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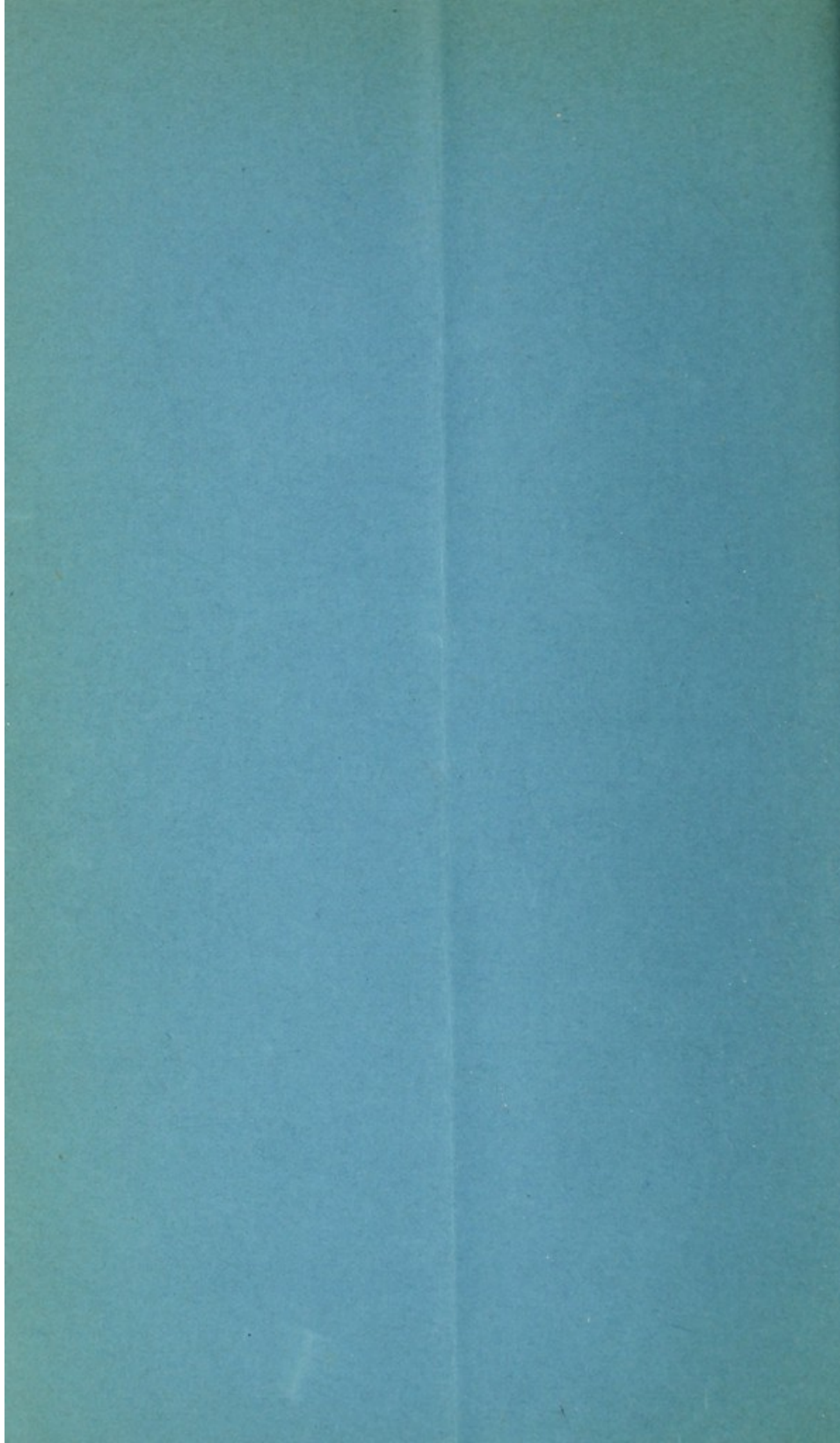
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LECTURES ON THE EARLY STAGES IN THE DEVELOPMENT OF MAMMALIAN OVA AND ON THE FORMATION OF THE PLACENTA IN DIFFERENT GROUPS OF MAMMALS. By ARTHUR ROBINSON, M.D., *Hunterian Professor*. (Plates XVII. and XVIII.)

(Delivered at the Royal College of Surgeons in November 1903.)

Lecture I.

MR PRESIDENT, Fellows and Members of the College, Ladies and Gentlemen,—Twenty-eight years ago, many years before I had the good fortune to enter his department as a student, my most esteemed master, Sir William Turner, delivered a series of lectures on the Comparative Anatomy of the Placenta in this College, and much as, for its own sake, I appreciate the honour of being elected by the Council to lecture here, that honour is increased by the fact that I am thus permitted to follow in the footsteps of a great teacher, to whose stimulating influence and ever-ready advice and help I shall always remain indebted.

In the lectures which he delivered, in 1875, Sir William Turner described more especially his own observations, and many of the statements which he then made stand unchallenged to-day, more especially those which referred to the formation of the crypts in the decidual mucous membrane, and to the distribution of the decidual vessels; but in this subject, as in others, great advances have been made in the methods employed, and by their means new facts have been disclosed. With continually advancing methods the number of workers has also increased, and if I were merely to read a list of the names of those who have made investigations on mammalian ova and the formation of the placenta during the last twenty years, and the titles of their monographs and papers, it would occupy the greater part of the time at my disposal. This, however, is quite unnecessary, and it is my intention to place before you, as shortly and as clearly as I can, a summary of the work which has been done, selecting examples of development which seem to me most pertinent, and including in the summary the results of my own work, which was commenced some years ago in the Anatomical Department of the Owens College, where I had the active sympathy and help of Professor Young, and which has been continued, as the opportunity occurred, in the Anatomical Departments of the Middlesex Hospital and King's College, to whose authorities I am indebted for the facilities placed at my disposal.

At first sight it might appear that the consideration of placental formation alone is more than sufficient to occupy the three hours devoted to these lectures, and whilst that is undoubtedly the case, yet such a consideration, which did not take into account the early

changes in the ovum, would be insufficient, for it is in these early stages that Nature, whilst she is preparing the rudiments of an embryo, is still more strenuously preparing the means for its maintenance, and in this respect, at all events, she acts in the most business-like manner, preparing the means before she proceeds to the end. It is advisable, therefore, to consider the early changes in order that the later may be properly grasped, and as the first step in the early stages commences with the ovum itself, it is obviously necessary to consider its structure and capabilities.

Mammalian ova are germ cells which have probably descended, in direct line, from the most primitive animal cell. During their descent they have acquired capabilities additional to those possessed by the original cell, partly on account of the effects of the continually changing circumstances in which they have been placed, and to which they were able to adapt themselves, and partly on account of the repeated separation of parts of their substance with its associated potentialities and their subsequent fusion with germ cells of the opposite sex, whereby new potentialities have been acquired. They contain, therefore, within their substance the basis of the essential capabilities of their ancestors and the main possibilities of their descendants.

When subjected to our present powers of inspection, their structure does not differ greatly from that of an ordinary animal cell (Pl. XVII., fig. 6)—that is, each ovum possesses a body in which are enclosed a nucleus and its contents, an attraction sphere and centrosome are possibly present, and a number of granules called yolk granules. In addition, the ovum is surrounded by certain coverings which are called the zona pellucida and the membrana granulosa.

The nucleus is a spherical vesicle of comparatively large size. It contains one or more nucleoli, a reticulum of unstainable substance called achromatin, which enmeshes a more fluid nuclear juice, and connected with or embedded in the achromatic network are a number of masses or rods of chromatin, which, under certain circumstances, may run together into skeins, and again separate into rods or loops. Each portion of chromatin is believed to consist of innumerable, almost unimaginably small particles, and in these are supposed to reside the inherited powers and the future possibilities of the embryo which will be formed as a result of the union of the mature ovum with the essential parts of the male germinal cell. The fate of the protoplasm or idioplasm of the particles is a matter of very considerable and practical importance in association with the capabilities of the segments into which the fertilised ovum divides, and to this subject it will be necessary to return subsequently.

The outer boundary of the nucleus, in certain stages, is a distinct membrane, which appears to be continuous with the reticulum of the nucleus, and when the ovum is dividing during maturation, or segmentating after fertilisation, the membrane entirely disappears; probably, therefore, it is the optical result of an aggregation of the peripheral ends of the fibrils of the reticulum, which is resolved, in association with the active changes which occur at certain definite periods.

The nucleolus is a spherical vesicle which disappears and reappears simultaneously with the nuclear membrane, and little or nothing is known with regard to its functions or capabilities.

The body of the ovum, like the nucleus, consists of a firmer portion, the cyto-reticulum, whose meshes are filled with a more fluid cytolymph, and in the latter are embedded the yolk granules, which increase in number as the ovum becomes more mature, and which are, in all probability, food material stored against the time when food will be less readily obtainable, or specialised food stored against the time when the ovum will be capable of using it. The yolk granules vary in size and number in different ova, and they are situated between the nucleus and the periphery of the cell, the larger granules forming an intermediate stratum with the smaller granules to their inner and outer sides.

The remaining content of the cell body, the attraction sphere with its centrosome, is not always visible, and, indeed, its existence in the mammalian ovum has been denied, but as it is present in the majority of animal cells and in the ova of lower forms, presumably it ought to be present in mammalian ova, and possibly the bodies which have been described as the body of Balbiani, the yolk nucleus, and the sphere, are to be looked upon as of the nature of attraction spheres. They are not visible at all stages in the development of the mammalian ovum, and, so far as my own observations go, are most readily seen before the space appears in the Graffian follicle. Then the body in question lies in the immediate neighbourhood of the nucleus. It consists of a central lighter sphere (Pl. XVII., fig. 6), enclosing one or two smaller spheres, and surrounded by a more darkly staining protoplasm. Fig. 6, Pl. XVII., is a camera lucida drawing of a ferret's ovum, and in the same ovary there are other ova, in some of which the clear sphere encloses only one smaller sphere, which then resembles the centrosome of an ordinary attraction sphere. I am inclined to think, therefore, that the body in question may represent an attraction sphere, but it disappears in the later stages of the growth of the ovum, and it is possible that it is only a special body in some way associated with yolk formation: if so, the presence of an attraction sphere and centrosome in the ovum is wanting, and in this respect the mammalian ovum would differ from an ordinary animal cell.

The Coverings of the Ovum.

The coverings of the ovum are (1) the zona pellucida, which is also known as the zona striata, and as the oolemma; and (2) the corona radiata, which is derived from the cells of the Graffian follicle.

The Zona Pellucida under comparatively low powers of the microscope appears a structureless homogeneous covering of glassy appearance. It stains readily with carmine and picric acid. On section it sometimes presents indistinct concentric striations, and occasionally shales are stripped from its surface (Pl. XVII., figs. 4, 9, 10); these may be taken as indications of its formation in concentric layers. There is no indication of it around the youngest ova, but it appears

shortly after the cells of membrana granulosa have assumed a cubical form, and it increases in thickness till the ovum is ejected from the ovary. Under high powers of the microscope numerous radial striations are seen in its substance, and these appear to be caused by fine radial canals which pass from its inner to its outer surface. There is some doubt as to whether it is formed by the ovum and is therefore a vitelline membrane, or whether it is formed by the cells of the membrana granulosa. Certainly if it is formed by the ovum it is not formed at the expense of the ovum, for the latter increases in size as the zona increases in thickness (compare figs. 1-5, Pl. XVII.), consequently the zona must be expanded as it is formed. Further, although the outer surface of the ovum is closely applied to the inner surface of the zona, it is not usual, when the method of preparation of the specimen has caused contraction of the body of the ovum, for portions of the latter to remain attached to the zona; therefore, if the zona is formed from the ovum, it is more probably a secretion than a condensation of the peripheral parts of the ovular substance. On the other hand the cells of the corona radiata are closely attached to the outer surface of the zona, and seem to be intimately blended with its substance (Pl. XVII., figs. 8 and 9). Indeed the inner extremities of the majority of the cells of the corona appear to abut against and blend with the outer surface of the zona, much in the same manner that the spongioblasts of the spinal cord blend with the external and internal limiting membranes. There is also reason to believe that processes of the cells of the corona pass through the radial canals of the zona and attain intimate contact with the surface of the ovum. So far, therefore, as mere relationships of contact and continuity can be taken as indications, there seems to be a greater probability that the zona pellucida is formed from the cells of the corona radiata than that it is secreted by or formed from the substance of the ovum. Moreover, as after death, and presumably during life, it is a fairly resistant membrane, it would seem to be rather more easy for the growing ovum to expand a membrane on which deposits are being formed externally—such deposits being probably of softer character than the finished membrane—than it would be to expand the more finished layer whilst depositing a second and softer layer on its inner surface.

The Corona Radiata.

In the early stages of ovular growth the cells of the Graffian follicle are flat plates which can scarcely be distinguished from the surrounding cells of the stroma of the ovary. Gradually they assume a cubical shape, and so become very distinct, forming a single layer round the periphery of the ovum (Pl. XVII., fig. 1). Then they proliferate, and ultimately form a large mass of columnar and cubical cells in which the ovum is embedded. Spaces appear between the cells of the mass on one side of the ovum; these gradually enlarge and stretch the cell bodies into a fine reticulum, which is ultimately ruptured, a space being formed (Pl. XVII., fig. 3). This fills with fluid, the liquor folliculi, and gradually extends till the ovum, with

some granulosa cells around it, is separated from the wall of the follicle, except over a small area, where continuity is still maintained by a pedicle of cells. At the apex of this pedicle the ovum and the immediately surrounding granulosa cells, which now constitute the corona radiata, project into the cavity of the follicle.

When the ovum is mature and leaves the ovary, it is still surrounded by the corona radiata and the zona pellucida. The former soon disappears, and in some animals—the dog, the rabbit, and the opossum—is replaced by a homogeneous sticky substance, called the albumen, which is possibly formed from the cells of the corona. This may serve as nutriment which is absorbed through the pores of the zona, and possibly as a means whereby temporary adhesions may be formed with the maternal mucous membrane; but even in the cases where it is formed, it only exists at very early periods, and though the albumen and the zona pellucida have been termed the primitive chorion, they only foreshadow in a very dim way the functions of the permanent chorion.

The Functions of the Coverings.

With regard to the corona radiata it probably takes a part in increasing the thickness of the zona, whilst the ovum still remains within the ovary. Very probably also processes of its cells are utilised as food by the ovum; indeed, there is no other source whence pabulum for the growing ovum can be derived, unless it be that the cells of the corona secrete material which passes through the zona to the ovum in its anterior. Finally it most probably takes part in the formation of the albumen in those animals in which albumen is formed, and in other cases its cells degenerate and are cast off, or are utilised as food whilst the ovum is in the highest parts of the Fallopian tube.

The zona pellucida differs very greatly in thickness, not only at different periods of its growth, but also in different animals (compare figs. 1-10, Pl. XVII.), and it also persists for a longer time in some animals than in others.

In *Tupaja javanica*, according to Hubrecht, it may disappear by the two-cell stage. In the macaque monkey Selenka states that it has disappeared by the four-cell stage. In the mouse and rat it has usually gone by the eight-cell stage, though it may persist till a later period. In the hedgehog and in bats it disappears before the blastocyst is formed. In the mole and the shrew it does not disappear till the embryonic ectoderm appears on the surface of the ovum. It disappears at a somewhat earlier stage in the sheep and pig, whilst it persists in the dog and the ferret until the formation of the primitive streak and the commencement of the formation of the mesoderm.

Its function is not quite obvious, and the suggestion that it is to protect the ovum from injurious pressure is contradicted both by the difference in the time at which it disappears in different animals, and by the fact that it disappears early in those animals in which the ova

become embedded in the decidua at an early period, and which are more likely, therefore, to be injured by contractions of the uterine wall than the ova which lie free in the uterine canal.

It probably prevents the contact and union of the chorionic cells on the outer surface of the developing ovum with the decidua until the ovum has attained a sufficient size. It also probably serves to prevent contact of the embryonic ectoderm with the uterine wall until the differentiation of the embryonic ectoderm cells has taken place to such an extent that they are no longer disposed to fuse with the decidua. The latter probability attains support from the fact that in ova such as those of the mouse, rat, guinea-pig, hedgehog, bats, and probably in the ova of the apes, and in human ova, in which the embryonic ectoderm never attains a surface position, but is separated from the maternal tissues by other cells or by a fluid-filled space of considerable size, the zona disappears at an early stage of development, that is, before the blastula stage is attained. It is true that the zona also disappears at a comparatively early stage in the ova of pigs, sheep, and deer, but in these animals there is no tendency for the union of the outer surface of the ovum with the uterine decidua, at all events after the disappearance of the zona, whilst in the carnivora, in which the tendency of the outer surface of the ovum to unite with the decidua is well marked, the zona persists till a much later stage of development, that is, till the embryonic area is well differentiated and the mesoderm formation has commenced. Moreover, in the latter animals the zona disappears considerably earlier from those regions which take part in the formation of the placenta, and which, therefore, fuse with the uterine wall, than it does in the embryonic area, where it can be distinguished for a considerable period after the commencement of the union of the chorion with the maternal tissues.

The Maturation of the Ovum.

Before the ovum is ready to proceed to the formation of a normal embryo it must become mature, and the maturation which commences whilst the ovum is in the Graffian follicle may be completed there or in the upper part of the Fallopian tube. It consists of a double mitotic division, whereby, in typical cases, the ovum is divided into four cells, one of which is much larger than the others and constitutes the mature ovum. The other three are small and are called the polar bodies. In some cases, however, as in the mouse, according to Sabotta's observations, only one maturing division may occur and only one polar body appear, whilst in other, more numerous, cases only two polar bodies appear, this being due to the fact that the polar body formed at the first division does not divide when the second polar body is separated from the ovum.

When maturation is completed the nucleus of the larger segment, or mature ovum, is known as the female pronucleus. In association with this process of maturation a significant fact must be noted. It is known that the nuclei of all animal cells contain a definite number

of chromatic masses or chromosomes. This number for the germ cells of the human female is believed to be sixteen, but whilst for the moment the exact number is a matter of little import, it is of the greatest significance that before maturation commences half these chromosomes have disappeared. Exactly when this disappearance occurs, and how it takes place, is not known. Most probably it occurs during those early divisions of the primary germ cells of the ovary which precede the formation of the definite ovum. The importance is great, however, if it is admitted that in the constituent parts of the chromosomes are contained the tendencies and capabilities inherited from preceding generations. Further, it must be noted that in the process of maturation the number of chromosomes present at the commencement of the process is increased by the division of each into four parts, and by the subsequent divisions the number of segments thus produced is reduced to one-fourth. Obviously, therefore, if the tendencies and capabilities lie in the chromosomes the possibility of variations from the preceding type is greatly increased by this process of casting-off, multiplication, and reduction.

The Sex of the Ova.

It may perhaps, at first sight, seem ridiculous to speak of the sex of ova, inasmuch as they are situated in the ovary, nevertheless there seems to be reason for believing, as Dr Beard has shown, that some of the ova will only produce male embryos after their union with a spermatozoon, and that others will only produce females, the spermatozoon and the conditions which follow fertilisation having no influence on the sex of the embryo, which was probably determined when the chromosomes were being reduced, in the periods which preceded maturation. If this is the case, although every well-developed ovum is destined under favourable circumstances to develop either into a male or a female embryo, the sex of the embryo is determined before the ovum leaves the ovary, and no combination of subsequent circumstances can alter it; consequently it follows that the ova as they lie in the ovary are either male or female, but there is no means yet available whereby we can distinguish, in mammals, which ova are male and which are female, neither do we know whether or not ova of different sexes are cast off alternately or in series.

Fertilisation.

Fertilisation is the union of the male and female germ cells, and the most essential part of the process is the restoring of the number of chromosomes in the ovum to that at which it previously stood before the reduction which precedes maturation had occurred. The union of the mature ovum with the spermatozoon may take place in the ovary or in the upper part of the Fallopian tube, and the spermatozoon may enter the ovum between the formation of the first and second polar bodies. It is generally believed that only the head and body of the spermatozoon pass through the zona pellucida,

and that after entering they rotate on their long axis through half a circle, but Van der Stricht has shown that in one of the bats the whole spermatozoon enters the ovum and does not rotate after entrance, but the head and body form a pronucleus, and the tail ultimately disappears, but may persist till the first segmentation spindle has formed.

Whether part or whole of the spermatozoon enters, it eventually becomes transformed into the male pronucleus, by the side of which an attraction sphere appears. Gradually the male pronucleus and the female pronucleus approach each other, and ultimately they fuse together to form the first segmentation nucleus, which contains as many chromosomes as the original germ cell. After the formation of the first segmentation nucleus by the union of the pronuclei, two attraction spheres and centrosomes are found in its immediate neighbourhood, and it is not yet decided whether they are both derived from the centrosome and the attraction sphere which appeared in the neighbourhood of the male pronucleus, or whether one belongs to the male pronucleus and the other is the female attraction sphere, which has now reappeared.

Segmentation.

After fertilisation is completed segmentation of the ovum commences. An achromatic spindle forms between the two attraction spheres. The chromosomes accumulate at the equator of the spindle and separate into apparently equal parts, which migrate to the opposite poles of the spindle. Thereafter the ovum divides into two equal or unequal portions, each of which contains the ordinary constituent parts of a cell.

The question which now arises is: Are these two portions of equal value? That is, has each part during the division received an equal amount of idioplasm, and, at the same time, is it the same kind of idioplasm; in other words, do the two daughter cells possess equal capabilities? in which case the first division has merely been quantitative; or does each daughter cell possess special characteristics by which it may be distinguished from its fellow, the division having been qualitative as well as quantitative?

The observations which have been made by Roux, O. Hertwig, and Morgan on frogs' ova, and by Wilson on amphioxus ova, for the purpose of settling these important practical points, are somewhat contradictory, but Roux deduces from the results of his own experiments the conclusion that the divisions which occur during segmentation are qualitative as well as quantitative. He believes that special parts of the idioplasm are allotted to the descendant cells at each division, and that, as a consequence, each descendant is only capable of following its allotted course in the further history, and that its place cannot be taken by other cells. Segmentation according to this plan is known as mosaic segmentation, for by it is produced a kind of mosaic-work, in which each element has its own definite destiny, which it cannot escape or override, and apparently such segmentation does to a greater or less extent occur in some

of the invertebrata—for example, in *Nereis*, according to Wilson's observations.

According to Roux's experiments, at the first division the ovum is separated into right and left halves, and the second divides the two halves into anterior and posterior segments, so that of the first two cells each can only produce its own half of the body, and of the first four each is the predecessor of one quarter of the body, a fore or a hind quarter of the right or left side according to its position, and undoubtedly such half and quarter embryos have been produced. If segmentation is strictly qualitative as well as quantitative, it follows that each cell of each tissue of the adult body can neither revert to a more primitive condition, nor can it by any possibility produce cells absolutely similar to those of another tissue; that is, a mesodermal cell, be it either an endothelial cell of a blood-vessel or a cell of ordinary connective tissue, cannot, by abnormal proliferation, produce anything but a mesodermal tumour, whilst, on the other hand, entoderm and ectoderm would only be capable of giving rise to tumours whose cellular constituents were similar to the parent cell. Roux admits, however, a process which he calls post-generation. In this, half an embryo having been formed from one of the first two cells produced by the first segmentation, the other half can be produced, at a later stage, from the parts of the already formed half, if a suitable nidus is present, into which the margins of the various parts and organs in the already formed half can grow. Such a suitable nidus in the form of a dead cell or an indifferent mass of protoplasm being present, then each part and organ in the already formed half can produce its counterpart, the half spinal cord producing the other half, the half notochord its counterpart.

As an explanation of this phenomena of so-called post-generation, it has been suggested that at each cell division each daughter cell receives not only its special idioplasm, but also a portion of unaltered original idioplasm. The latter remains latent unless stimulated into activity by injury or other abnormal circumstances, but being stimulated, it enables the cell in which it lies to produce its counterpart.

There are, however, facts which seem to negative, or, at all events, to greatly modify, Roux's hypothesis, and whilst Hertwig in the first instance entirely denied the correctness of Roux's results, Morgan has shown that each of the first two daughter cells may either produce half an embryo, or a whole embryo of small size; and Wilson's experiments with *amphioxus* ova prove that whilst even at the eight-cell stage, each cell, if separated from its fellows, will attempt and will succeed in forming a rudiment which will develop as far as the blastula stage, it is not capable of developing into a complete embryo, though each of the four cells of the four-cell stage can, under favourable circumstances, produce a complete embryo of small size. It is to be further noted, in association with this subject, that segmentation does not proceed so regularly in mammals as it does in lower forms, and the two-cell stage may be followed by a three-cell stage, and the latter by a five-cell stage, instead of the regular, the

two-, four-, eight-, and sixteen-cell stages of orthodox segmentation. Moreover, the cells so produced are not necessarily of similar size. Under these circumstances it is scarcely possible to believe the results obtained in amphibia and amphioxus can be applied to mammals, at all events in the earliest stages, where there is no definite indication of bilateral arrangement or mosaic work. But, be this as it may, it is perfectly clear that in amphioxus, as segmentation proceeds, qualitative changes are taking place in the cells, for whilst each of the first two daughter cells of the ovum is capable of producing a living embryo, the cells of the four-cell stage can only do so with increasing difficulty, and the cells of the eight-cell stage, though they may travel a certain distance in the right direction, are incapable of reaching the wished-for end.

There are also indications of qualitative changes in the higher forms as segmentation proceeds, and such qualitative changes undoubtedly increase with each succeeding division of the cells, but the opponents of the mosaic plan of segmentation ascribe these changes not to differences in the idioplasm of the various cells produced at the cell divisions, but to physiological changes which take place in the cells after each division, which are due to the interaction of the cells of the cell complex upon each other, and to the action of surrounding influences. According to this view the specialisation which occurs is not due to any differences in the constitution of the idioplasm of the cells, but is a physiological differentiation, some portions of the idioplasm being utilised and stimulated into activity, whilst others remain latent, and this is due to the fact that in every compound organism the parts develop in relation to each other, and that the development of the parts is dependent on the development of the whole. Naturally, therefore, according to this view also, after each succeeding division, as the organism becomes more complicated, the specialisation of the cells becomes more and more perfect, the capabilities of the cells being more and more specialised in certain directions, according to their positions in the organism, and at the same time their other capabilities are thrown more and more into the background, but the idioplasm of all the cells is essentially the same. If this is so, it must be possible that under favourable circumstances any cell incapable of taking on the functions of any other cell, and one cell may replace another in the organism, and, to push the matter further, it should be possible under these circumstances for a connective tissue cell to produce an epithelial tumour, or, *vice versa*, for an epithelial cell to produce a connective tissue tumour.

So far as I am aware no evidence is yet forthcoming to prove that a cell can thus change its nature, and, in any case, we are pushed towards the conclusion that, as segmentation proceeds, different cells receive different portions of idioplasm, or that the idioplasm of each cell or group of cells can be so changed by the interactions that occur between it and adjacent cells and surrounding influences that its original characteristics and capabilities are profoundly modified, and it is unable to return to its primitive condition.

We know from Wilson's experiments on Annelids that qualitative changes become evident during the segmentation of invertebrate ova at an earlier period than they do in vertebrates, but in the latter, also, indications are not wanting that specialisation occurs. At one time it was believed that such specialisation was indicated by differences in the size and colour of the earlier-formed cells, but as Assheton's observations on rabbits, sheep, and pigs have shown, cells of larger and smaller size, and of lighter and darker colour, are so intimately mingled together that little reliance can be placed, in mammals, upon such differences as indicating differences of function, and it is impossible at present to speak with any certainty of distinct differentiation of the cells of the mammalian ovum till in the mass of cells resulting from the segmentation an outer layer and an inner group are recognisable. This differentiation is sometimes evident, as in the rabbit and in bats, before the cavity appears and transforms the solid mass or morula, which is present at the end of the segmentation, into the vesicle or blastocyst which succeeds the morula; and in other cases, as in the rat, not until the blastocyst is developed; but when once the distinction is apparent, the fate of the cells of the two groups is fixed within certain limits, as subsequent development shows. The cells of the outer layer are all called ectodermal cells, but, as we know from the observations on the later stages of development, ectodermal cells have not all the same functions. Some take part in the formation of the embryo, and they constitute the embryonic ectoderm. Others enter into the constitution of the amnion, which forms a protective covering for the embryo, and the remainder, which, in the first instance, are by far the more numerous, constitute the ectoderm of the chorion, and play an important part in the formation of that very essential nutritive organ, the placenta. Indeed, it is upon the latter cells that the nutrition of the embryo and the ovum depends in the early stages of development, before the placenta is formed, and for these cells collectively Prof. Hubrecht has suggested the convenient name of trophoblast. Under circumstances which have been outlined, it becomes important to inquire what the nature of the cells which form the outer layer of the morula of the early blastocyst is? Are they trophoblast cells alone, or trophoblastic cells together with amniotic and embryonic ectoderm cells? The answer to this question varies according to the animals dealt with, and before it can be answered the developing ova of various animals must be examined.

Opossum.—Selenka's observations on the ova of the opossum show that at the four-cell stage all four segments are of about equal size and shape. When the blastocyst is formed, it is at first unilaminar, and becomes bilaminar by the in-wandering of cells from a definite area of the surface. The cells which thus gain the interior become the entoderm; therefore the outer wall contains the rudiments of the embryonic ectoderm, the amniotic ectoderm, and the trophoblast.

The zona pellucida disappears before the germinal area is distinctly differentiated.

Pig.—In the pig after the segmentation is completed a blastocyst is formed whose outer wall consists of trophoblast, to the inner surface of which, at one pole of the ovum, an inner mass of cells is attached. The latter certainly contains the rudiments of the embryonic ectoderm and the entoderm, and it is possible that it

may also contain the rudiments of the amniotic ectoderm. The entoderm cells are differentiated from the more internal cells of the inner mass as in the bat (Pl. XVIII., fig. 21), and they rapidly migrate round the inner surface of the trophoblast, converting the originally unilaminar into a didermic blastoderm. When the separation of the entoderm is completed, a cavity appears between the trophoblast and the embryonic ectoderm. The roof of this cavity gradually disappears, the embryonic ectoderm thus attains a surface position, and its margins blend with the surrounding trophoblast. At this period the embryonic area of the ovum forms a relatively small part of the surface of the ovum, and as the ovum usually contains but little fluid in its interior, it assumes the form of a flaccid folded tube, although, if it were fully distended, it would have an almost spherical contour.

In the earliest stages the tube-like ovum is of no great length, but it grows until it frequently attains a length of 39 inches.

The zona pellucida disappears before the embryonic area attains its typical form.

Sheep.—In the sheep also, when the blastocyst stage is attained, the outer wall of the vesicle is formed by trophoblastic cells, and the inner mass represents the embryonic ectoderm and the entoderm. The cells of the entoderm separate off from the inner part of the mass, and, as they proliferate, they extend round the inner surface of the trophoblast. Ultimately, without the appearance of a cavity, the trophoblast over the inner mass disappears, and the embryonic ectoderm becomes superficial and forms a small round area on the surface of a long tubular ovum, which terminates in bulbous extremities.

The zona pellucida vanishes before the trophoblast has disappeared from the surface of the embryonic ectoderm, but not until the ovum has attained a considerable size.

Roe Deer.—The stages of segmentation and morula formation in the roe deer do not differ essentially from those of the pig and sheep. The blastula (Pl. XVIII., figs. 14, 15 and 16) closely resembles the blastula of the pig and mole at a similar stage, the outer wall of the vesicle consisting entirely of trophoblast, and the inner mass representing the rudiments of the embryonic ectoderm and the entoderm. The latter separates from the ectoderm and gradually extends over the inner surface of the trophoblast, whilst the trophoblast over the embryonic ectoderm disappears. The latter thus attains a surface position, and its margin becomes indistinguishably blended with the adjacent trophoblast cells.

Cat.—In the cat the stages between the morula, which consists of larger inner and smaller outer cells, and the didermic blastoderm have not been traced. The greater portion of the wall of the bilaminar blastoderm is trophoblastic, the embryonic and amniotic regions forming but a small section of the citron-shaped vesicle.

The exact stage at which the zona pellucida disappears is not known, but it is before the neural groove is well established.

Dog.—The stages which precede the formation of the bilaminar blastoderm have not been traced in the dog, and when the latter is formed it is separable, as in the cat, into embryonic, amniotic, and trophoblastic or chorionic areas, all of which are bilaminar. The ovum at this stage is citron-shaped, and the embryonic area forms only a small part of the whole surface, the trophoblast constituting the major part.

The zona pellucida does not disappear till after the formation of the primitive streak. Even after the embryonic area is well differentiated the zona pellucida is covered externally by a layer of albumen, from which numerous villous processes project.

Ferret.—In the ferret in the four-cell stage I have not been able to distinguish any very great difference between the cells. My later stages of morula formation are not yet quite satisfactory, and in the youngest blastocysts I have obtained the embryonic ectoderm forms small part of the surface of a fairly spherical ovum, the greater part of which consists of trophoblast. The entoderm covers the under surface of the embryonic ectoderm, and extends a short distance beyond it on the inner surface of the trophoblast.

The zona pellucida persists until shortly after the formation of the primitive streak in the embryonic area, but it disappears in the region of the trophoblast at an earlier period.

Rabbit.—In the rabbit, at the end of segmentation, the outer layer of the morula consists of trophoblastic and possibly amniotic ectoderm cells, and the inner mass contains the rudiments of the embryonic ectoderm and the entoderm. The same conditions persist during the early stages of the blastocyst, but after the differentiation of the inner mass into embryonic ectoderm and entoderm, and as the latter extends round the inner surface of the wall of the vesicle, the external ectoderm which covers the embryonic ectoderm and which is known as Rauber's layer gradually disappears.

When this disappearance is completed the embryonic ectoderm attains a surface position and its margins become continuous with the inner margin of the amniotic ectodermal layer.

The zona pellucida disappears as the embryonic area forms.

Rat and Mouse.—The conditions in the rat and the mouse differ from those met with in the rabbit, for, in the cases of both the former animals, when the blastocyst is formed not only does its outer wall consist entirely of trophoblastic cells, but the inner mass also contains the rudiments of a further portion of the trophoblast as well as the rudiments of the amniotic and embryonic ectoderm and the entoderm (Pl. XVIII., figs. 17, 18, 19 and 20). The young blastocysts of these animals are somewhat spherical or ovoid in form (Pl. XVIII., fig. 17), but as development proceeds they become distinctly cylindrical (Pl. XVIII., fig. 18). The core of the cylinder is formed by the inner mass, which is gradually differentiated into an outer segment, the internal trophoblast, continuous with and of the same nature as the superficial trophoblast, an intermediate part of amnio-embryonic ectoderm, and a small number of internal entodermal cells which gradually extend over the inner surface of the trophoblast.

When they are first differentiated the internal trophoblast and the amnio-embryonic ectoderm are both solid masses of cells, but soon they both become hollowed by the appearance of a cavity in the midst of each mass. These cavities enlarge, and ultimately they perforate the adjacent sections of the internal trophoblast and the amnio-embryonic ectoderm. In the meantime the entoderm has covered their external surfaces and has wandered over the inner surface of the external trophoblast. After their cavities have united the inner margin of the internal trophoblast and the outer margin of the amnio-embryonic ectoderm are in contact with each other, but they do not become continuous (Pl. XVIII., fig. 19), as was the case in the animals previously considered. On the contrary a clear line of demarcation is always visible between them. At a later stage, as the mesoderm appears and extends, both the inner trophoblast and the amnio-embryonic ectoderm again become closed vesicles, which are separated from each other by the coelomic cavity which appears and rapidly extends in the mesoderm (Pl. XVIII., fig. 20).

It is at this period of the development of the rat and the mouse that they, in common with several other rodents, present that peculiar relationship of the layers of the blastodermic vesicle which is known as inversion. There is no inversion in the true sense of the word, that is, in no place does the inner take the position of the proper outer layer of the ovum, but the ovum consists of a series of closed tubes, an outer tube of trophoblast closed at each extremity and continuous at one extremity, which may be termed the upper, with the inner trophoblast. Inside the external trophoblast is a tube of entoderm, closed at the lower end, and continuous at the upper end with an internal tube of entoderm, which, in its turn, encloses an internal tube of three sections, an upper of internal trophoblast, a middle of mesoderm, and a lower of amnio-embryonic ectoderm (Pl. XVIII., fig. 20).

The condition thus produced is merely a kind of invagination which enables the ovum to form a greater surface than would otherwise have been possible with the space at its disposal, and it is obvious that it might have easily been produced in a blastocyst of the common type by a force acting on the central part of the embryonic area, which pushed the embryonic area, and afterwards the surrounding embryonic and trophoblastic ectoderm, into the interior of the ovum.

The zona pellucida disappears at or before the eight-cell stage, and before the ovum is embedded in the decidua.

Guinea-pig.—The guinea-pig is even more peculiar than the rat and the mouse, for, although its young blastocyst corresponds closely with the blastocysts of the

latter animals, the internal trophoblast and the amnio-embryonic ectoderm soon become separated by a wide space which occupies the position of the primitive coelom of the rat and mouse, but which, in the guinea-pig, is bounded externally by entoderm alone, and is devoid of a mesodermal lining until a much later period.

The zona pellucida disappears as the ovum is embedded in the decidua and before the inner cell mass is differentiated.

Mole.—In the mole the segmentation is somewhat similar to the segmentation of the rabbit, and when the blastocyst stage is reached, as in the latter animal, the inner mass consists of cells destined to form the embryonic ectoderm and the entoderm, and its superficial surface is covered by a mass of cells which correspond with the cells of Rauber's layer in the rabbit. These gradually disappear, the embryonic ectoderm enters into the formation of the surface of the ovum, and its margins become continuous without any line of demarcation with the remainder of the surface ectoderm.

The zona persists till the embryonic ectoderm becomes superficial.

The Shrew Mouse.—The segmentation of the shrew mouse does not differ essentially from that of the mole, and at its close a blastocyst is formed containing a small inner mass of cells, from which embryonic ectoderm and entoderm are differentiated. As in the mole, the surface cells over the inner mass disappear, and the embryonic ectoderm, which is evolved in the usual way from the outer portion of the inner mass, passes to the surface, where it forms a small embryonic area, the greater part of the blastocyst wall consisting of trophoblastic ectoderm which is lined internally by a layer of entoderm that spreads peripherally from the inner mass.

The zona persists till the embryonic ectoderm becomes superficial.

Tupaja javanica.—In *Tupaja javanica*, an East Indian insectivorous animal, described by Hubrecht, the conditions met with during the blastocyst stage are practically similar to those described in the pig. The whole of the outer wall of the primitive vesicle consists of trophoblast, and the inner mass contains the rudiments of the embryonic ectoderm and the entoderm. Also, as in the pig, the embryonic ectoderm reaches the surface by the disappearance of the trophoblast over the inner mass.

The zona disappears early.

Hedgehog.—In the hedgehog the conditions of the blastocyst stage, as described by Hubrecht, differ in some important points from those met with in the other insectivora, and the differences are of interest, for it is just in these points of difference from the other insectivora that the hedgehog seems to approach the conditions which are probably present in the early stages of the development of the human ovum, and the ova of the higher apes. The wall of the blastocyst, which is of ovoid form (Pl. XVIII., figs. 11, 12 and 13) consists of a comparatively thick layer of nucleated protoplasm, thicker at one pole than at the other, and in the interior of the vesicle there is a small mass of cells which ultimately becomes the entoderm.

As the blastocyst enlarges, the thicker pole of the vesicle becomes relatively still thicker and bulges into the interior, where it forms a mass of amnio-embryonic ectoderm, and, at the same time, the internal mass of entodermal cells does not extend, as in most other animals, round the inner surface of the wall of the blastocyst by peripheral extension, but it itself becomes a hollow vesicle, which gradually expands till its outer surface is in contact with the outer wall of trophoblastic ectoderm (figs. 12 and 13). After a time, as in the mouse and rat, a cavity appears in the amnio-embryonic ectoderm, and its outer wall is continuous with the trophoblast, from which it is not separated till the mesoderm of the amnion folds appears.

The zona disappears very early, before the expansion of the entoderm.

Bats.—In bats the outer wall of the young blastocyst consists entirely of trophoblast, and the inner mass represents the rudiment of the embryo-amniotic ectoderm and the entoderm. As the blastocyst increases in size, a cavity appears in the inner cell mass; and in the early stages, at all events, in all bats the roof of this cavity is formed both by trophoblast and amniotic ectoderm, but according to Duval's figures, this roof disappears after a time in the central part of its extent, and the amniotic cavity is closed by the uterine tissues. In *Hippocrepis vulgaris*, however, Van Beneden notes the persistence of the roof of the amniotic cavity, and

he points out that the trophoblast divides in the whole of its extent into an outer syncytial layer, which he terms the plasmodiblast, and an inner cellular layer for which he proposes the term cytoblast.

In the fruit bats, also, according to Dr Gohre's observations, the cavity which appears in the inner cell mass does not open on to the surface of the ovum, but remains separated from the uterine tissues by the amniotic ectoderm and the trophoblast.

The zona pellucida disappears early, before the differentiation of the embryonic area.

Tarsius spectrum.—Hubrecht has shown that in *Tarsius spectrum* no special peculiarities are seen during the segmentation, and the blastocyst which succeeds is similar to that of *Tupaja*, the outer wall consisting of trophoblast, and the inner mass containing the rudiments of the embryonic ectoderm and the entoderm. The latter separates from the embryonic ectoderm, and gradually extends round the inner surface of the trophoblast, whilst the trophoblast over the inner mass disappears, and thus the embryonic ectoderm attains a surface position.

The zona pellucida disappears before the embryonic ectoderm reaches the surface.

Monkey, Apes and Man.—The segmentation stages of the ova of apes and men are unknown, except the four-cell stage of *macacus nemestrinus*, which is figured by Selenka, and in this stage no special characteristics are noticeable, except that the zona pellucida has already disappeared, such an early disappearance only being noted in other animals amongst the bats and the insectivora.

In the next youngest known stage both in the ova of monkeys, anthropoid apes and the human subject, the outer surface of the ovum is formed by trophoblast. This is lined internally by mesoderm, and in the interior of the ovum is an ectodermal vesicle, one wall of which is formed by the small but relatively thick embryonic ectoderm, and the remainder by the amniotic ectoderm. This ectodermal vesicle is attached to the inner surface of the trophoblast by a mesodermal stalk into which a prolongation of the amnion passes, and the entoderm forms a small vesicle which is surrounded by mesoderm and attached by that layer to the under surface of the embryonic ectoderm.

It follows from what has preceded that in all mammals which have been investigated the greater part or the whole of the surface of the ovum consists in the blastocyst stage of trophoblast. In all cases the rudiment of the entoderm is contained in the inner cells of the inner mass, and in many, if not in all, cases the inner mass also contains the rudiments of the amniotic ectoderm, this being the condition in the rat, mouse, guinea-pig, hedgehog, and bats. In other mammals the exact situation of the rudiments of the amniotic portion of the ectoderm are not at first distinguishable, and after they have differentiated it is not certain whether they were derived from the original outer layer or from the cells of the inner mass. It is clear, however, that whilst cells occupying similar positions in the earlier stages of development of the segmenting ovum do not necessarily possess similar functions, nevertheless a very considerable degree of specialisation has occurred at the end of segmentation, and again we are brought back to that question of qualitative and quantitative divisions which to the surgeon and physician are of such great practical importance. For if the qualitative changes have proceeded to such an extent by the time the blastocyst stage is reached, whence and how are going to be formed those germ cells on which the lives of future generations depend? The answer to this question, which has hitherto been deemed quite satisfactory, is that after the mesoderm or third layer of the germ has been secondarily differentiated from the ectoderm some of its cells, occupying a position called the

germinal ridge, which is situated on the dorsal wall of the abdominal cavity, proliferate and display the characters of germinal cells. If this is the case, then we must admit either that cells whose more remote ancestors took part in the formation of the ectoderm, and their more immediate ancestors in the formation of the mesoderm, have retained the capabilities of the original germ cells, in spite of the varying influences to which they must have been subjected, or that, having played many parts before they entered the germinal ridge, the influences there brought to bear upon them are such as to resuscitate the original capabilities derived from the parent cells. It would apparently follow, as a necessity, that every cell which attains a position on the surface of the germinal ridge is capable of becoming a germ cell. But, as only some cells which attain positions in the germinal ridge are capable of becoming germ cells, the idea naturally arises that it may be possible that during the segmentation of the fertilised ovum certain cells remain practically unchanged, receiving idioplasm similar to that of the fertilised ovum and carrying it unaltered through all succeeding generations, and that certain of these cells ultimately become located in the germinal ridge, which is the only situation favourable for their further growth and maturation, and the only situation whence they can pass to union with a germ cell of the opposite sex. This idea cannot at present be based upon any known facts of mammalian development, but it is not devoid of support, for Rabl and Beard have shown that, in elasmobranch fishes, germ cells which are distinguishable by characteristic features appear outside the embryonic area of the developing ovum and wander into the embryo, many of them ultimately reaching and entering the germinal ridge, whilst others, less fortunate in their wanderings, pass into the other parts of the body, and, being unfavourably situated, they degenerate and disappear under ordinary circumstances. It is possible, however, that such germ cells which have wandered into other parts of the body than the germinal ridge may persist and lie quiescent amidst the surrounding tissues until circumstances arise which facilitate their development, when they spring into activity and produce tumour formations, which, as they are derived from a germ cell, may have the characteristics of any of the tissues of the body, and may vary from a simple collection of undifferentiated cells to a more or less completely-developed embryo. Under these circumstances, as it has already been shown that one of the earliest and most active structures which a developing germ cell forms is the ectodermal trophoblast, it is not altogether unlikely that, given the requisite conditions, an abnormally-placed germ cell should develop until it produces a mass of cells similar to the trophoblast and at the same time similar to if not identical with cancer tissue. Indeed, Dr Beard strongly urges that carcinomata and other tumours also are the result of the more or less modified development of aberrant germ cells which, during embryonic life, wandered into other parts of the body than the germinal ridge. Certainly this contention receives support from the pathological observations of Wilms and Pick; nevertheless, although it may be accepted as the explanation of some

malignant tumours, it can scarcely account for all, and personally I can see no reason why any ectodermal should not under favourable circumstances proliferate more rapidly than ordinarily and why its descendants should not invade the adjacent tissues, just as we shall find the ectoderm of the ovum invading the uterine decidua. Unfortunately there is as yet no known method of recognising the germ cells of the mammalian blastoderm from the cells of the soma, nor of tracing them from the exterior into the body of the embryo, neither are there any means at our disposal whereby we may distinguish such cells during the formation and growth of the human embryo in any place except the ovary. It is possible that, as Dr Beard suggests, they arise at the thirty-two cell stage of segmentation, and at that period one cell is a germ cell and the remaining thirty-one are already differentiated to form the membranes and appendages of the embryo, but of this we have no absolute proof. Granting that it is the case, then, it may be that the descendants of the germ are all germ cells, one of which and its descendants become differentiated to form the embryo, and under these circumstances it becomes clear, as Dr Beard urges, that the germ cells are the most important structures and that the embryo and the adult into which it may develop constitute but a transitory nidus in which the germ cells attain that maturity which is necessary before they can combine with other germ cells for the production of a succeeding generation. According to this theory the germ cells are the alpha and omega, and they pass from a beginning of which we have no knowledge to an end which we cannot conceive, whilst the individuals in which the cells are located, though interesting in themselves, are not the end to which the cells are moving but merely the means by which they move to an unseen future.

Dr Beard's speculations are still unproved, but they point the way to a solution of many difficult pathological problems, and at the same time the fact that germ cells can be distinguished in the lower forms makes it very probable that they exist also in the higher forms, and, if this is the case, it is not sufficient to suppose that they arise from modified epithelial cells of the peritoneal cavity which penetrate the germinal ridge and become ova or spermatozoa. It is much more probable that the cells which form the germinal elements are the direct descendants of cells which during the period of segmentation receive portions of the primitive idioplasm from the fertilised ovum and transmit it unchanged to their descendants, and that the latter during the evolution of the embryo become located in the neighbourhood of the germinal ridge.

The most striking feature, however, in the early development of the mammalian ovum is the great preponderance of the trophoblast over the other portions, and as the trophoblast is the chief means by which provision is made for the nutrition of the ovum and the embryo, not only in the early but also in the later stages of development, it is clear, as before stated, that Nature is careful to make ample provision for the maintenance of the embryo before she proceeds to elaborate it.

In the majority of cases the trophoblast is situated entirely on the surface of the blastocyst, but in the rat, mouse, and guinea-pig a portion of it is located in the inner cell mass in the interior of the ovum, from which it is subsequently differentiated. Obviously, therefore, mere position at the end of segmentation is no absolute indication of the future fate or functions of a cell, except in the cases of the cells of the external trophoblast, for in some mammals the inner mass is the rudiment of the entoderm and the embryonic ectoderm alone, in others it also includes the rudiment of the amniotic ectoderm, and in the rat, mouse and guinea-pig, it contains, in addition, the rudiment of a portion of the trophoblast, which may be termed the internal trophoblast.

It is interesting to note that the internal trophoblast of rodents retains its cellular character, whilst the external trophoblast of rodents forms a nucleated syncytium, as in the hedgehog. The cellular character is retained until a comparatively late stage of development, and the internal trophoblast, therefore, resembles the cytoblast or inner layer of the trophoblast of bats, whilst the external trophoblast of guinea-pigs, rats, and mice corresponds with the plasmodi-blast of the bats. Compare figs. 17, 18, 19 and 20 with figs 21, 22 and 23.

EXPLANATION OF PLATES.

<i>B.</i> Body of Balbiani.	<i>MM.</i> Mesoderm.
<i>C.</i> Cytoblast.	<i>P.</i> Plasmodiblast.
<i>EAE.</i> Embryo-amniotic ectoderm.	<i>S.</i> Spaces which become the cavity of the Graffian follicle.
<i>EN.</i> Entoderm.	<i>T.</i> Trophoblast.
<i>IM.</i> Inner cell mass.	<i>Z.</i> Zona pellucida.
<i>IT.</i> Internal trophoblast.	
<i>M.</i> Membrana granulosa.	

Fig. 1. Section of the ovum of a ferret surrounded by a single layer of membrana granulosa cells. The zona pellucida has begun to appear. $\times 190$.

Fig. 2. Section of a ferret's ovum when the membrana granulosa possesses in some places two, and in others three, rows of nuclei. The zona pellucida is very little thicker than in the preceding stage, but the bodies of some of the surrounding membrana cells have become fibrillar and their inner ends are fused with the outer surface of the zona pellucida. $\times 190$.

Fig. 3. Section of a ferret's ovum when the surrounding membrana granulosa contains five rows of nuclei, and the rudiments of the cavity of the Graffian follicle have begun to appear as small fluid-filled spaces between the bodies of some of the cells. The zona pellucida is now distinctly thicker than in the preceding stages, and it is still closely connected with the inner extremities of some of the cells of the membrana granulosa. $\times 185$.

Fig. 4. Section of a ferret's ovum which lay in an almost mature Graffian follicle. The zona pellucida is much thicker than in the preceding stages, and it presents a laminated appearance. Its outer surface is closely connected with the cells of the corona radiata, some of which are represented in the upper and left part of the figure. $\times 185$.

Fig. 5. Section of a segmenting ovum of a ferret from the upper end of the uterus. The zona pellucida is thicker than in any ovum still lying in the ovary, and after staining and hardening it had become brittle, and was fragmented by the cutting razor. There was no trace of an albumen layer upon it. $\times 185$.

Fig. 6. Section of a ferret's ovum showing the body of Balbiani. $\times 400$.

Fig. 7. Section of a portion of the ovary of a half-grown mouse. The membrana granulosa in some of the follicles contains from three to five rows of nuclei, but there is no distinct evidence of a zona pellucida. The dark lines in

the figure round the bodies of the ova and the inner boundaries of the membranæ granulosa are merely limiting lines, and are not intended to indicate membranes. $\times 250$.

Fig. 8. Section of the ovum of a mouse from an almost mature Graffian follicle. This ovum is at the same stage of development as the ovum represented in fig. 4, but the zona pellucida is extremely thin; nevertheless it is closely connected with the inner ends of some of the cells of the membrana granulosa. $\times 250$.

Fig. 9. Ovum of a cat at a stage of development intermediate between the stages of ferrets' ova represented in figs. 2 and 3, but the zona pellucida is much thicker than in the latter ova, and it presents a laminated appearance. On its outer surface there is a granular layer with which the cells of the membrana granulosa are continuous. $\times 200$.

Fig. 10. Ovum of a cat at the same stage of development as the rat's ovum represented in fig. 8. The zona pellucida of the cat at this stage is much thicker than that of the rat, but it is equally closely connected with the inner ends of the cells of the membrana granulosa. $\times 150$.

Figs. 11, 12 and 13 are diagrams representing different stages of the development of the hedgehog.

Figs. 14, 15 and 16 represent three stages of development of the ovum of the roe deer.

Figs. 17, 18, 19 and 20 are diagrams representing four stages in the development of the ovum of the rat.

Figs. 21, 22 and 23 are diagrams representing three stages in the development of the bat *Hippocrepis vulgaris*.

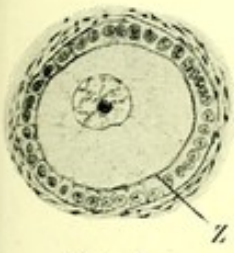


FIG. 1.

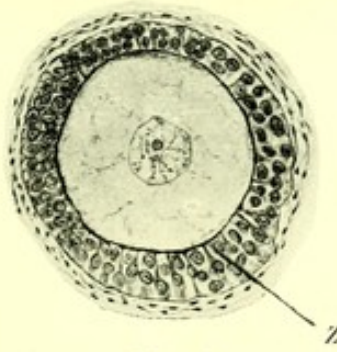


FIG. 2.

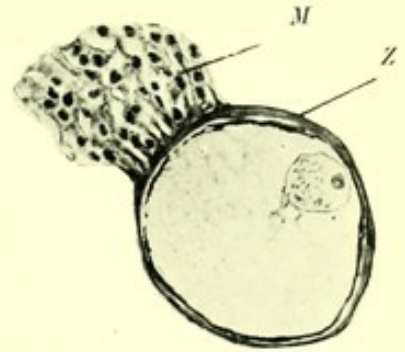


FIG. 4.

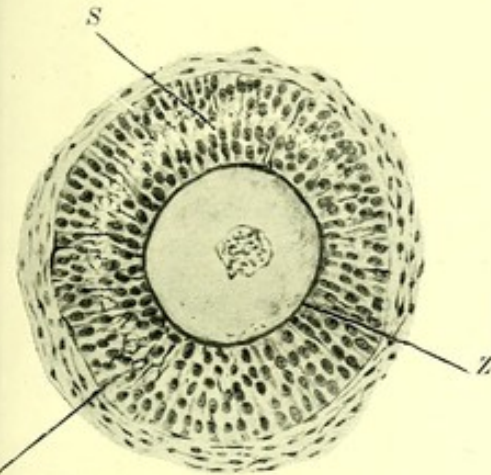


FIG. 3.

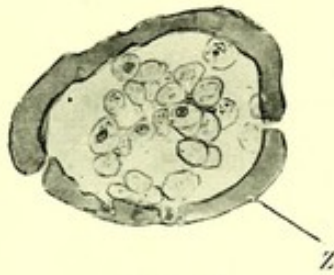


FIG. 5.

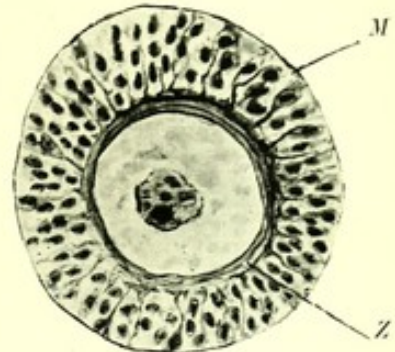


FIG. 9.

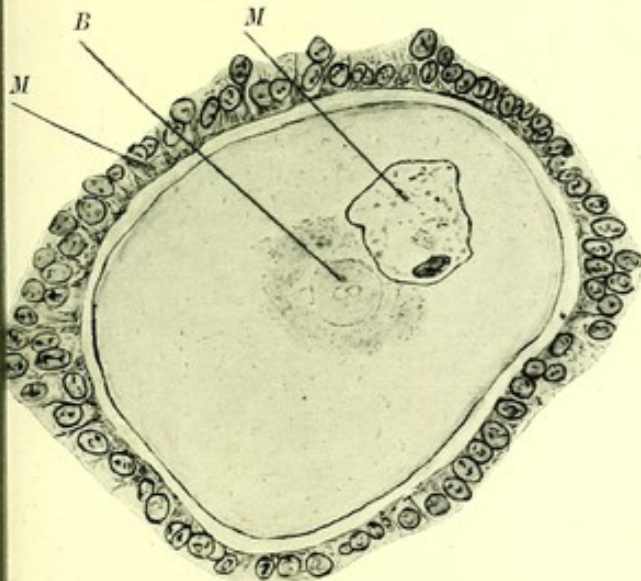


FIG. 6.

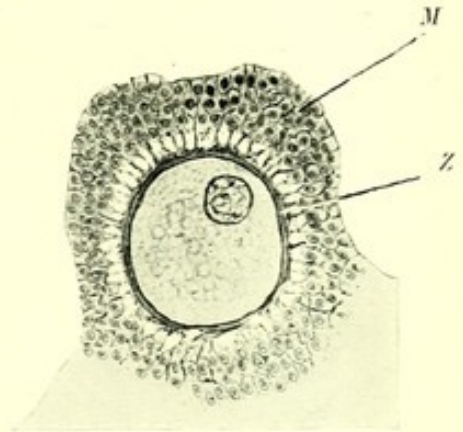


FIG. 10.

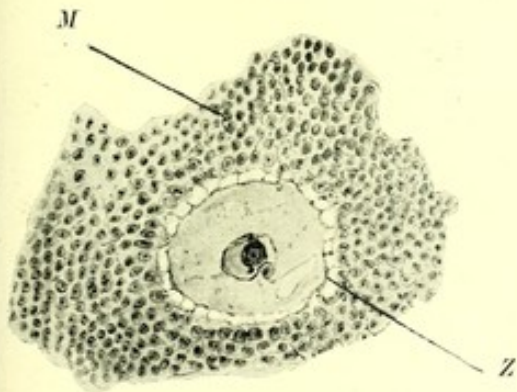


FIG. 8.

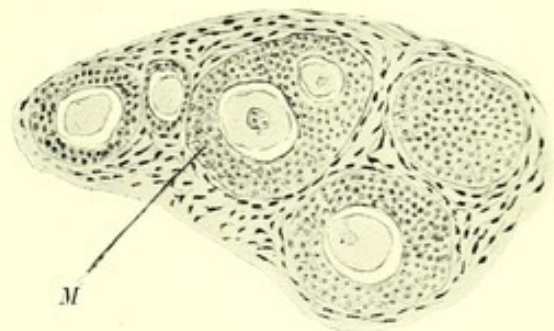


FIG. 7.



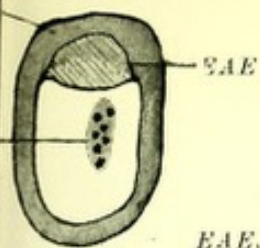


FIG. 11.

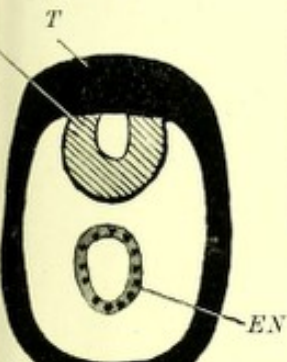


FIG. 12.

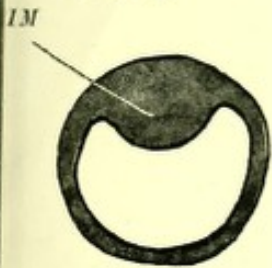


FIG. 17.

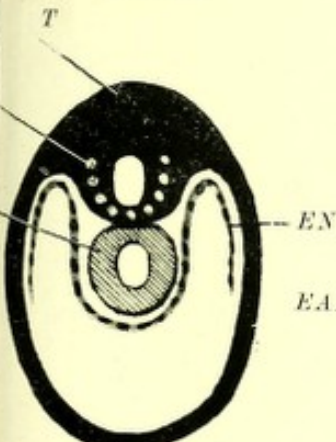


FIG. 18.

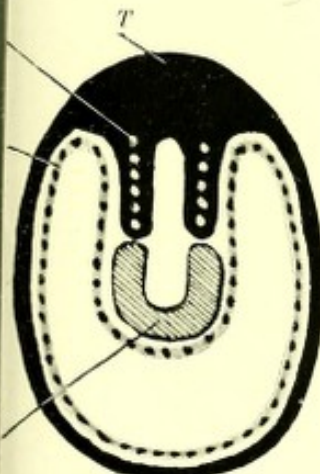


FIG. 19.

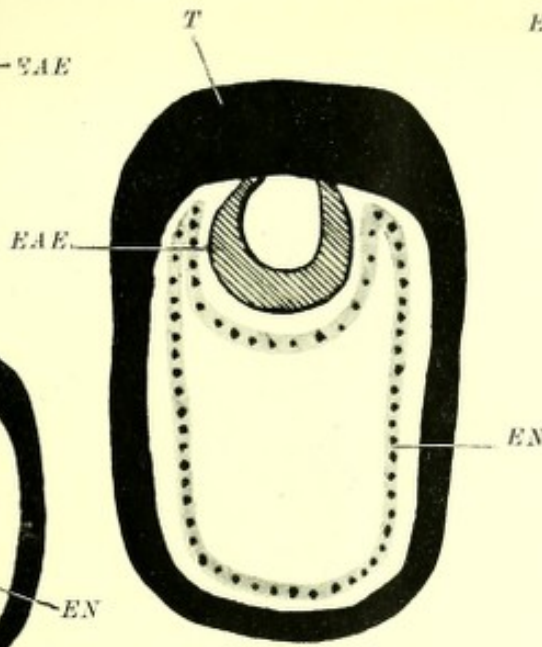


FIG. 13.

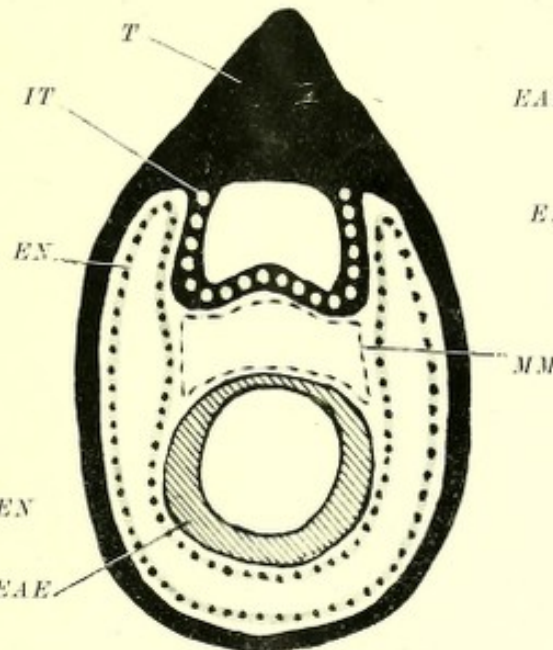


FIG. 20.

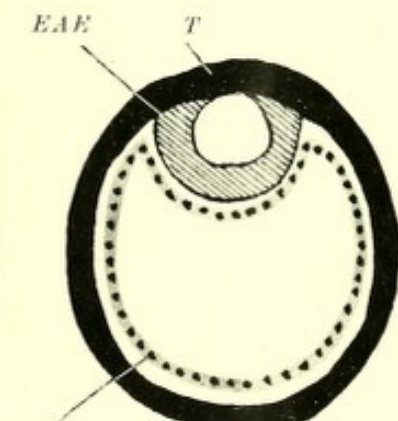


FIG. 22.

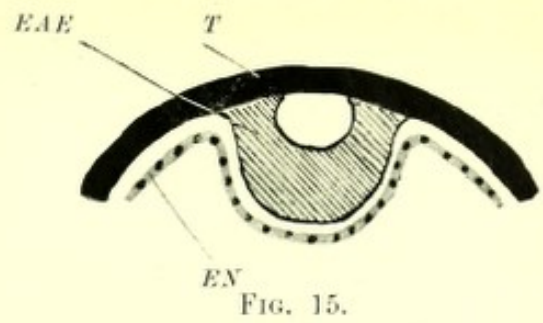


FIG. 15.

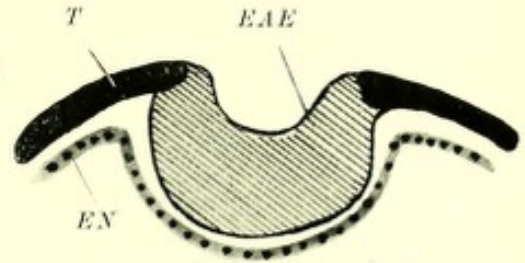


FIG. 16.

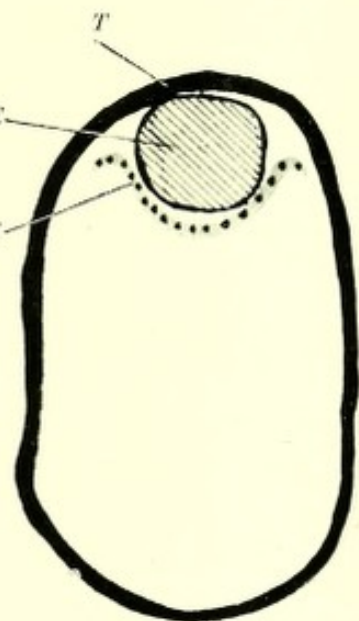


FIG. 14.

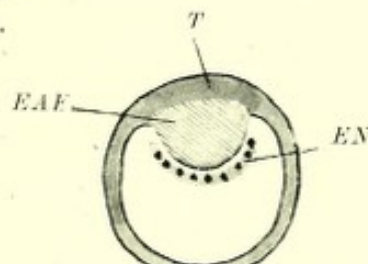


FIG. 21.

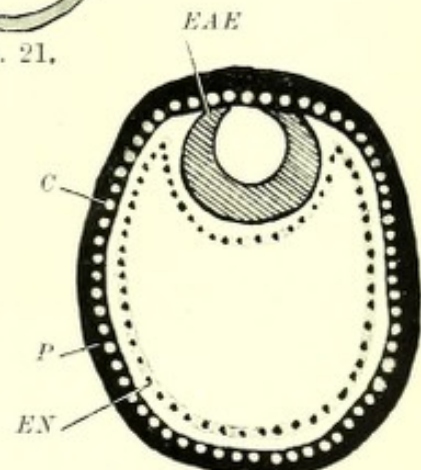


FIG. 23.

