

THE EFFECTS OF CARBON DIOXIDE ON THE EGGS OF ASCARIS¹

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FIFTEEN TEXT FIGURES AND THREE PLATES

The material upon which the present study is made was placed in carbon dioxide on July 14, and removed on October 9, of the same year (1913). Professor Boveri, in an attempt to keep the eggs of *Ascaris* for a long time without allowing them to undergo development, had a number of smears² from one female placed in a stoppered glass jar. The air of the jar was then replaced by passing a current of carbon dioxide through it for an hour and a half and, after sealing it carefully to prevent the escape of the gas, it was placed in a basement room in the Zoölogical Institute at Würzburg.

At the time the eggs were placed in the gas, no ill effects were anticipated, consequently, no control smears were preserved to show the exact nuclear condition in which the eggs were at that time; and, after they were removed from the gas, they were placed directly on ice where they remained until used. When a few smears were allowed to undergo full development later, it was found that only part of the worms were normal. Professor Boveri called my attention to the fact and placed the material at my disposal with the suggestion that I determine the cause of the abnormal development which part of the worms showed.

Although the material for this study was obtained in Würzburg, the greater part of the work has been done since my return

¹ A preliminary note on this subject has been published by the author (1914).

² The smears were made on ordinary microscope slides according to Boveri's well known method.

to America. I take this occasion to express my thanks to Professor Boveri for suggesting the problem and for placing the necessary material at my disposal.

In preserving the eggs, a mixture of 4 parts 95 per cent alcohol and 1 part glacial acetic acid was used. They were stained *in toto* and mounted in glycerine.

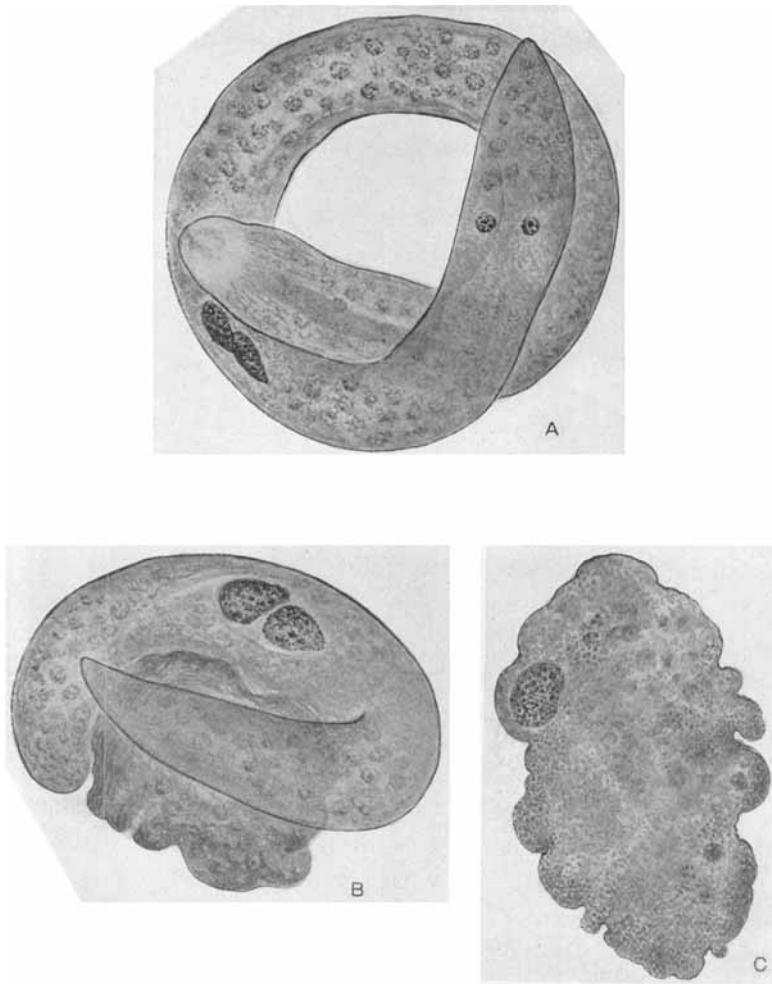
DESCRIPTIVE

Among the embryos which had been allowed to undergo full development, one finds perfectly normal specimens; specimens in which only one end of the body is developed; and lastly, totally disorganized embryos. The problem was primarily to determine the causes which produced the abnormalities observed, but as the work went forward a number of other questions of general interest came up which will be touched upon in the following paper.³

A drawing of a normal worm, which developed from an egg exposed for three months to carbon dioxide, is given in figure A. A blunt anterior end with the pharynx and the pointed posterior end may be seen, and, in addition, towards the posterior part of the body the large deeply-staining nuclei of the primordial germ cells. Typically there are only two of the latter but occasionally more occur; in one case six were present.

The abnormal embryos are of two general types. One of these has the posterior end of the body fully developed while the anterior end is disorganized. The second type is characterized by the absence of organization in its blastomeres. The first type of embryos is shown in figure B. This occurs in roughly 33 per cent of the embryos (in 54 cases out of 165 examined for the point). The pointed posterior end is clearly seen together with the primordial germ cells, but there is much variation in the degree to which the posterior part of the body is developed. We

³ A brief description of the normal development of *Ascaris* is given on page 367. Any one not familiar with the cleavage, or with the nomenclature, in this worm, will find it helpful to read this over together with a glance at the schematic diagrams given. The nomenclature of Boveri has been used throughout the present work.



Text figures A to C

may, in rare cases, find fully seven-eighths of the worm normally formed, or we may find nothing but a pointed stump; figure B gives a fairly typical case.

An example of the second type of embryo is given in figure C. Such individuals are found in about 40 per cent of the cases (66 cases in 165). The greatest variation is noted in the appearance

of such embryos. Typically they consist of a mass of cells, among which one may distinguish the primordial germ cells, but no organization exists and quite frequently it is evident that no cleavage cavity was ever formed in the embryo, consequently, that gastrulation had not taken place.

Eggs preserved as they were taken from the ice chest, where they had been since their removal from the CO₂, showed a slight amount of development. All of them had divided at least once, and something less than half (227 out of 505 eggs counted) had reached the 3-cell stage. Occasionally, in such a preparation, a 4-cell stage is found.

An examination of these 3-cell stages (fig. 1) shows that the S₁ blastomere has divided to form the A and B cells, while the P₁ blastomere is in a 'resting stage.' The nuclear condition of the A and B cells is normal: as may be seen by the figure, waste chromatin occurs in one or both of the cells showing that the diminution process has taken place, but the two cells do not always lie pressed against each other as is normally the case (compare fig. 1 with text fig. F). In 50 eggs out of 78, examined at random for this point, the A and B blastomeres were separated, in extreme cases the two cells lying on opposite sides of the P₁ cell.

Among the eggs in the 2-cell stage, 101 out of 278 cases showed the S₁ cell dividing, with the chromosomes in the equatorial plate phase. The remainder showed resting nuclei in both the S₁ and P₁ blastomeres. A close examination of the equatorial plates in the dividing cells shows an abnormal condition of the chromatin (figs. 2a to 2d). Figures 2b, 2c, and 2d are drawn at a higher magnification. In practically every case (96 out of 101 eggs examined for the point) the chromosomes were found fused together. This fusion seems to affect the ends principally (figs. 2a, 2d) leaving the middle portion free, but in extreme cases even the middle parts are involved and all four chromosomes are clumped together into one mass (figs. 2b and 2c). One very constant feature of this fusion is the formation of what appear to be vacuoles in the fused ends. It is also to be noted that when only the ends of the chromosomes are involved the middle

portions have the normal clumped or lumped appearance which precedes diminution (compare fig. 2d with fig. 9).

In rare cases, both the S_1 and the P_1 cells were dividing at the same time (fig. 3). When this occurred, the chromatin of the S_1 blastomere showed the fused condition, while the chromosomes of the P_1 cell were normal.

In the 2-cell stages with the nuclei in the resting phase, no departures from the normal could be distinguished.

When the eggs are allowed to develop a short time before they are preserved, the P_1 blastomere begins to divide. The elongated chromosomes, so characteristic of the primordial germ cells, are always found and aside from the axis of division, the cell appears normal. Here and there a tendency for the four chromosomes to break up has been noted (text fig. J and L), but this is not to be regarded, I think, as an effect of the CO_2 . The point will be taken up in detail later under the heading 'Anomalies.'

If the eggs are allowed to develop further until half are in the 4-cell stage, a variety of conditions are found in the 4-, 3- and 2-cell stages. Needless to say, these various conditions arise out of the 2- and 3-cell stages described above.

Among the 4-cell stages we find three different types of embryos. One of these (fig. 5) is perfectly normal, both in the position of the blastomeres and in their nuclear conditions. A second type is characterized by the failure of the four blastomeres to form a rhombus, as they normally should do (fig. 4).⁴ Most frequently the planes connecting the two pairs of blastomeres lie at right angles to one another, but this is variable, every imaginable condition being met with in a large number of eggs. The third type is one where, in addition to an abnormal position of the blastomeres, we find an unequal distribution of the chromatin between the A and B blastomeres (fig. 6; this drawing is of a 3-cell stage, selected because it shows particularly well the

⁴ It is not to be thought that these various positions which the A and B blastomeres occupy are just phases of the normal shifting which certain cells of the egg undergo about this period. As will be seen, these positions are retained in later cleavage.

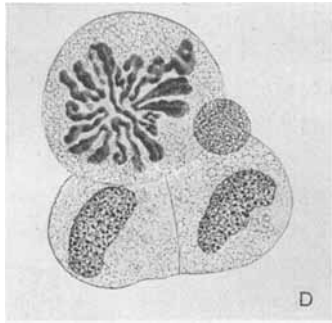
unequal distribution of the chromatin). Here we fail to find the usual waste chromatin in either the A or B cell. On the other hand, one of the cells in such eggs always contains a very large amount of deeply-staining reticular chromatin (fig. 6) while its mate lacks this. This is a very constant feature of eggs in which there has been an unequal distribution of the chromatin between the A and B blastomeres.

Among the 3-cell stages there is always a certain proportion of eggs which are perfectly normal, as far as can be determined. The S_1 cell is divided, the blastomeres lie pressed up against each other, and the waste chromatin lying in the cytoplasm of these cells indicates that diminution has taken place. There is a second type of 3-cell stage which is very striking (figs. 7 and 8). In the egg shown in figure 7, the P_2 and the EMSt blastomeres have separated—are in a stage of division, in fact while the S_1 cell is undivided and contains a tetraster. Figure 8 shows the same condition of the S_1 cell and in addition we note the enucleated protoplasmic ball lying on top of it. Eggs showing tetrasters usually, if not always, possess this protoplasmic ball. In figure 7 it is present, but lies under the other cells and is not shown in the drawing. The amount of chromatin between the four centrosomes of the S_1 cell is very large. It may be undergoing diminution or we may find the elongated chromosomes still present. In the latter case, one may usually count eight chromosomes lying in the spindles. In text figure D, an egg is shown with a history similar to that of the tetraster eggs, but for some reason, the centrosomes have failed to divide. In this respect it is exceptional, but eight chromosomes and the characteristic protoplasmic ball are both clearly seen.

The 2-cell stages usually show the S_1 and the P_1 blastomeres undergoing division (fig. 9). Except for the axes of division, which frequently do not occupy normal planes with regard to each other, the division figures are typical of the untreated eggs; and the lumpy condition of the chromatin of the S_1 cell, which precedes diminution, is seen.

A number of smears of eggs were preserved when the embryos were in or just beyond the 4-cell stage, and in these, we find a

tremendous variation in the relative proportions in which the various abnormalities described above occur. In one preparation over 50 per cent of the eggs showed a tetraster in the S₁ blastomere, or showed the later effects of it. In other slides only a very small proportion of the eggs showed this abnormality. The proportion of the eggs which showed an unequal distribution of the chromatin between the A and B blastomeres, showed the same variation in different slides. On the other hand, the failure of the blastomeres to form the rhombus is an abnormality found in a large per cent of cases on every slide.



Text figure D.

In the large amount of material which was preserved at frequent intervals after the 4-cell period, it has been possible to follow the results of the abnormal conditions described through the later development. Of course, as cleavage progresses, it becomes increasingly difficult to follow the course of the individual blastomeres, except in the case of the primordial germ cell. The latter is usually conspicuous on account of the large size of the nucleus. An analysis of the later stages has been facilitated by the use of clay models of the eggs and a comparison of these with the excellent figures given by Boveri ('99). In this way it has been possible to determine very exactly the plane in which a given cell is dividing, and the relation it will have to the rest of the blastomeres.

For the sake of clearness, the course of the different types of eggs will be followed separately through later cleavage in the following description. These will be taken up in the following order: (a) The effects of the abnormal positions which the S_1 derivatives take in cleavage. (b) The result of the unequal distribution of the chromatin between the A and B blastomeres. (c) The fate of the tetraster eggs.

Among the treated eggs there is always a certain per cent which are perfectly normal. The 4-cell stage, such as shown in figure 5, is followed by the division of the A and B blastomeres in a plane approximately at right angles to the plane of the paper upon which the drawing is given (compare with fig. G). Following this, the P_2 and EMSt cells divide in the median plane of the embryo (compare with fig. 1), and throughout the later cleavage, the analyses with models show that the normal development is continued.

The development of embryos in which the A and B blastomeres occupy abnormal positions in the 4-cell stage, may be followed with ease up to the time when the S_1 derivatives number 16 cells. From this point on, such eggs are not to be distinguished from normal embryos. Figure 4 shows a typical case of the positions which the A and B cell take. In figure 10, we see these two cells dividing. The diminution process is taking place normally, but the planes which the dividing cells occupy, instead of being parallel (compare with the normal as shown in fig. G) are at right angles to each other. The result of such a division is shown in figure 11. In the egg shown in figure 12, the ectodermal cells are eight in number and the EMSt cell has divided in the median plane of the embryo. The P_2 cell is in the equatorial plate phase of division. The elongated chromosomes characteristic of the primordial germ cell are clearly seen. In figure 13, we see a somewhat later stage. Both the P_2 and EMSt blastomeres have divided. It is especially to be noted that the MSt blastomere does not lie in the median plane of the embryo (the EMSt cell divides into an E and an MSt cell; these normally lie in the median plane of the embryo, compare with fig. 1). It is very rare that we find the EMSt cell dividing in any plane but the

median, but in eggs examined *after* the division, we frequently find the MSt blastomere lying outside of the median plane. In these cases, the A and B derivatives are very asymmetrically distributed over the dorsal portion of the embryo and it seems that these cells push the MSt blastomere out of its normal position. This is more apparent on models of the eggs, of course, than in figures. The probable significance of this will be taken up later.

Many hundreds of eggs similar to those shown in figures 10 to 13 have been analyzed. The abnormal positions of the A and B cells are retained, there is no shifting to form the rhombus, at least it is not usually realized, the ectodermal cells derived from the S_1 blastomere take up positions on almost any part of the egg. Thus the bilateral symmetry of the embryo may be completely lost and, what is for our study more significant, the members of the ventral family, especially the MSt blastomere, may be moved out of the median plane.

The most striking results of the unequal distribution of the chromatin between the A and B blastomeres is shown in figure 14. Aside from the positions which these two cells have, we see that one is dividing early and that it contains only a small number of somatic chromosomes in the spindle. The mate, on the other hand, shows no sign of division, and it will be noted that it contains a very large nucleus (compare fig. 14 with fig. 6). This result always follows the unequal distribution of the chromatin, apparently, and the early division of the one cell upsets the cleavage rhythm of the S_1 derivatives. If the distribution has been very uneven, then one of the cells may divide twice before its mate cleaves. With a more equal distribution the rhythm is not so markedly upset, but in any event the end result is the same: the S_1 derivatives become scattered irregularly over the surface of the embryo, the symmetry or balance of the embryo is upset, and probably members of the ventral family are pushed out of their normal positions, as was the case with the egg shown in figure 13.

The development of the embryos which showed a tetraster in the S_1 blastomere, is extremely variable, both in the number of cells formed by the division and in the distribution of the

chromatin. A typical case is shown in figure 15. Here there are six cells besides the P_2 and EMSt blastomeres. One of these is evidently an enucleated protoplasmic ball, so characteristic of the tetraster eggs. The remainder came from the division. It will be noted that one of the small blastomeres contains a large amount of waste chromatin. This is a very common phenomenon exhibited by such eggs after division. In this egg, we also see that the MSt blastomere is not dividing in the same plane as the P_2 . Such a condition is seldom met with.

The later development of these eggs which have had a tetraster in the S_1 blastomere, is extremely abnormal. The S_1 derivatives divide irregularly; they become scattered over the surface of the embryo or lie in one heap; and there is every indication that they very rarely or never form a cleavage cavity and gastrulate. In later cleavage stages, the abnormalities caused by these tetraster eggs is very striking. One of the marks of such eggs is the presence of the small cell with the large amount of chromatin (fig. 15). This disorganization, however, does not extend to the primordial germ cell, for we find it dividing normally in later stages when the embryo is otherwise totally abnormal.

At the time of gastrulation, a majority of the embryos appear normal or exhibit minor irregularities, such as a slight asymmetry of shape. The cleavage cavity is present in such eggs, however, and there seems to be no reason why they should not gastrulate normally. Among such embryos one finds a large number which have no cleavage cavity. This sometimes appears to be due to the fact that the ectodermal cells are too scattered or are too few in number to form it. But in every case the primordial germ cell nuclei may be clearly seen.

To sum up the foregoing description, we find that the abnormal eggs are of three types: (a) Eggs in which the A and B blastomeres have abnormal positions in the 4-cell and later stages. (b) Eggs in which there has been an asymmetrical distribution of the chromatin between the A and B cells. (c) Eggs with a tetraster in the S_1 blastomere. We have now to inquire how these three abnormal conditions arose from the eggs just removed from the CO_2 . And, secondly, what relation these ab-

normalities in cleavage bear to the embryos which had been allowed to undergo full development.

Taking up the first question, a close examination of the material has shown that the tetraster condition of the S_1 blastomere and the irregular distribution of the chromatin in the A and B cells, is due to the same cause, that is, the fusion of the chromatin in the S_1 cell (figs. 2a to 2d). A glance at these figures will show that in part of the eggs, only the ends of the chromosomes were involved and that the middle portions were free. In such eggs a division of the S_1 blastomeres occurs but, owing to the fused condition of the chromatin, an equal distribution of it can not take place. Diminution of the chromatin occurs and one blastomere receives a number of small 'diminished' or somatic chromosomes, while the other cell receives, in addition to the somatic chromosomes, the whole mass of fused chromatin of the equatorial plate. If the fusion involved part of the chromatin which would normally go to form somatic chromosomes, then one cell would receive this together with the waste chromatin. When this fused mass goes to one cell, it does not undergo degenerative changes but (probably because of the presence of some somatic chromatin) it becomes resolved into a reticulum and fuses with the normal nucleus of the cell. In this way, one cell comes to contain more chromatin than its mate, and in later stages, the cell with the least chromatin divides earlier. Various stages of this process have been observed in my material.

When, however, the fusion involved all of the chromatin, as in figures 2b or 2c, then division appears not to take place. Apparently, the fused condition of the chromatin is responsible for this, but whether this prevented the centrosomes from going apart, or whether the fused mass kept the cell wall from cutting through, is not known. Stages that would decide this point have not been seen, but various other steps in the process have been observed. Thus the one cell comes to contain all the chromatin which should be distributed between blastomeres A and B. At the next division cycle, such eggs showed a tetraster in the S_1 cell and eight chromosomes are found in the spindles (fig. D).

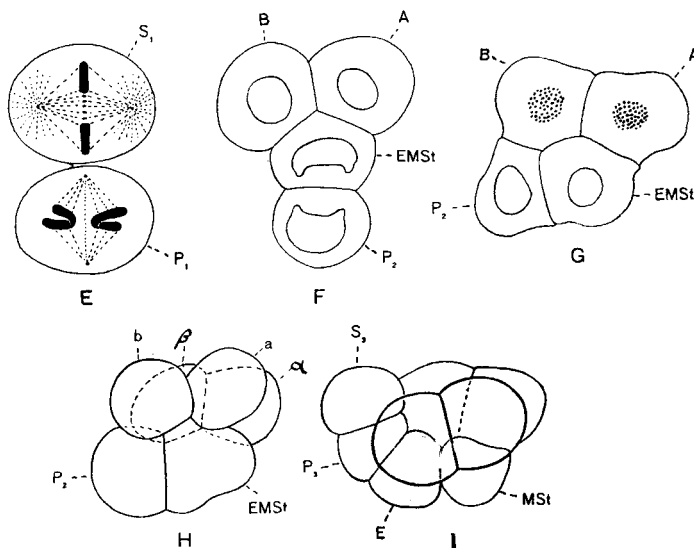
One very constant feature of the eggs showing a tetraster is the occurrence of a protoplasmic ball which lies on the blastomere showing this condition. There seems to be an intimate connection between the formation of the ball and the failure of the egg to divide. This point will be touched upon again.

The abnormal positions which the A and B blastomeres take up is to be traced, in part at least, back to the 3-cell stage, when the A and B cells separated (fig. 1). It is probable, however, that this is not the only source of the abnormality, for not infrequently, one finds eggs like those shown in text figure N. As may be seen in the figure, the A and B cell lie pressed against each other, and are dividing. The interesting thing is that the P₂ cell, is dividing in an abnormal plane. This condition could come about only in one of two ways. Either there has been a rolling or shifting of some of the cells, or, their polarity has been changed. Of the two possibilities the former seems the most probable since in the normal egg, a shifting process is involved which brings the B and P₂ blastomeres together (compare with figs. F and G). As we know nothing definite about the cause of the shifting in the normal egg, it is useless to speculate over the matter at this time. It seems worth while, however, to point out that the separation of the A and B cell might come about by an increase of surface tension. These cells normally lie pressed against each other, but were the surface tension of them increased, as through some action of the CO₂, then they would separate. If the P₁ cell were also affected, the effect (that is, the separation) might be still more marked.

Before we take up the relation between the abnormalities found in cleavage and those exhibited by the fully developed worms, it will be necessary to describe a few of the more striking points in *Ascaris* development. Thanks to the works of Boveri ('99) and Zur Strassen ('96), we know the origin and fate of practically every cell in the young worm.

A few schematic sketches of the normal development are given in figures E to I. The first division results in two blastomeres, S₁ and P₁ respectively, following Boveri's nomenclature. These two cells have different potentialities. In the next division

cycle, these two cells divide in planes at right angles to each other (fig. E), and furthermore, as is well known, the chromatin of the S_1 cell undergoes a process of "diminution" while the P_1 cell retains the elongated chromosomes. The result of the division is four blastomeres which form a T-like figure (fig. F.). Following this, the cell marked P_2 shifts around until it comes in contact with the B blastomere, forming in this way a rhombus. Up to this time, one can not speak of an anterior and posterior end of the embryo, but after the shifting, these parts are marked out. The



Text figures E to I

anterior end lies to the right in the figure, that is, at the A and EMSt side, while the posterior end is indicated by the P_2 blastomere. The A and B blastomeres lie dorsally, as in the figure, the P_2 and EMSt ventrally, the median plane of the embryo being parallel to the paper and passing through all four blastomeres.

The A and B cells now divide in a plane approximately at right angles to the median plane (fig. G), while the P_2 and EMSt cells divide in the median plane (fig. I). The A and B cells give rise to the ectoderm covering the dorsal and anterior end of the body. The EMSt cell will give rise to the entoderm, part of the

mesoderm, and the cells of the stomodaeum. The P_2 cell, after giving off several generations of ectodermal and mesodermal cells, forms the primordial germ cells. The most important points for us to remember are the following: During later development the A and B cells grow over the dorsal and anterior end of the body of the embryo. The EMSt and P_2 cells lie ventrally and posteriorly and form the most important organs of the body. Nearly all of the posterior part of the body of the young worm comes from the P_1 derivatives.

We can now turn to the question of the relation of the normal cleavage of the treated eggs and the worms which resulted from them.

Since a certain percentage of eggs always developed in a perfectly normal fashion during cleavage, it is clear that the fully developed normal worms, such as shown in figure A, arose from this source.

It is equally clear that the masses of totally disorganized cells which one finds in figure C are due, in part at least, to the formation of the tetraster in the S_1 blastomere, with the subsequent abnormal development. No doubt other sources contributed to this class of embryo.

The embryos in which the posterior end is only partially differentiated are undoubtedly to be traced to the eggs with the A and B cells lying in abnormal positions, but the details of how this condition affected the later development are uncertain because we have so little knowledge in how far the later shifting of the blastomeres in *Ascaris* is due to internal organization, and in how far to simple mechanical relations, such as mutual pressure, etc. Admitting this uncertainty at the start, we may give a very simple explanation which appears to agree with all of the observations recorded.

A glance at figure I will show that the derivatives of the P_1 cell form a sort of half keel on the ventral and posterior end of the embryo, and the ectodermal cells, (derivatives of the S_1) by their division form a more or less symmetrical covering for this. The works of Boveri ('09) and Miss Stevens ('09) have shown that this ventral keel may take place when the A and B derivatives

are absent (as when the S_1 is killed by ultra-violet light). This indicates a high degree of internal organization in these cells. Here it may be remarked that in these experiments there were no ectodermal cells which might move the members of the ventral family out of the median plane. Were, however, the A and B derivatives present but in positions which would destroy the symmetry of the embryo (for example, were they lying only on one side of this keel, and I have observed cases which approached this) to unbalance the system, so to speak, then it seems very probable that some of the ventral cells would be moved out of the median plane.

In the later development of the eggs in which the A and B cells occupy abnormal positions, we seem to see this unbalancing taking place. Quite frequently in such eggs, the majority of the A and B derivatives lie on one side of the keel, and in the later stages, as shown in figure 13, one of the blastomeres (the MSt in this case) is moved out of the median plane. Prior to the division, the EMSt blastomere lies in the median plane and even in the metaphase, it holds this position. I have noted only one or two exceptions to this. After division, however, the MSt cell is frequently found lying out of the median plane, and the most probable explanation is that it has been moved out of this plane by the overlying ectodermal cells. It may well be that other causes were operating to produce the same end effect. In a few cases, the EMSt blastomere divided in an abnormal plane, figure N shows such a case, or, disorganization may have come later.

As cleavage went forward in these eggs, the A and B derivatives formed the cleavage cavity by mutual pressure and gastrulation took place. It was not until organ formation began that the effects of the shifting of the MSt cell could be observed. Looked at from a theoretical point of view, since the MSt blastomere forms the cells of the stomodaeum (after several divisions) we should expect that were this cell pushed out of its normal position, the resulting embryo would lack this part. The embryos in which the anterior end is disorganized but in which the posterior end is normal, seem to fulfill these expectations. The

posterior end, coming from the other end of the keel and more or less independent of the derivatives of the S_1 cell, would be normally formed, since all the necessary elements were present. It is not to be expected that simply the MSt cell would be affected. No doubt the failure of this cell to take up the proper position causes the whole anterior end to be disorganized, and when the A and B derivatives were very irregularly distributed (when they, for example, lay on top of the P_2 cell without touching the EMSt, and I have observed such cases) the disorganization probably extended to the posterior end. It seems likely that the degree to which the posterior end was differentiated is to be correlated with the positions which the A and B derivatives took, and thus we have a series of stages from worms which are seven-eighths normal, to worms in which only the stump of the posterior end is differentiated.

In this way we are able to explain the production of the half embryos following the treatment of the eggs with CO_2 . There is, of course, another way of explaining their production, but this has not been advanced because it did not harmonize, as it seemed to me, with the facts which have been discovered by Boveri and his students. We have assumed that the ectodermal cells coming from the S_1 blastomere, were more or less indifferent in their nature. This is indicated by the normal development, for the cells divide rhythmically and form the general ectodermal covering for the anterior end of the body. In contrast to these, the cells of the ventral family (P_1 derivatives) divide very irregularly and possess a high degree of specificity, that is, one forms the entoderm, another the mesoderm, or primordial germ cells, and so on. Were we to attribute specificity to the ectodermal cells, then the explanation for the half embryos would be that they were formed since the cells destined for the anterior end were scattered and this part of the embryo was undifferentiated.

The latter view does not seem tenable since all work points to the indifferent nature of the S_1 derivatives. In any event, however, the production of the half embryos is to be traced to the abnormal positions which the A and B blastomeres take in the 4-cell stage.

While the great majority of the eggs followed the different types of development outlined above, a small per cent could usually be found in any slide which were abnormal for no apparent reason. One condition rather common, is that seen in figure N, where we find the A and B cells undivided even after the P₂ and EMSt cell have nearly completed their division. What the fate of such eggs is can not be definitely stated, but since we may find the A and B cells undivided in later stages, it seems probable that such eggs never gastrulate. The percentage of eggs of this type is small, in any event.

We now come to the question, why are some of the eggs affected by the treatment with the CO₂, and why do others develop normally under the same conditions? And why do we find different proportions of abnormalities in the smears of the same female? It was for the solution of these questions that two series of experiments were planned and attempted, but since these were unsuccessful, the working hypothesis upon which they were based, will be given. This explanation is only tentative.

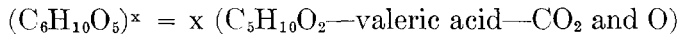
It is well known that in any mass of *Ascaris* eggs, some develop more rapidly than others. When the eggs were placed in the CO₂ they underwent a certain amount of development before the oxygen available was exhausted. When the supply of oxygen lasted until the nuclei were in the resting stage, no ill effects resulted except such as might arise from a shifting of the blastomeres. If, however, the eggs were in the equatorial plate phase when the supply of oxygen gave out, then they remained in this state until brought into the air again. Whether the fusion of the chromatin took place in the CO₂ or whether it resulted later, was to have been determined.

The variation in the proportion of the abnormal types is, without doubt, due to the following reason. All the eggs for the present study were taken from the fresh uterus of a single female. Such eggs removed from the end of the uterus have given off their polar bodies and the male and female pronuclei lie side by side until oxygen is admitted. Eggs lying farther back in the uterus are not so far advanced as those lying at the tip, con-

sequently, some of the smears are advanced farther than others; a larger proportion of eggs reach the equatorial plate phase in one slide than in another, and we find as a result a larger proportion of tetrasters. It seems probable that in the smear with over 50 per cent of the eggs showing tetrasters in the S_1 cell, the S_1 blastomere had been able to bring its division only to the equatorial plate phase. However, on all these points more experimental evidence is needed and the author hopes to fill in the gaps as soon as suitable material is available.

It is of further interest to ask how the eggs were able to live and develop to a slight degree, in an atmosphere of CO_2 . Especially, when they had been kept at a temperature where they would normally have developed in some three weeks. These questions are taken up in the following discussion.

It has long been recognized that intestinal parasites live under anaerobic conditions, but Weinland ('01) was the first to show the mechanism by which they obtained the oxygen necessary for their existence. It had been known previously that *Ascaris* contained a large amount of glycogen, and Weinland was able to show that when these animals were kept in a medium without food, this glycogen disappeared and he obtained CO_2 and valeric acid. He suggested that the glycogen had been broken down by some animal ferment. Glycogen is one of the complex sugars with the empirical formula of $(C_6H_{10}O_5)^x$. According to Weinland's ideas, this is broken down in essentially the following way:



A number of authors (Brault and Loepers '04; Buschs '05, '06; Kemnitz '11; and Brammertz '13) have shown that the eggs of *Ascaris* contain large amounts of glycogen. Brammertz was able to show that during the formation of the polar bodies, the amount of glycogen diminished in the region where these bodies were being formed. The reason for this was that the glycogen was broken down to furnish the oxygen necessary for this process. For further development the oxygen of the air seems to be essential, although the glycogen in the egg is

slowly used up as development goes forward. Brammertz regards this glycogen as a sort of reserve to tide the embryo over unfavorable conditions. He cites one experiment in favor of this view, which is quite similar, in its conditions, to the experiments recorded above. He found that if eggs were placed in 70 per cent alcohol, part of them developed as far as the two cell stage before they were penetrated by the alcohol and killed. He regards it as improbable that the eggs could have gotten the oxygen necessary from the alcohol, and thinks there is proof here that the glycogen was used for this purpose.

The author has not made any experiments with the eggs treated with CO₂ but the conditions are so similar with the experiment cited that it seems very probable that the eggs used in my experiments were able to live over this period of three months because of the presence of the glycogen stored in their protoplasm.

ANOMALIES

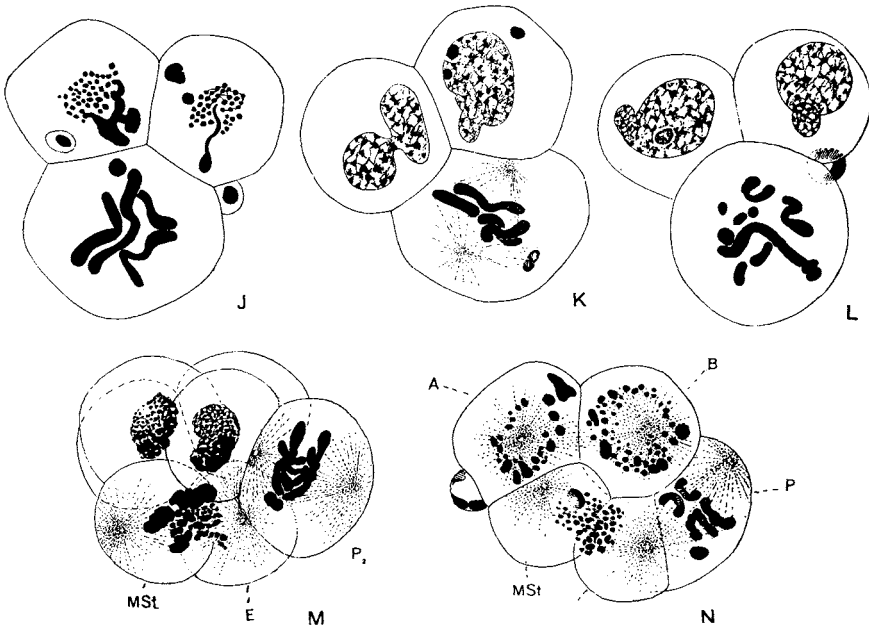
Under this heading I wish to record some observations made on the treated eggs, which have a bearing on the problems of sex determination and the cause of diminution in *Ascaris megalocephala*.

In the dividing primordial germ cells, occasionally eggs have been seen in which there were, besides the four chromosomes, additional elements (in 13 cases out of 123 eggs taken at random). Typically there is only one additional chromosome, as in figure J, but cases with two, three, four, and even eleven fragments have been seen (fig. L). The way in which the single element behaves during division is shown in figures K, M, and N. Cases with more fragments could not be followed through division.

The presence of one or more chromosome fragments in the primordial germ cells of *Ascaris megalocephala* have been described by a number of authors, and at the present time two views have been advanced to explain them. According to Boring ('09) and Boveri ('09), they represent the accessory chromosomes in this species. This is the view generally accepted. Kautsch ('13), however, has shown that another interpretation

is possible. After an extensive study of anomalies in *Ascaris*, he comes to the conclusion that these fragments are probably bits of chromatin brought into the egg when the polar bodies did not receive their full share of chromatin.

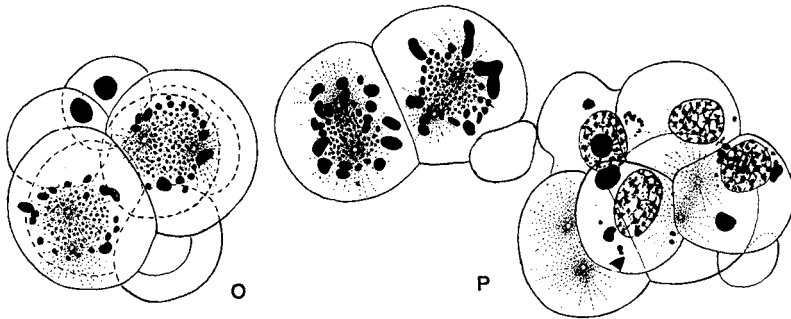
Kautsch approached the question of the accessory chromosome in *Ascaris* in another way. He counted the number of somatic chromosomes in eggs in which there was only one chromosome,



Text figures J to N

and he found that they fell into two numerical groups; one centering around 27, the other around 36, somatic chromosomes. These, he suggests, may be male and female numbers. The counts made by Kautsch are too few in number to be conclusive, but it is interesting to note that his view falls into line with the work done by Edwards ('10) on *Ascaris lumbricoides*. The latter author, as is well known, found that the accessory chromosome in this species, is represented by a group of five small chromosomes.

The observations recorded above, have a certain bearing on the question, since it is clear from them that genuine fragmentation may take place in the chromosomes of *Ascaris megalocephala*, in some cases all four chromosomes being affected. The fragmentation of the chromatin in figure L, however, certainly has nothing to do with the accessory chromosome, and since a series may be formed in which one, two, or even all four chromosomes may be broken up, it becomes a question whether or not we can interpret such cases as shown in figures K, M, or N, as having any relation to the accessory chromosome. Just what relation the fragmentation in the normal eggs has to do with that observed in the eggs treated with CO₂, is uncertain, but it does not



Text figures O and P

seem improbable that they are both expressions of a tendency residing in the chromosomes.

A second anomaly found is shown in figures O and P. In figure O, the P₂ and EMSt cells are both dividing, and it will be seen that both are undergoing diminution. It is especially to be noted that the spindles of the two cells are not parallel. In figure P, another egg is shown in which both the P₂ and EMSt cells are undergoing diminution. A detailed drawing of the two cells is seen to one side. It is especially to be noted that a protoplasmic ball lies on one of the two cells.

In figure 16 is shown a rare case where the P₂ and EMSt cells are fusing after division. Note particularly the protoplasmic ball coming from the fusing cells. (The S₁ cell is seen lying be-

neath; it is clearly undergoing diminution.) In figure 17 is a later stage in which the fused P_2 and EMSt cell is undergoing division. It will be seen from the figure that there are four small cells present which contain waste chromatin, besides two protoplasmic balls without any chromatin. The presence of a large amount of chromatin in one small cell indicates that the four small cells have arisen by the division of a tetraster. Compare this egg with that shown in figure 15. The presence of the protoplasmic balls indicates the same thing. One lies on the fused P_2 and EMSt cells, and has arisen, probably during division, as in figure 16. The other protoplasmic ball was undoubtedly formed when the S_1 cell failed to divide. The interesting thing about this egg is, that the cell which I interpret as coming from the fusion of the P_2 and EMSt blastomeres, such as we see in figure 16 is undergoing division; shows a tetraster and as may be seen in the figure, the chromatin is undergoing diminution. Only one case of this sort has been seen, but it has been very carefully studied and there can be little doubt of the correctness of the interpretation given.

I do not propose to take up a detailed discussion of the question, "What causes the diminution in the somatic cells of *Ascaris*?" And yet the question can not be fully omitted since the observations recorded in the foregoing pages throw some light on the subject, even though they do not give a final answer. It is well known that two views have been held with regard to this subject. Zur Strassen ('06) invoked what was essentially a qualitative division of the chromosomes, in order to explain the phenomenon. This view has been contested by Boveri ('10), who, by his masterly analysis of dispermic and centrifuged eggs in *Ascaris*, showed that the explanation advanced by Zur Strassen was untenable. Finding it impossible to explain the cause of the diminution to factors residing in the chromosomes themselves, Boveri turned to the cytoplasm and advanced his 'Schichtung' hypothesis. This author, convinced of the heterotropic nature of the protoplasm in *Ascaris*, conceives of the various substances being arranged in layers. The blastomeres receiving certain layers of protoplasm, undergo diminution while

other cells not receiving these layers retain the elongated form of the chromosomes. A considerable body of evidence has been produced by Boveri to substantiate this view.

From time to time, various authors have noted exceptions to the rule that the germinal cells never undergo diminution. Most recently we have the work of Kautsch who has given drawings of a number of cases and points out that in all of the eggs observed by him, the axes of the spindles in the P_2 and EMSt cells were parallel. This the author took as indicating that the protoplasm of the two cells was similarly structured. How the condition arose, he does not state. A glance at either figure O or P will show exceptions to this rule. In fact, in the number of eggs in which I have observed this anomaly, I have been unable to find any two spindles which were parallel.

Viewed in the light of Boveri's hypothesis, the two eggs shown in figures 16 and 17, prove very interesting. Here we have the P_2 and EMSt blastomeres fused together after division, and, if the diminution process depends on a qualitative division of the chromosomes, we should find some somatic and germinal chromosomes in the spindles. But as will be seen from figure 17, this is not the case. On the other hand, if the presence of the elongated chromosomes depends on some specific substance carried in the cytoplasm of the primordial germ cell, then we should expect to find eight complete chromosomes in the spindles.

A glance at figure 17, shows that the chromosomes are undergoing typical diminution. How is this to be explained by the hypothesis advanced by Boveri? Can it be that the germ path determiner (or however we choose to think of this postulated substance) has lost its potency over the protoplasm which it had controlled in the preceding division? Or, is the explanation for the diminution process to be sought in some other theory? The answer to this question is, I think, given by the protoplasmic ball which almost invariably is found in eggs showing diminution in the germinal cells.

If we conceive of the presence of the elongated chromosomes in the germinal cells as being due to some finely balanced qualitative or quantitative chemical inter-reactions, then it is easy

to imagine that any process which would remove part of the protoplasm or otherwise disturb the relations, would upset the reaction. Or, if we think of some specific substance, a true germ path determiner, as being present in the germinal cells, the loss of part or all of this substance would produce diminution in such cells. Which of these two views will prove the correct one, it is impossible at the present time to say, but the evidence to be presented can be equally well interpreted for either case.

In the eggs treated with CO_2 the quantitative relations are frequently upset by the formation of protoplasmic balls. These balls are apparently formed by the giving away of the cell wall when the cleavage pressure is at its height. It is invariably found when the A and B blastomeres fail to separate. More rarely, it may be given off from the dividing primordial germ cell. In figure P we have such a case, associated with the diminution process in the germinal cells. Most of the eggs which have shown diminution in the germinal cells, have been characterized by the presence of such a ball. In glancing over the figures given by Kautsch, I notice also the frequent appearance of such a ball, although Kautsch does not mention them in his description of these abnormal eggs. Finally, in the eggs shown in figures 16 and 17, we see a large mass of protoplasm has been cut off from the germinal cell. The constant occurrence of the protoplasmic ball with the diminution going on in the primordial germ cell has convinced me that the two have a close relation. Here we seem to have an explanation for the diminution process, for, when this mass of protoplasm is thrown out of the germinal cell, substances (such as the germ path determiner itself or some material necessary for its action) are either removed, or the balance between the inter-reacting chemical substances is upset. Either of these causes would probably be sufficient to bring about the diminution. In the case of the ectodermal cells, A and B, the formation of the protoplasmic ball has no effect on diminution since the substance inhibiting this process is not present.

In advancing this explanation, it is not my intention to imply that diminution in the germinal cells takes place only after the formation of the protoplasmic balls. In fact, I have myself found cases in which diminution was plainly taking place, but I was unable to find any ball. Such eggs, however, were invariably abnormally retarded in their development and the explanation for the diminution is probably to be found in another cause. The author ('15) has shown that in the sea urchin egg, after fertilization, progressive changes are going on in the cytoplasm of the egg which are independent of the cleavage process and thus we may have the formation of the micromere, which normally comes in the 16-cell stage, in the 8- or 4-cell stage. It is very probable that this process is characteristic of all eggs. Thus in the germinal cells of *Ascaris* we may imagine series of changes are going on, only here they tend toward different results. Were the germinal cell long delayed in its division it may be that the somatic tendency becomes so strong as to suppress the germinal one, and thus diminution takes place.

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PLATE 1

EXPLANATION OF FIGURES

All drawings made with camera lucida, and $\gamma_{1\frac{1}{2}}$ oil immersion. Figures 2b, 2c and 2d are drawn at a higher magnification than the remainder of the figures.

- 1 A 3-cell stage showing the separation of the A and B blastomeres.
- 2a A 2-cell stage showing the peculiar fusing which the chromatin in the S₁ blastomeres.
- 2b, 2c, 2d Detailed drawings of the fused chromatin.
- 4 Showing a 4-cell stage with the A and B cells occupying abnormal positions.
- 5 A 4-cell stage, normal after treatment with CO₂.

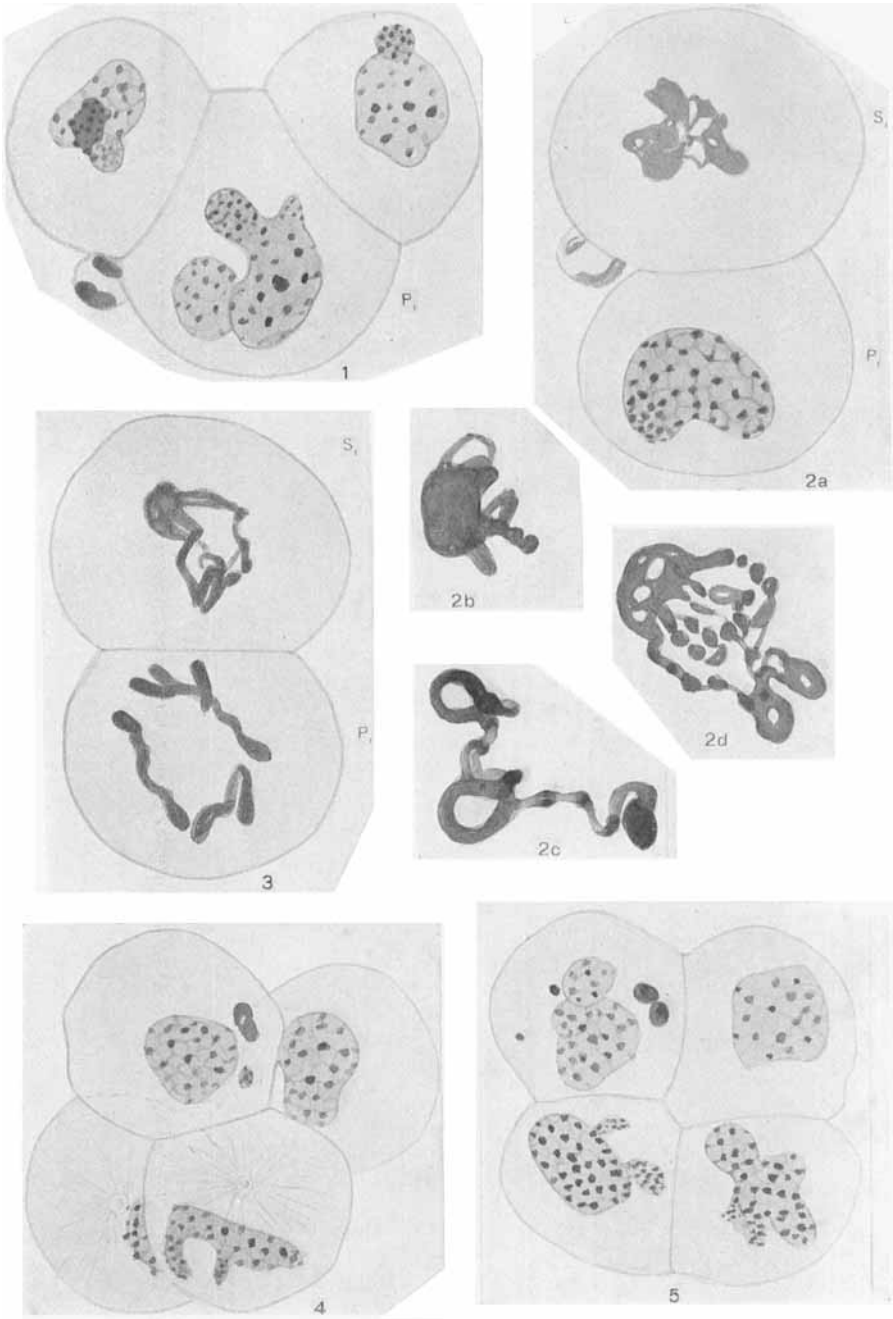


PLATE 2

EXPLANATION OF FIGURES

- 6 A 3-cell stage showing the unequal distribution of the chromatin between the A and B cells.
- 7 Showing the tetraster in the S₁ blastomere.
- 8 Showing the tetraster in the S₁ blastomere.
- 9 Showing normal 2-cell division after treatment with CO₂
- 10 Showing the A and B blastomeres dividing in abnormal planes.
- 11 Showing the result of the division when the A and B cell occupy abnormal positions.

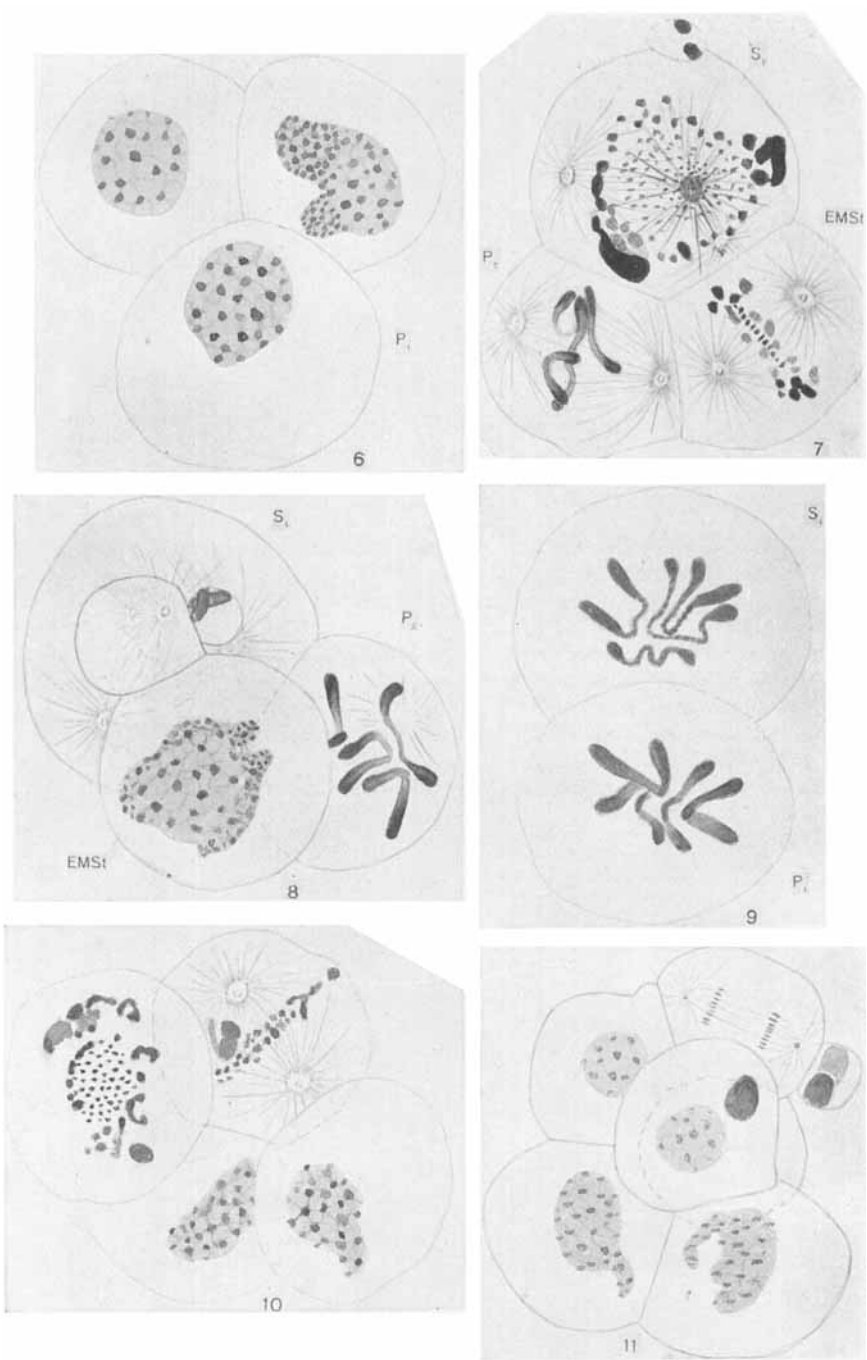


PLATE 3

EXPLANATION OF FIGURES

12 Showing the effect when the A and B cell divide in abnormal planes; the ectodermal cells number 8 in this egg.

13 A slightly later stage of the same; note that the MSt cell does not lie in the median plane of the embryo.

14 An egg showing the effect of the unequal division of the chromatin between the A and B blastomeres.

15 An egg showing the effect of the tetraster formation in the S₁ cell.

16 Showing the P₂ and EMSt cell fusing; note particularly the protoplasmic ball projecting from the fusing cells.

17 Showing the later effect of the fusion of the P₂ and EMSt cells; note that diminution is taking place in this cell.

