 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 usually 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 particular 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 this locality.

Lots B<sub>1</sub> and B<sub>2</sub>, 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<sub>3</sub> 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<sub>1</sub>, 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<sub>2</sub>, at 7°C., was in much the same condition as lot B<sub>1</sub>, 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<sub>3</sub>, 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<sub>1</sub>, 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 B<sub>2</sub> 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.

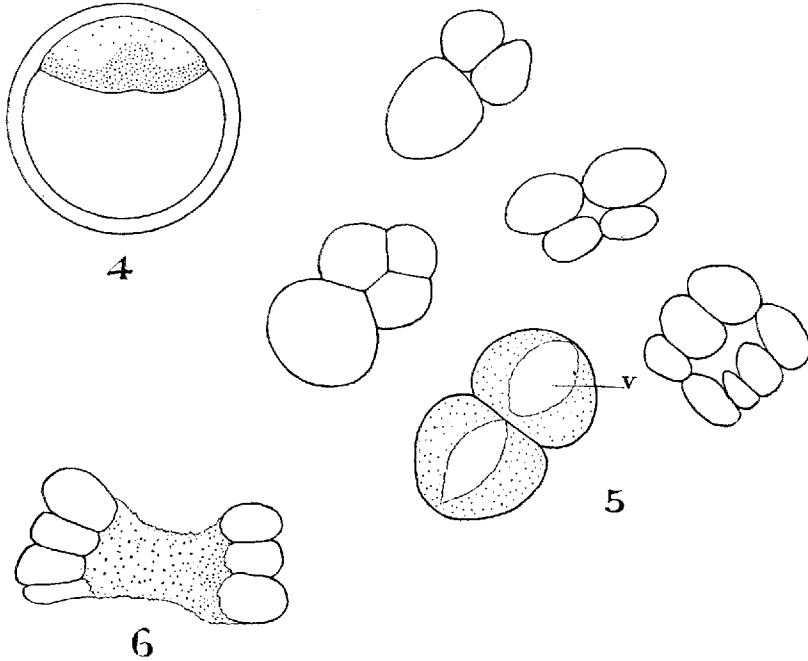


Fig. 4 A control embryo 48 hours old, the germ-ring only one-quarter over the yolk, far behind the usual stage on account of the cool season.

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 B<sub>3</sub> 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<sub>1</sub> 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<sub>2</sub>, 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<sub>3</sub>, after 24 hours at room temperature, were in an even worse condition than those in B<sub>2</sub>, 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<sub>1</sub> and B<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>. 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<sub>1</sub>, after 3 days at room temperature, con-

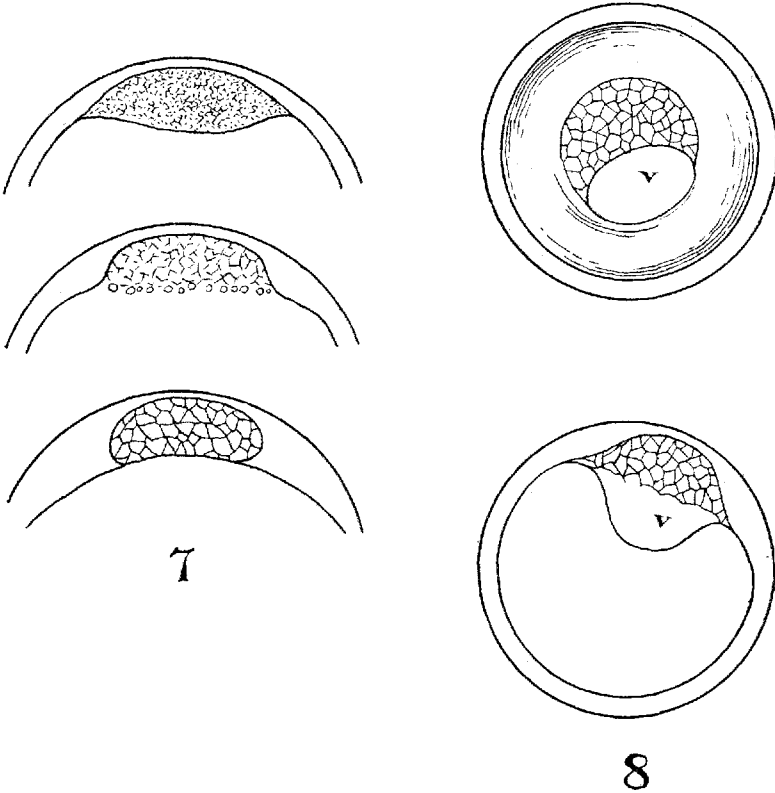


Fig. 7 Three specimens 4 days old, having been 24 hours at room temperature following a stay of 70 hours at 7°C. The upper outline shows a disc-like cap flattened down on the yolk-sphere; the middle one, a high segmentation cap; and the bottom specimen has a cell mass comparable to a normal 12-hour stage.

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<sub>2</sub> 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<sub>1</sub>.

The Lot B<sub>3</sub> 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 B<sub>1</sub> 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 B<sub>2</sub>, 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 B<sub>3</sub> 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 B<sub>1</sub> 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 B<sub>2</sub> showed no circulation, many eggs did not develop, and almost all were readily seen to be abnormal. The lot B<sub>3</sub> 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 B<sub>1</sub> 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 B<sub>2</sub> 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 B<sub>3</sub> contained a few eggs with early cell-masses similar to those in lot B<sub>2</sub>. 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<sub>3</sub> 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<sub>1</sub> 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<sub>2</sub> 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<sub>3</sub> 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<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> 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<sub>1</sub> none had hatched and several more had died. In B<sub>2</sub> none had hatched and a few more also had died. In B<sub>3</sub> 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 21 days old, in lot B<sub>1</sub> 2 more had died and 3 had hatched, in B<sub>2</sub> 2 had hatched, and in B<sub>3</sub> many had hatched.

The control at 22 days old showed 47 hatched and 18 unhatched, although all were normal. In lot B<sub>1</sub> 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<sub>2</sub> 4 had hatched and 36 were unhatched, of these 11 were grossly deformed and 25 seemed normal in structure. In lot B<sub>3</sub> 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<sub>1</sub> there were 35 hatched and 20 unhatched. Lot B<sub>2</sub> contained 27 hatched and 12 unhatched. In lot B<sub>3</sub> 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<sub>1</sub> 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<sub>2</sub> 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<sub>3</sub> 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<sub>1</sub> lot finally had 9 specimens which were unable to hatch, all of them were deformed.

Lot B<sub>2</sub> showed 6 unable to hatch, all deformed and without a blood circulation. In lot B<sub>3</sub> 4 failed to hatch. It must be recognized that a great many specimens in each of the lots B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> 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<sub>2</sub> 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 hardest 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 c'

*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<sub>1</sub> and C<sub>1</sub> at 5°C., B<sub>2</sub> at 7°C., and B<sub>3</sub> 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<sub>1</sub> and C<sub>1</sub> 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<sub>1</sub> lot almost all were 8 cells. In many the 8 cells were arranged into two groups of four (fig. 9).

Lot B<sub>2</sub> 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<sub>3</sub> 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 out-pushing had not yet arisen.

In lots B<sub>1</sub> and C<sub>1</sub> 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<sub>2</sub> 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<sub>3</sub> 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

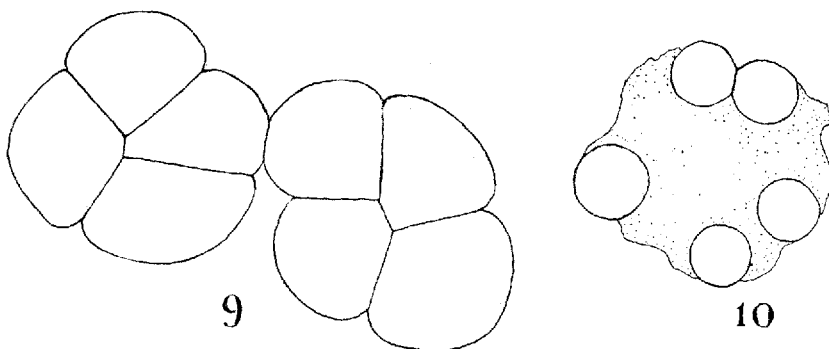


Fig. 9 A cell group 24 hours old, having been in a temperature of 5°C. since three hours after fertilization. The 8-cells are peculiarly arranged into two groups of 4 each, such specimens may give rise to ordinary single individuals.

Fig. 10 A large unsegmented protoplasmic mass with blastomeres around the periphery. A frequent specimen in lot B<sub>2</sub>, 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<sub>1</sub>, 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<sub>1</sub> 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<sub>2</sub>, 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<sub>3</sub>, 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<sub>1</sub> 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<sub>2</sub> were in very nearly the same condition as B<sub>1</sub>.

In B<sub>3</sub> the great majority were developing and the germ-rings were here also about half over the yolk-sphere.

Lot C<sub>1</sub> 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<sub>1</sub> 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<sub>2</sub> 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<sub>3</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>2</sub> 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<sub>3</sub> seemed normal and well, although far behind the control.

In lot C<sub>1</sub> 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<sub>2</sub> 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<sub>2</sub> and 3 in lot B<sub>3</sub> had hatched, though none in B<sub>1</sub> and C<sub>1</sub>.

At 18 days old, the controls still had a few unhatched.

In lots B<sub>1</sub> 6 were hatched and 2 were not; in B<sub>2</sub> 21 were hatched and 20 were unhatched; in B<sub>3</sub> 33 were hatched and 27 were not; in C<sub>1</sub> 13 were and 12 were not hatched.

When 24 days old, lot B<sub>1</sub> contained one badly abnormal specimen still unhatched. In lot B<sub>2</sub> 17 were still unhatched, 5 of these were grossly deformed. In lot B<sub>3</sub> 12 were unhatched, though seemingly normal in structure. These were all far behind the control in time and manner of hatching. In lot C<sub>1</sub> 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  $9^{\circ}\text{C}$ . 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, bifid, 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 development and its later progress. An egg developing very slowly but still continuing the process during the early cleavage stages apparently 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, development 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 cleavage 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 contain 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<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub> 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<sub>4</sub> and C<sub>5</sub> 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<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>, 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.

*e. 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 bifid 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 irregularly through them. Along with these variously defective individuals 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.) during 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 progress 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 MONSTERS' BY AN EARLY ARREST OF THE DEVELOPMENTAL RATE

One of the earliest accomplishments in experimental embryology 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 produced by separating the two primary blastomeres so that they were no longer in their usual intimate relation, each then developed 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 cohesion 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 operandi* for a well-known natural phenomenon, has undoubtedly held back our real understanding of the phenomenon and strikingly 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 the 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 experimental 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 fourteen 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 development 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 gastrulation, but just here the degree of energy necessary for normal developmental processes is insufficient and a single embryonic bud is not formed with that normal rate of growth which suppresses 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 somewhat 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<sub>1</sub> and A<sub>2</sub>, 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  $A_1$  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  $A_2$  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  $A_1$  and  $A_2$  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<sub>1</sub> and even the 2.3 per cent of doubleness in group A<sub>2</sub> 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<sub>1</sub> 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<sub>1</sub> 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

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<sub>1</sub> lot, after being out of the refrigerator for 24 hours, had high segmentation 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<sub>1</sub> 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<sub>1</sub> group were carefully surveyed for deformities when 9 days old. Four eggs had yolk-sacs containing blood-cells and chromatophores, 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 specimens. 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 temperature 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  $\text{CO}_2$  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 actually 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 separate 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 occurred 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 somewhat 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 apparently 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 unusual 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-

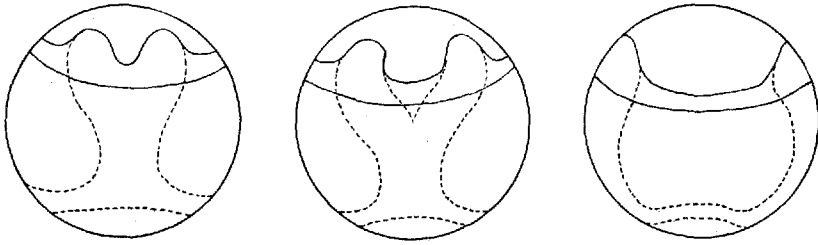
pletely developed twins attached to the common yolk-sac. It will be noted at once that each of the final twin individuals is equally as large and perfect in form as is the single specimen at the beginning of the series. This fact is of importance in showing that up to this stage of development and growth 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  $5^{\circ}$  to  $10^{\circ}$  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  $90^{\circ}$  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  $90^{\circ}$  and on up to  $180^{\circ}$ , 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  $180^\circ$  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  $20^\circ$  apart. When the germ-ring has descended further over the yolk-sphere, the dotted line indicates how the two embryonic



# 11

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  $90^\circ$  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  $90^\circ$  apart, and the resulting duplicity extends throughout the upper half of the body. In the figure on the right the embryonic shields are  $180^\circ$  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  $180^\circ$  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 embryonic 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  $180^\circ$  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), Burkhardt, Daresté, 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 blastoderm 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 process 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 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 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.

*e. '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 separate-ness 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.

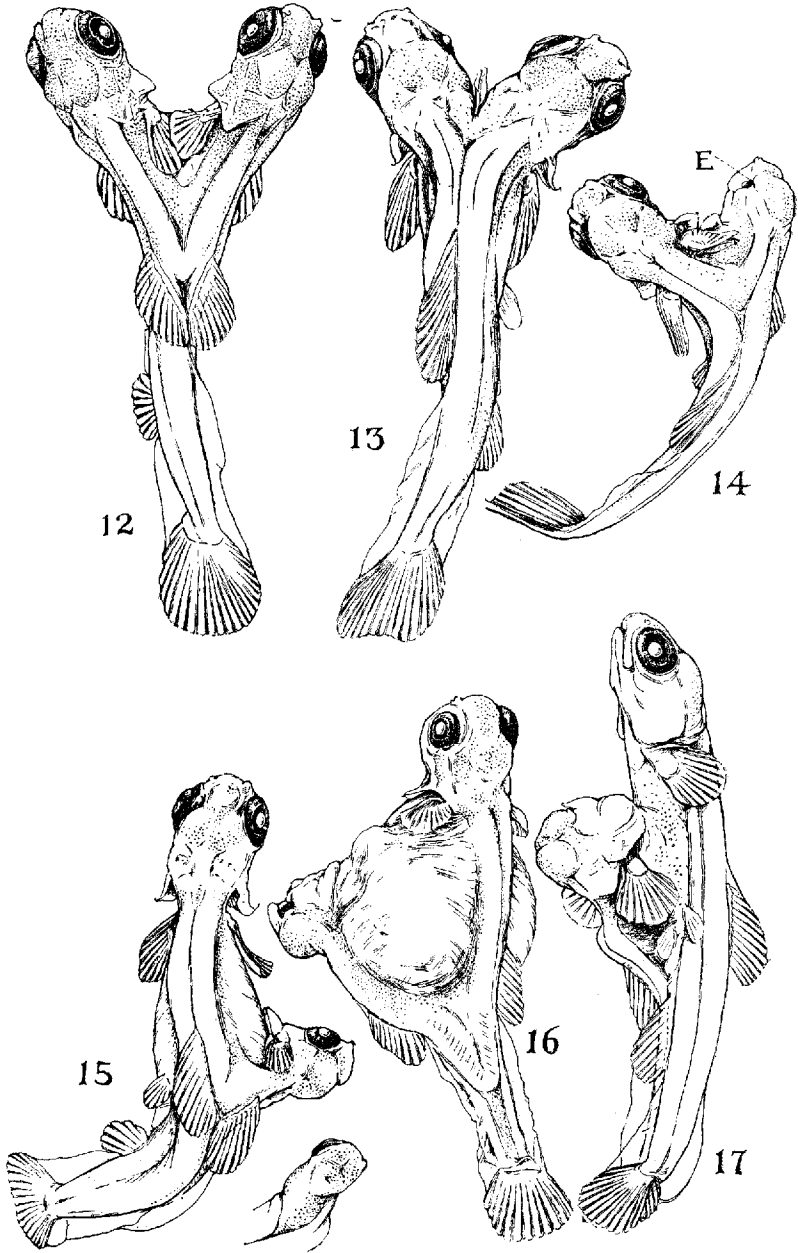
##### *a. Double individuals with identical or equal-size components*

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.

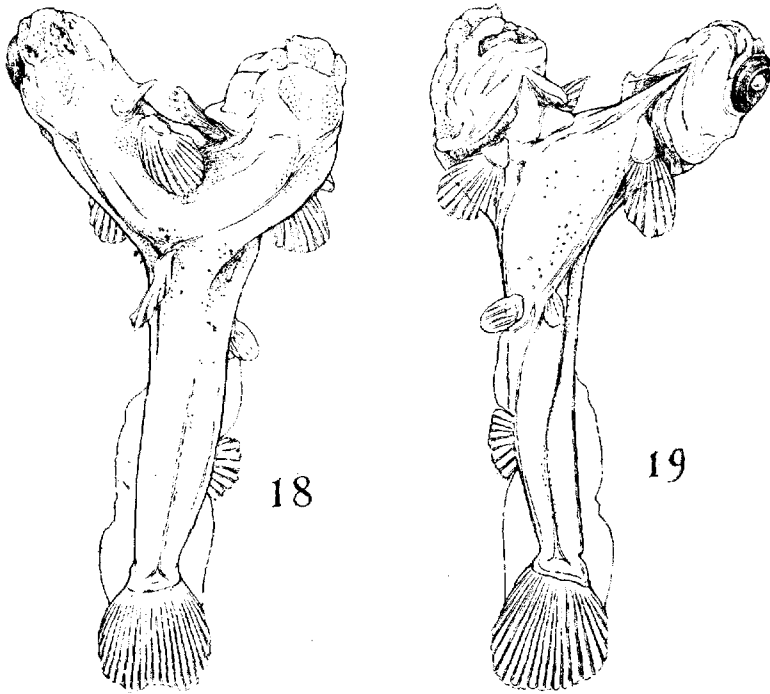
Finally, figure 17 shows only an amorphous mass representing the smaller head on a perfectly normal larger component. This head mass contained no ophthalmic structures at all, the brain was entirely distorted and the mouth was completely absent, with the gill structures greatly deformed. Behind this 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 uncommon 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.

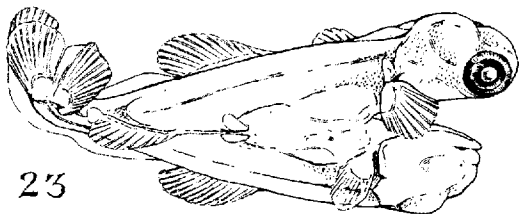
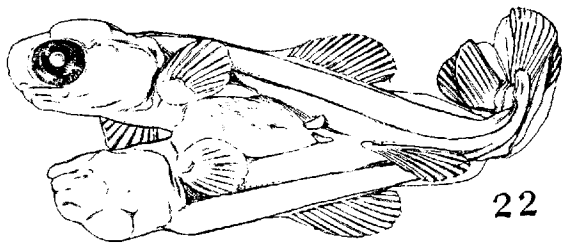
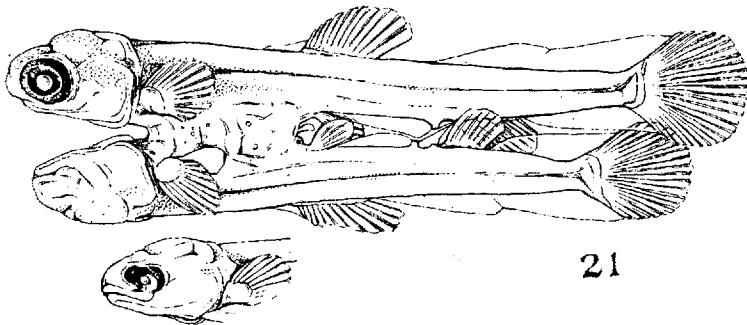
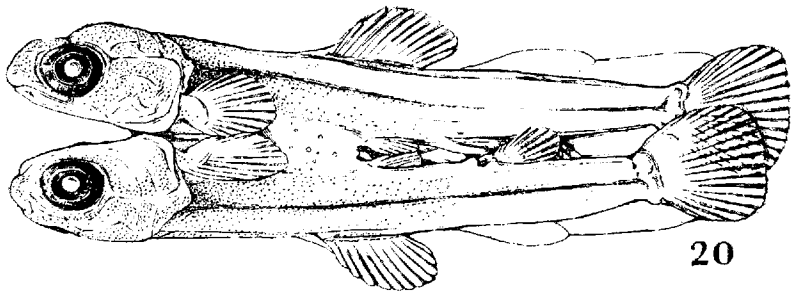
Two views of another double individual are illustrated by figures 22 and 23. The larger component is perfectly normal except for the fact that its tail is somewhat unusually bent. The smaller component is completely anophthalmic and its brain presents a very abnormal contour.

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



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.

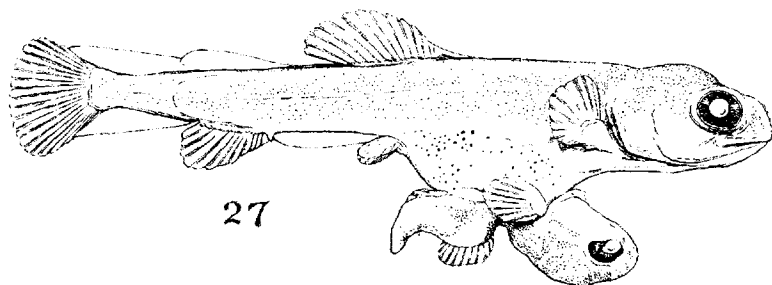
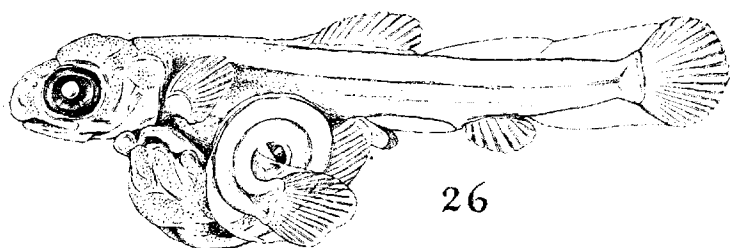
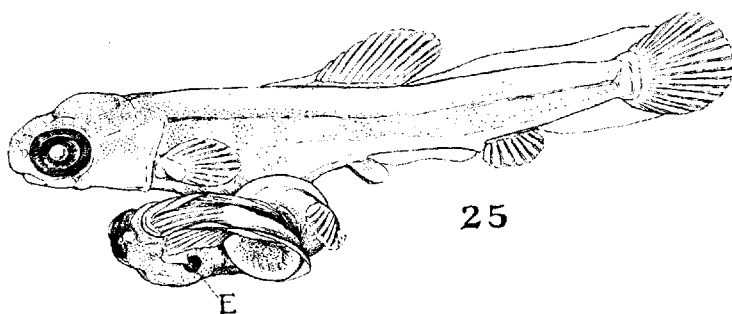
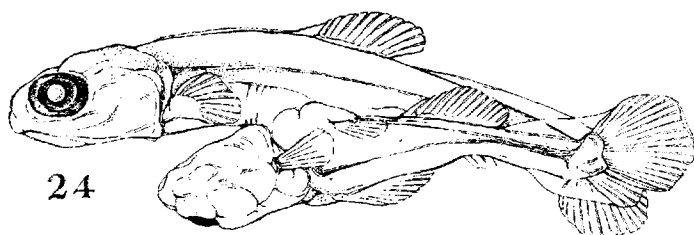
In connection with such probabilities the condition of situs inversus viscerum is of interest. Morrill ('19) has found in an examination of certain of these double fish that a reverse arrangement of the viscera occurs in one of the components with a far greater frequency than has ever been known to occur 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.



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 frequency 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 developmental processes of the two. And, further, since the malformations 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 presenting 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  $180^{\circ}$

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

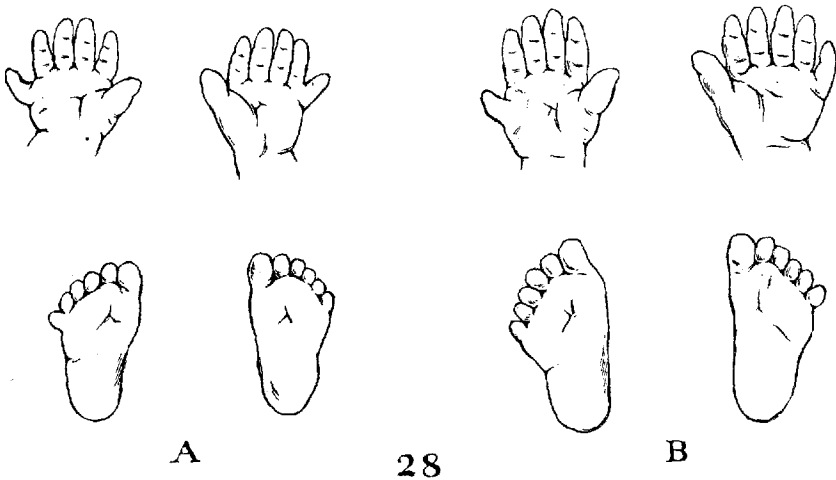


Fig. 28 Drawings of the ventral surfaces of the hands and feet of the identical human twins shown in plate 4. A, from one individual, and, B, from the other. The four hands are all polydactylous, having an accessory finger on the ulnar side, and the four feet are similarly polydactylous, all having an 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  $6\frac{1}{2}$  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 duplicities 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 malformation 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 condition presented by the combination of a larger normal twin developing in actual union with variously deformed lesser individuals. 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 component 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 collateral 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.

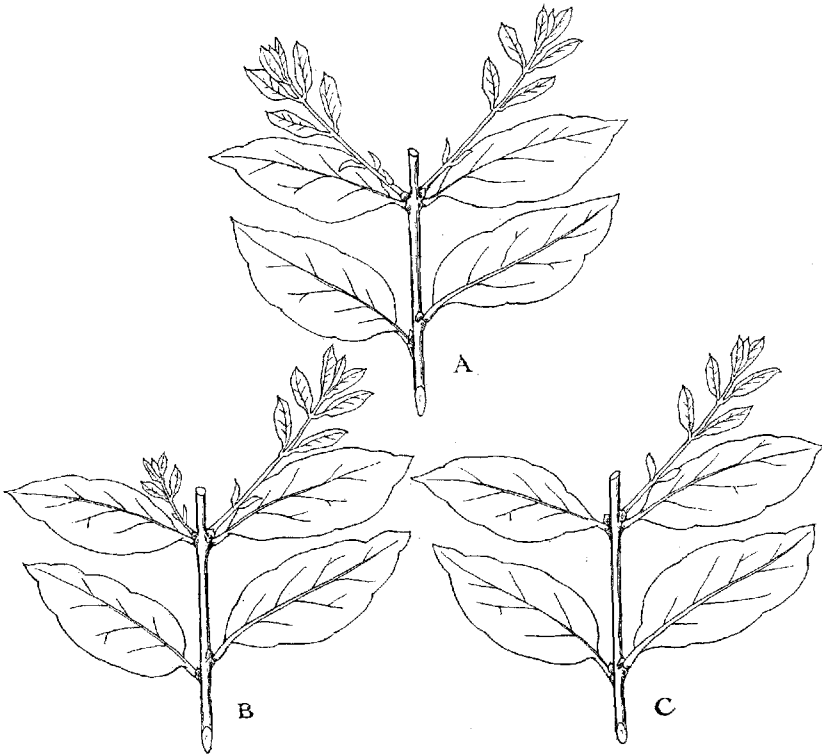
There must, however, be some sort of competition between the two components other than a competition for the appropriation of ordinary yolk material. Much evidence suggests that an interaction exists between the components similar to, if not identical with, the interaction between two plant buds growing 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.

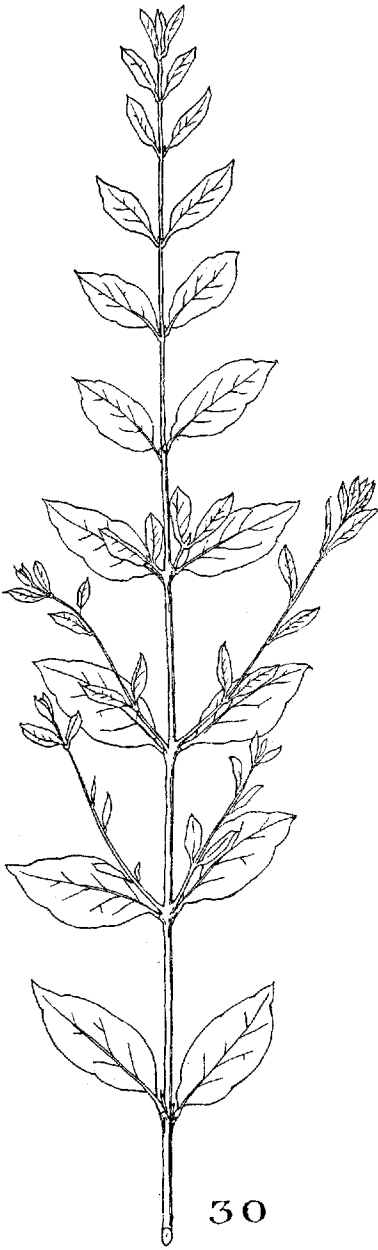
The two embryos developing from a single blastoderm compete in a comparable way, and the results of the competition are also similar to the case of the plant buds. In the present state of our knowledge, it is impossible to say what the primary cause is that gives one of the growing parts an advantage 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.



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 anomalies, 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 membranes containing a nodular embryo; fifth, of cylindrical embryos; 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. Unquestionably, all members of such a series have suffered developmental arrests of the severest types. In some cases the arrest has come at an early stage and been followed by a disorganization 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 developmental 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, monophthalmia, 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.

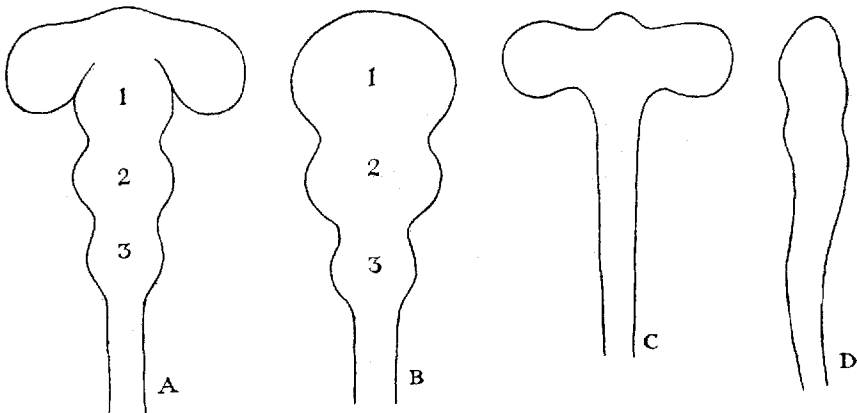
Specimens have again been recorded from my experiments, and these also may be induced in a great number of ways, showing either two poorly formed eyes, cyclopia, or anophthalmia, accompanied by a narrow tubular brain. Here the arrest or slowing in developmental rate has affected the optic outgrowths 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; Spemann, 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  $K_3FeCy_6$  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

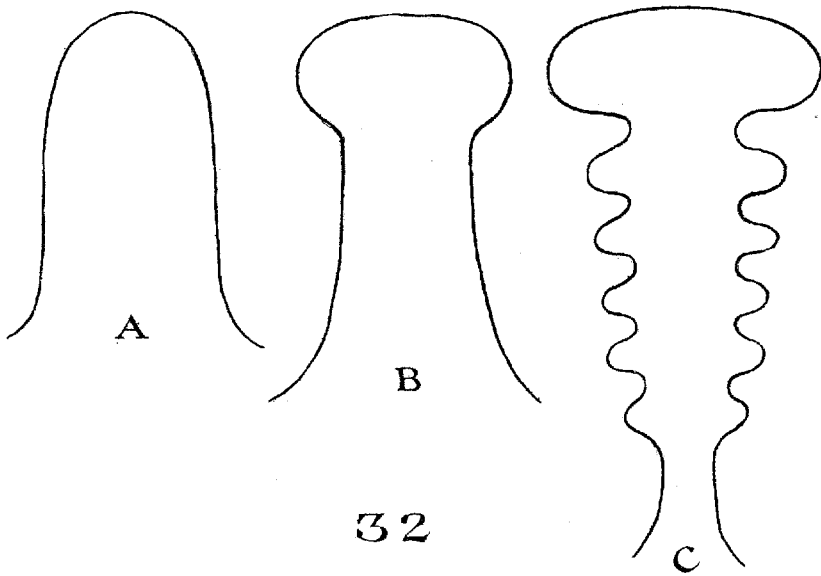


Fig. 32 A series of outlines indicating the primary linear, or cephalad, growth of the foregut, and the subsequent lateral branches or outgrowths from it. A, outlines the simple forward growths of entoderm to form the foregut. B, lateral outgrowths have begun from the forward end to form the mandibular 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-

finer 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 germinating 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 anomalies 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 genitalia, etc. It is evident that such anomalies could not occur unless there was a certain moment of specific and peculiar susceptibility on the part of each organ during which any unfavorable 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 discarded. A strong point of evidence is the fact that a typical defect of one organ can be induced by a great number of treatments, 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 substance used. As repeatedly shown, the primary effect in all instances is simply a slowing in the rate of development.

*c. Developmental rate and postnatal changes*

The relations between the rate of growth and particular developmental 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 diplopaga 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 Wilder'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 individual 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 experimentally produced by an identical physical change in the environment, 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 during which the slowing in rate was effective.

#### 10. THE NECESSITY OF A CONTROLLED OR REGULATED ENVIRONMENT 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 vertebrate embryo, and sudden lowering of the surrounding temperature 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 development 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 existence, 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 condition.

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, however, 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 cancerous 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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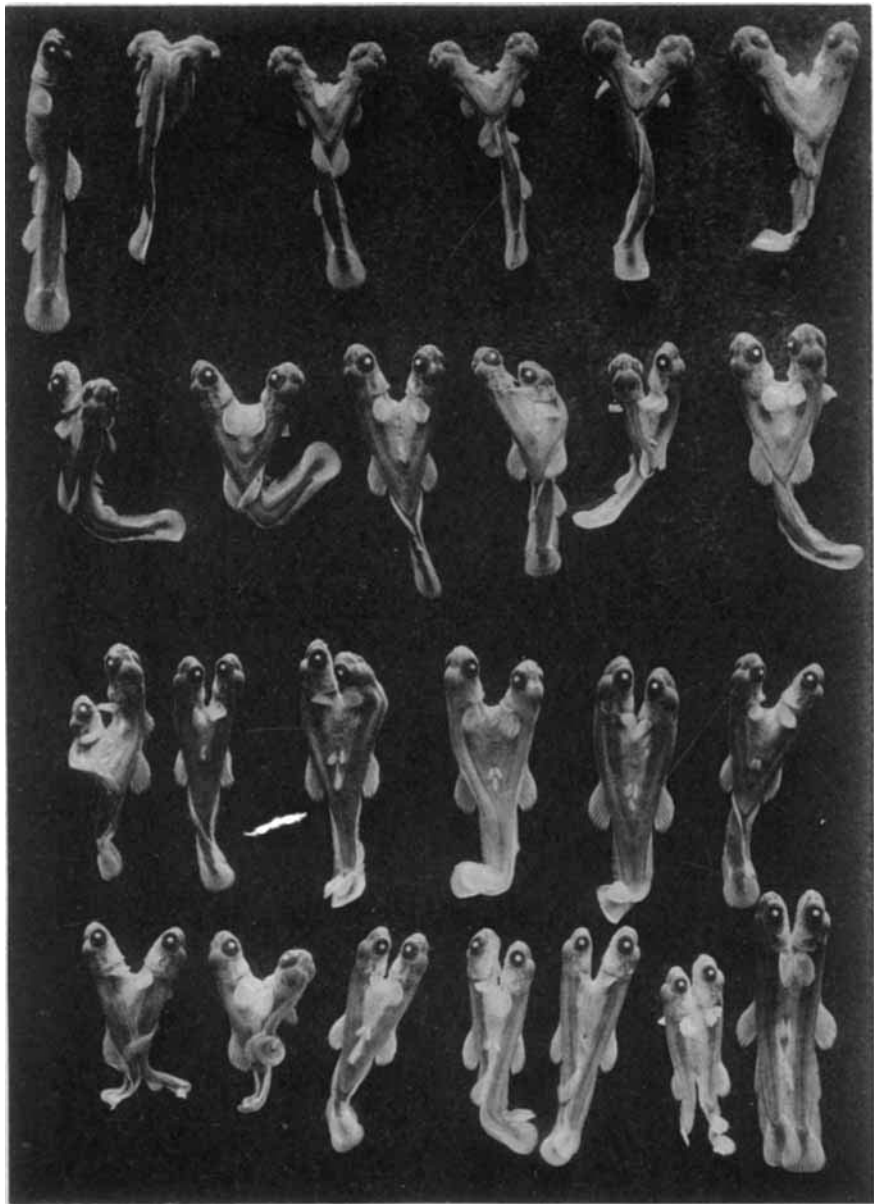
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## PLATE I

### 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.

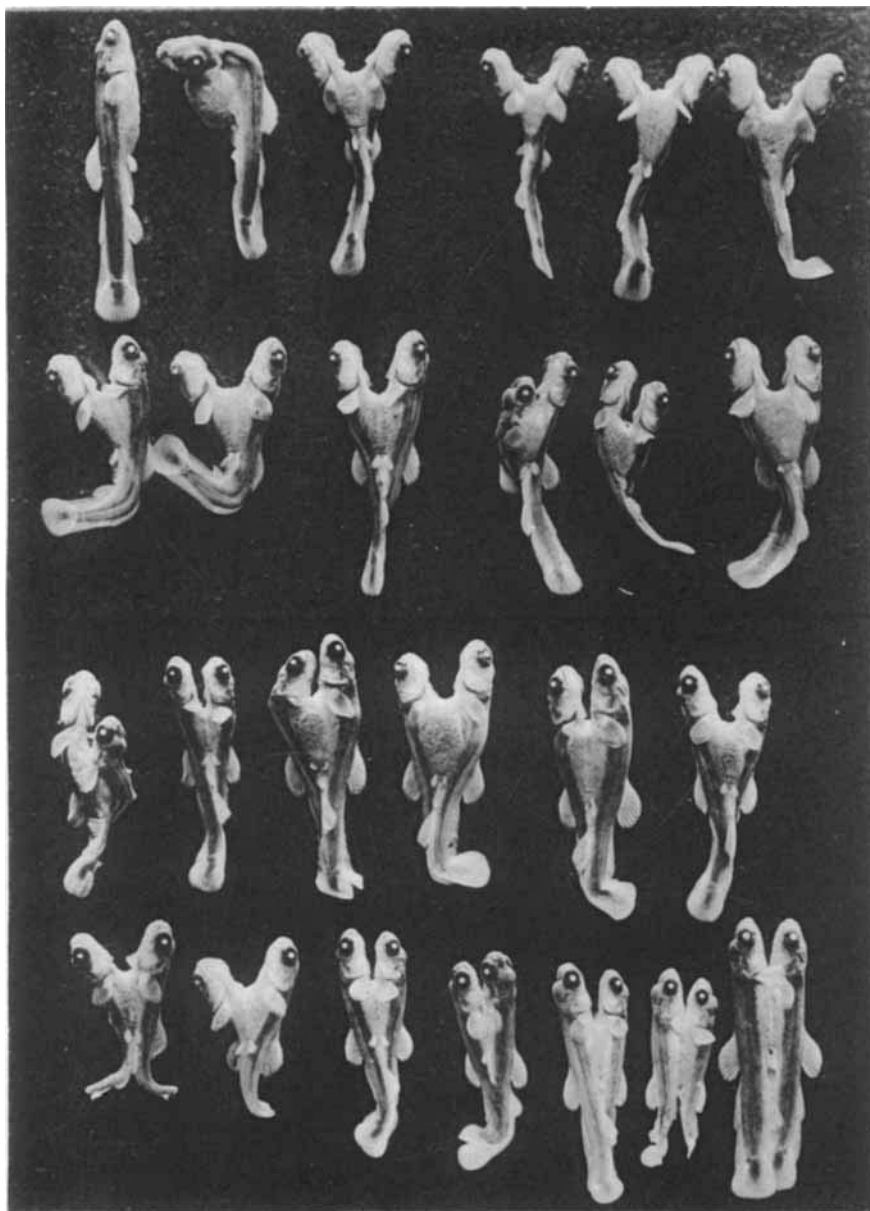
The photographs were all made at one magnification and show as nearly as possible the dorsal aspect of each specimen. On careful examination it will be found that in every specimen the two components are practically identical in size, and when the anterior halves are considered all heads are found to be normal in structure.



## PLATE 2

### EXPLANATION OF FIGURES

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.

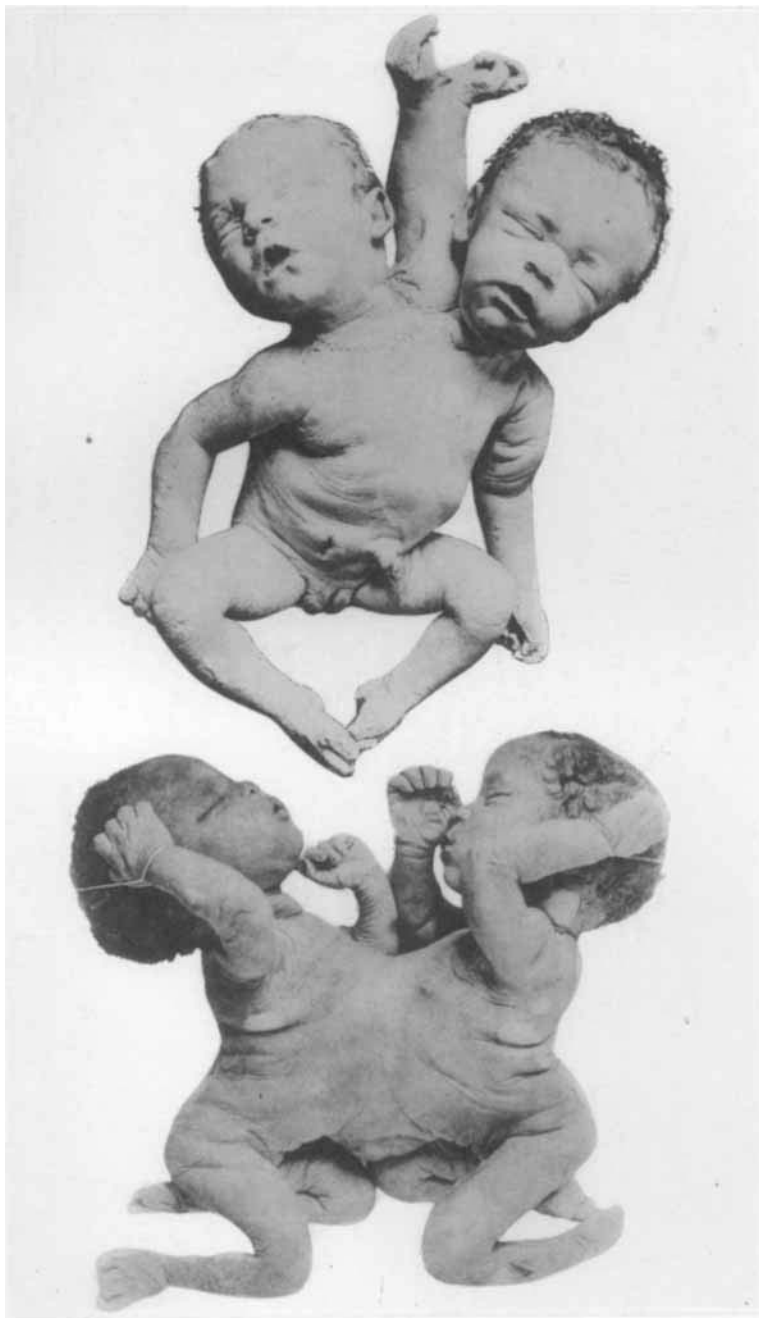


### PLATE 3

#### 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.

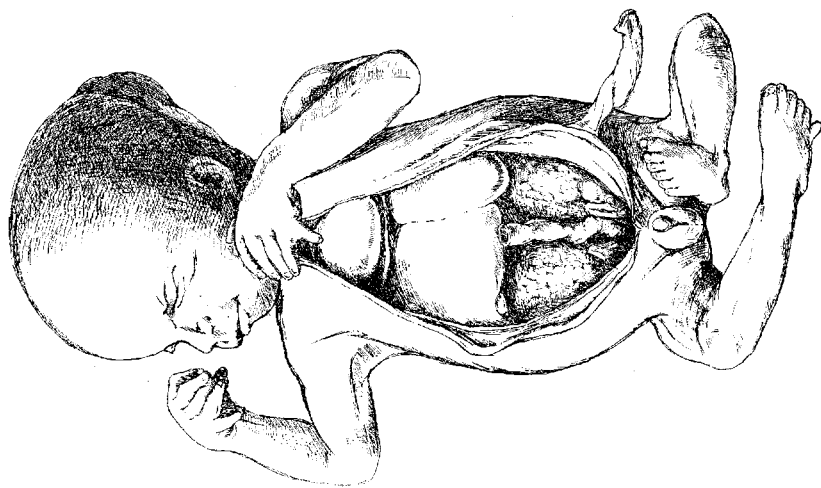
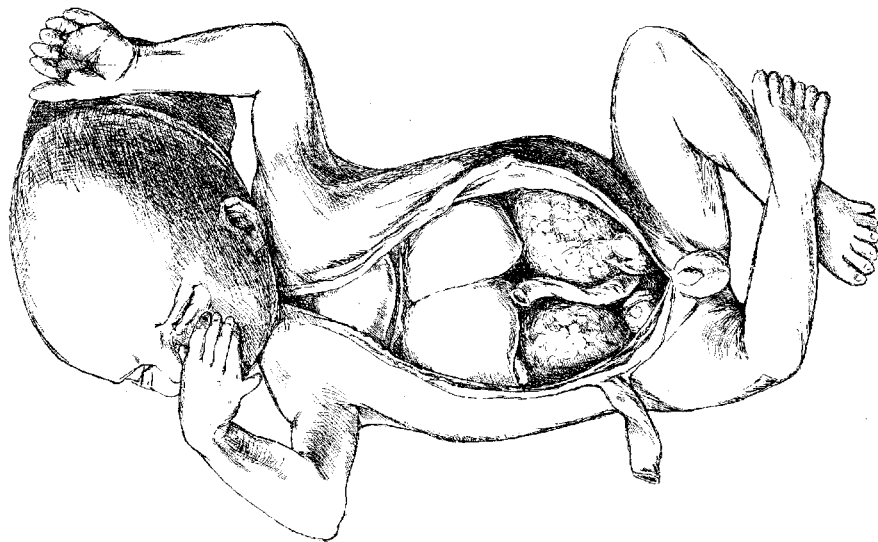
In both of these specimens the components are of equal size and their structures are normal throughout. In all specimens of human duplicities examined or found recorded in which the components were of equal size they were both structurally normal.



## 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 5

### EXPLANATION OF FIGURES

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.

