XVII.—*On the Development of the Ova and Structure of the Ovary in Man and other Mammalia.* (Plates XXVII.—XXXI.) By JAMES FOULIS, M.D. (Edin.) Communicated by Professor TURNER.

(Read 21st December 1874.)

CONTENTS.

	PAGE		PAGE
Introduction,	345	Corpuscles in the Stroma of the	
The Ovary of the Calf,	349	Ovary,	361
The Ovary of the Kitten,	352	(d.) Development of the Egg Clusters, .	363
The Human Ovary,	357	(e.) The Development of the Membrana	
(a.) Nature of the germ epithelium, .	357	Granulosa,	365
(b.) The Relation of the germ Epi-		General observations on the Development	
thelium to the peritoneal epi-		of the Membrana Granulosa in adult	
thelium,	359	Ovaries,	368
(c.) The manner of inclusion of the Pri-		Structure of the Ovum,	375
mordial Ova and germ Epithelial		General conclusions,	376

In the month of August 1872, Professor TURNER suggested as a subject of investigation the structure of the ovary, and development of the ova, more especially with reference to the recently published observations of WALDEYER.

In the month of April 1874, I handed in to the Medical Faculty of the University of Edinburgh, as my graduation thesis for the degree of M.D., an essay entitled "Contributions to the Normal and Pathological Anatomy of the Ovary and Parovarium."

Accompanying this thesis, were numerous microscopic preparations of the human foetal ovary, by means of which I was able to demonstrate my descriptive remarks on the anatomy of the organ. Since that date I have made many additional observations on the anatomy of the ovary, especially in connection with the development of the ova, and the formation of the membrana granulosa; and in the present memoir the result of my observations is given. During the last eighteen months, I have made numerous microscopic preparations of the ovaries of various foetal and adult animals, most of which I now possess, and a careful examination of these verifies my earlier statements on the development of the ova, and the structure of the ovary.

It is not my intention to give a lengthened account of the views of earlier observers on the development of the ova and the structure of the ovary. It may, however, be interesting to notice the following points in the history of this subject.

Previous to the time of DE GRAAF, the ovary and testicle were considered

identical in structure. In 1671, DE GRAAF introduced the word ovary, and described the Graafian vesicles as ova, which he said were produced in the ovary. The distinction between the true ovum and the Graafian vesicle was not made out till the year 1824, when Messrs PREVOST and DUMAS, by numerous observations, were led to the conclusion that the ova were contained in the Graafian vesicles before conception. The mammalian ovum itself was discovered by VON BAER in 1827, and in 1834 COSTE and others discovered the germinal vesicle in the mammalian ovum. In 1835, WAGNER demonstrated the germinal spot and established the doctrine of a general uniformity in the structure and mode of origin of the ova of animals.

The discussions in the numerous works which appeared between the years 1835 and 1838 turned more or less on the question whether the germinal vesicle, or the ovum itself was first formed, or whether the Graafian vesicles were first produced, and the eggs subsequently developed in them. In MÜLLER'S "Archiv. für Anatomie und Physiologie," 1838, there is a paper by VALENTIN, on the development of the Graafian follicle in the ovary of mammals. He described the Graafian follicles as being formed within long tubes which originally constituted the special structures of the ovary, as the seminiferous tubes do of the testicles, but which become obliterated by the increasing growth of the Graafian follicles.

MARTIN BARRY, by his numerous observations, was led to the conclusion that the germinal vesicle is the part which first makes its appearance in the stroma of the ovary at the commencement of the formation of the ova.

VALENTIN, BISCHOFF, and others, held the view that the Graafian follicles may be detected in the stroma of the ovary before any part of the ovum can be distinguished.

It was with the appearance of PFLÜGER'S work that the views of the structure of the ovary and development of the ova took a new turn. VALENTIN, in 1838, as already observed, first demonstrated the branched and tubular glandular structure of the ovary; an observation which BILLROTH corroborated. Little attention, however, was paid to this observation until it was rediscovered by PFLÜGER, who in a detailed monograph developed his views respecting the structure of the ovary.

In the first place, PFLÜGER showed that the Graafian follicles with the eggs do not appear individually and independently in the stroma of the ovary. He described tubes as passing from the surface of the ovary down into the stroma, composed of a structureless membrana propria, by the inflections of which the several Graafian follicles are successively divided off.

PFLÜGER, (as quoted by WALDEYER),* stated that the tubes lying close beneath the surface of the ovary terminate by coecal extremities, and in these

* STRICKER'S "Handbuch der Gewebelehre," article Ovary, by WALDEYER, translated by Mr POWER.

the germinal vesicles originate surrounded by a diffuse protoplasm, that forms a more definite investment for each as they become more deeply situated with the tube. Thereupon a number of the cells become conspicuous by their more vigorous growth, whilst the rest remain unaltered, and form the epithelial lining of the tubes. The larger cells, which are primordial eggs, occupy the axis of the tube.

These subsequently increase in number by fission and budding, the products constituting the definite eggs that for a time remain connected with one another in the interior of the tubes in the form of a chain, by processes of protoplasm, constituting the "egg chains" of PFLÜGER. The membrana propria outside the epithelial lining of the tubes sends in processes between the individual eggs, in consequence of which the latter are gradually separated from each other along with a portion of the tubular epithelium; the latter forms the epithelial lining of the Graafian follicles in this way produced. PFLÜGER, in one of his figures (plate iii. fig. 1), has represented a connection existing between his tubes and the superficial columnar epithelium, and has frequently remarked that the contents of the egg tubes must proceed from the ovarian epithelium, which he always considers to be a serous epithelium.

The most important work published within the last few years on the development of the ova and ovary is that by W. WALDEYER, entitled "Eierstock und Ei." (Leipzig, 1870).

According to WALDEYER, the first appearance of the ovary consists of a thickened germ epithelium investing a small outgrowth rich in cells, which projects from the interstitial tissue of the Wolffian body on its median side. The thickened epithelium investing this outgrowth gradually forms the rudiments of the Graafian follicles and ova, and of the subsequently appearing epithelium of the ovary, whilst the outgrowth itself is destined to furnish the vascular stroma of the ovary.

In the embryo of fowls, WALDEYER states, the interesting observation may be made as early as the fourth day of incubation, that some among the germ epithelial cells have become conspicuous by their round form, their size, and the size of the nucleus they contain. We may conclude, from the regular arrangement of these structures and the constancy of their position, that they represent the youngest primordial ova, which thus, even during embryonic life, are formed by a simple process of growth from the epithelial cells of the germ organ. The further development of the ovaries depends on a peculiar mode of growth of the superficial epithelium on the one hand and of the vascularised stroma on the other. Certain more or less delicate processes of the connective tissue now shoot forth from the stroma, whilst coincidentally the epithelium increases by the continual production of new cells. The processes then penetrate between the epithelial cells, enclosing a variable number of them, which

thus by degrees come to be more or less deeply imbedded in the vascular stroma. From the plan and mode in which these changes take place it is evident that the several epithelial masses must be connected with each other in a plexiform fashion, and consequently that the ovary at this period of development is composed of a framework of connective tissue, the meshes of which communicate freely as in a cavernous tissue. Some, and sometimes many, among the imbedded epithelial cells become conspicuous by their size and the size of their nuclei, as we have already seen to occur amongst the superficial epithelial cells. Other cells remain of small size, and surround the larger cells as a kind of epithelium. The connective tissue stroma between the imbedded masses of epithelial cells constantly undergoes increase, and especially grows in between the several egg cells with their epithelial investment. Thus each epithelial ball is divided by these ingrowing vascularised trabeculae into as many cavities as it contains egg cells.

In describing the ovary of a newly born child, WALDEYER thus states, in reference to its tubular structure, "One sees long branching formations in the form of tubes, anastomosing with each other, as VALENTIN first described, and lying separate from each other at considerable distances. They pass upwards opening with narrow mouths into the epithelium, and appear as direct tubular gland-like processes of it.

"At the time in which the tubes described by PFLÜGER exist, that is, as far as I can find, from the ninth month till a short time after birth, they present the structure ascribed to them by PFLÜGER, with the exception already mentioned, that there is as little of *membrana propria* in them as there is in the primary follicles. In the tubes, and mostly in the middle of them, as PFLÜGER described, we meet with egg cells distinguished by their size and form, often immediately concatenated one behind the other. Whether in the tubes new egg cells are formed, I cannot decide; but I think it likely, because here, as well as on the surface epithelium, some epithelial cells may develop into egg cells. Division of the egg cells in the tubes PFLÜGER seems to have observed, but I have not seen it in fresh specimens."

Follicles are formed from the tubes as well as from the egg compartments, directly through the growth of interstitial tissue. At the lower end of the tube, as may be well explained from the want of a *membrana propria*, interstitial tissue grows into the tubes and encloses the individual egg cells along with a portion of the not fully developed epithelial cells which surround them, and in this way primary follicles are produced.

In summing up WALDEYER thus remarks:—

"As the chief result of my investigations, it must be stated that both the egg and the follicular epithelial cells are derived directly from the germ epithelium. There is a reciprocal growth of vascular connective tissue and germ

epithelium cells, in consequence of which large and small masses of the latter become imbedded more and more in the stroma of the ovary. The imbedded cells present a variety. Some of them, by simple increase in size, grow into ova, viz., primordial ova, while others keep to their original size, and by numerous divisions, at least as it appears to me, produce still smaller cells, viz., the follicular epithelial cells. A genetical distinction between primordial ova and follicular epithelial cells has consequently no existence. The germ epithelium is the common source of both. The first origin of eggs cannot be looked for in the ovary, but dates much farther back, even to the beginning of embryonic life.

The tubes of VALENTIN and PFLÜGER can lay claim only to a secondary importance, and are not essential for the egg and follicle formation; the greater part of the follicles have undoubtedly an earlier existence, long before these tubes are formed."

In the following description of my observations on the development of the ova and structure of the ovary, I shall in the first place state what I have seen in the foetal calf, then describe the ovary in young kittens, afterwards give an account of what I have observed in the human ovary, make some general observations on the development of the *membrana granulosa* in adult ovaries, and conclude with a few remarks on the structure of the ovum.

The drawings in illustration of this paper are all original, and have been carefully prepared, by the aid of the camera lucida, from microscopic preparations in my possession.

THE OVARY OF THE CALF.

If thin sections are made through the ovary of a foetal calf of about nine inches in length, the following appearances may be recognised in them. A section of such an ovary presents for examination two parts,—an external cortical or parenchymatous part (fig. 1, *a*), and an internal fibro-vascular part (*b, b*). The latter is directly continuous with the peduncle or stalk to which the ovary is attached at the hilum (*c, c*). On the surface of the peduncle, above and below, is a layer of epithelium (*d, d*) directly continued from the peritoneal epithelium towards the ovary; and when the layer is detached from the stalk or peduncle, and examined under high powers of the microscope, it is found to consist of small corpuscles, with large, clearly-defined, oval-shaped nuclei (fig. 2, *e*). Many of the nuclei contain granular matter in their interior. Each nucleus presents a sharply defined wall. In their largest diameter the nuclei measure about $\frac{1}{2500}$ th of an inch. The protoplasm round each nucleus is very clear and transparent. Between the nuclei are seen delicate lines, indicating apparently the surfaces of contact of the contiguous corpuscles. The clear substance round some of the nuclei is so small in quantity that the nuclei are almost in

contact; but in other instances it is in considerable quantity, and the nuclei are proportionally distant from each other.

When such a layer of epithelium is seen in profile (fig. 3, *f, g, h*), it consists of nuclei as before described, placed close to each other. Around each is a quantity of clear protoplasm, which abuts against that of its neighbour. The nuclei are arranged in a single layer, and as we approach the ovary they become placed closer and closer together, and more columnar in form, and the protoplasm investment of each becomes gradually less and less in quantity till we reach the actual surface of the ovary at the lower border, where we find the nuclei crowding together and continued on to the surface of the ovary in the form of the germ epithelium. As the nuclei pass on to the surface of the ovary they are no longer arranged in a single layer, but crowd one on the top of the other, and it is then not possible to say where peritoneal epithelium ends and germ epithelium begins.

All round the ovary the germ epithelium consists of a thick layer of corpuscles. Each corpuscle is a nucleus surrounded with a thin film of protoplasm.

On the upper and lower surfaces of the stalk, as seen in section, the peritoneal epithelium is continuous with the germ epithelium at the lower borders of the ovary in the manner described. The germ epithelium consists of corpuscles (fig. 4, *h, h, h*), arranged in the form of a thick layer passing round the ovary from one lateral border to the other, and gradually thinning off as it passes into and becomes continuous with the peritoneal epithelium.

The corpuscles of the germ epithelium vary considerably in size and shape; some are round, others oval or columnar, some are twice as large as their neighbours. The greater number are spherical, and within the larger ones a distinct spot or nucleolus is seen. Many of the corpuscles may be seen undergoing division into two or more parts. By this process of fission the corpuscles are ever producing new elements, which in their turn produce similar ones.

The largest germ epithelial corpuscles measure about $\frac{1}{2000}$ th of an inch in diameter. In several instances among the germ epithelial corpuscles on the surface of the ovary, I have observed some individuals flattened from above downwards, and the protoplasm round the nucleus drawn out at both ends in such a manner as to present the appearance of a fusiform corpuscle, or peritoneal epithelial cell seen in profile (fig. 4, *i*.)

Below the germ epithelium the cortical zone of the ovary consists for the most part of corpuscles which resemble very closely the germ epithelial corpuscles. A careful examination of this part of the ovary shows that from the deeper or fibro-vascular zone delicate bundles of young connective tissue with blood-vessels (fig. 4, *j, j, j*) pass upwards among the corpuscles in a radiating manner towards the germ epithelium; offshoots proceed in various directions from these bundles, and give rise to the formation of irregular-sized

meshes of vascular connective tissue, in which are enclosed large and small groups of corpuscles.

In the cortical zone such groups of corpuscles are found under the germ epithelium all round the ovary, and in many situations they are in connection superiorly with the corpuscles of the germ epithelium. At this stage of development the germ epithelium cannot be separated by any distinct line of demarcation from the masses of corpuscles which are below it, but at a later stage the germ epithelium rests on a thin irregular stratum of young tissue. This stratum is part of the fibro-vascular stroma of the ovary, and is formed by the growth of that tissue round the groups of corpuscles, which at an earlier stage of development are found in connection with the germ epithelial corpuscles all round the ovary.

Tracing downwards the bundles of vascular tissue which lie between the groups of corpuscles, we come to the fibro-vascular zone of the ovary (fig. 1, *b*). It must be understood that there is no boundary line between the cortical and fibro-vascular zone; the division is more or less artificial, but it is certain that the cortical, and by far the greater part of such a young ovary, consists of corpuscles with a few bundles of vascular tissue intermingled, while the deeper fibro-vascular zone, as its name implies, consists of very vascular tissue arranged in the form of a mesh-work, and in these meshes are included large corpuscles in various sized groups.

In this fibro-vascular zone we find numerous blood-vessels anastomosing freely with each other (fig. 5, *k, k*); the blood-vessels everywhere lie in the midst of bundles of young connective tissue. In the smallest meshes of fibro-vascular tissue generally one large corpuscles is found (fig. 5, *m*). The largest corpuscles consist of a central, large, bright nucleus with a nucleolus; the nucleus presents a sharply-defined double-contoured wall, and is surrounded by a considerable quantity of protoplasm, in contact with which are several very small corpuscles, forming a sort of wreath round the large corpuscle. Immediately outside this wreath is the vascular tissue of the stroma. Many of such large bodies as are now described lie imbedded throughout the fibro-vascular zone of the ovary, especially in its deeper parts; more superficially, many large bodies, but generally without the wreath of smaller corpuscles, are found in the meshes of the stroma; and as we pass upwards in our examination of the cortical zone, we find the fibro-vascular tissue decreases in quantity, but the corpuscles become more numerous, though smaller, till we reach the germ epithelial layer, which consists entirely of corpuscles.

From this description of the appearances presented in a section of the ovary of a very young foetal calf, we learn that the greater part of the parenchymatous zone of such a young ovary consists of corpuscles very similar in appearance to the corpuscles of the germ epithelium. From the deeper parts

of the ovary delicate bundles of vascular tissue proceed upwards in all directions among the corpuscles, which thus become included in groups in the meshes of the stroma. All round the ovary, under the germ epithelium, groups of corpuscles from the germ epithelium are being imbedded in the stroma by the growth of vascular connective tissue round them. The largest corpuscles are situated most deeply in the stroma, and are in an advanced stage of development, while those more superficially imbedded are in a less advanced stage of development; the youngest corpuscles are immediately under, and in connection with the germ epithelium. The most deeply imbedded corpuscles have already assumed the character of primordial ova, and among the others we can trace many in course of development into similar bodies. In each primordial ovum the nucleus becomes the germinal vesicle, and the protoplasm which surrounds it gradually forms the yolk of the mature ovum.

THE OVARY OF THE KITTEN.

I shall now describe the structure of the ovary and the development of the ova in a kitten of two or three weeks; and I may here remark, that I know of no animal better suited than the kitten to show the relation of the germ epithelium to the stroma of the ovary. A section of a young kitten's ovary presents a form similar to that of the young calf's ovary.

In a thin vertical transverse section of a two weeks' old kitten's ovary we may distinguish

- 1st, The germ epithelium.
- 2d, The zone of egg clusters.
- 3d, The fibrovascular stroma.

The germ epithelium consists of distinct corpuscles arranged in a layer which passes round the ovary from one lateral border to the other, and becomes continuous with the peritoneal epithelium which covers the stalk or peduncle.

The corpuscles of the germ epithelium (fig. 6, *h*, *h*) consist of clearly defined nuclei, all of which have a thin investing film of protoplasm. In some instances this protoplasm is very clearly made out, and is in considerable quantity, but in other cases it can scarcely be seen, even under very high powers of the microscope. As in the ovary of the foetal calf, there is a constant proliferation of the germ corpuscles by a process of fission. They are somewhat granular, and vary considerably in size; some are oval, but the greater number are spherical. In the ovary of a kitten of four weeks the corpuscles of the germ epithelium appear columnar in form and compressed laterally. In the round or spherical corpuscles (*l*, *l*), which are generally larger than the others, the nucleus is extremely well marked, and frequently possesses a bright nucleolus. The spherical form of the larger corpuscles appears to be produced

by the swelling out of the nucleus. In the two weeks' old kitten the corpuscles of the ovarian germ epithelium are several deep, and the layer itself is of irregular thickness.

As we examine the layer of germ epithelium as it passes round the ovary, we are at once struck by the fact that the corpuscles present a great variation in size and degree of development. Here and there we see large spherical nuclei (*l, l, l*), having round them a thin investing layer of protoplasm, while in other situations certain individual corpuscles stand forth prominently among their neighbours, and are conspicuous by their size and the size of their nuclei (*m, m.*) In these latter the protoplasm surrounding the nuclei is in the form of a thick layer. Between these largest corpuscles and the ordinary small ones every variety in size and form is to be met with. The largest corpuscles, which present so much protoplasm round the nucleus, are evidently individuals which have reached an advanced stage of development. These have been termed primordial ova, and there can be no doubt that a great number of the larger or spherical germ epithelial corpuscles are developing into similar bodies.

The ordinary sized germ epithelial corpuscles measure about $\frac{1}{2500}$ th of an inch, while the primordial ova measure about $\frac{1}{1000}$ th of an inch, but these measurements vary.

On carefully examining the primordial ova, in many instances two or more small fusiform corpuscles are seen in close contact with their yolk or protoplasm.

These fusiform bodies are about the size of the smallest germ epithelial corpuscles, and appear like connective tissue corpuscles, and in more than one case I have traced them in direct continuity with delicate bundles of minute fusiform bodies, which will presently be described as passing up from the deeper parts of the ovary towards the germ epithelium. In other cases, however, it appears to me that the bodies which lie in contact with the protoplasm of the primordial ova are germ epithelial corpuscles which have been displaced, pushed aside, or flattened out during the growth of the primordial ova. No cell wall can be made out round these primordial ova, but in certain sections one frequently sees a thin irregular stratum of a hyaline substance passing round the young ovum in contact with its protoplasm, apparently growing up from the young connective tissue subjacent to the germ epithelium. In this hyaline substance two or three oval-shaped nuclei are seen. In some preparations the primordial ovum has tumbled out of its hyaline girdle, which remains like a ring standing up among the germ epithelial corpuscles.

In favourable specimens, in a single section of a three to four weeks old kitten's ovary, I have counted as many as from thirty to forty large primordial ova among the ordinary germ epithelial corpuscles on the surface of the ovary. They may be recognised at once, for they appear like giants among their neighbours. Each consists of a large spherical nucleus, surrounded with a con-

siderable layer of protoplasm; it is this latter substance which gives magnitude to the young ovum, as may be seen by the fact that though many of the nuclei of the larger germ epithelial corpuscles are nearly as large as the nuclei of the primordial ova, yet, being without the extensive investment of protoplasm, they do not appear markedly conspicuous among the rest like the primordial ova. Occasionally, two large spherical nuclei or germinal vesicles are found in a single large primordial ovum among the germ epithelial corpuscles on the surface of the ovary.

Among the germ epithelial corpuscles in the deeper parts of the germ epithelial layer all round the ovary we meet with many large primordial ova, and in contact with their yolk or protoplasm are small fusiform bodies, each consisting of an oval-shaped nucleus, around which is an investment of protoplasm drawn out at either end in a fusiform manner. Besides these primordial ova, numerous germ epithelial corpuscles, in various stages of development into the same, are found in this situation.

Immediately subjacent to the germ epithelial layer is the zone of egg clusters. In osmic acid preparations this can be seen with the naked eye as a well-marked thick layer. The egg clusters are large oval-shaped and spherical collections of round corpuscles (*o, o, o*). The oval-shaped clusters lie close together, with their long axes directed from the centre of the ovary in a radiating manner towards the germ epithelium. Between the clusters, and separating them, are delicate bundles or strings of small fusiform corpuscles, with blood-vessels (*j, j, j*), which may be traced growing upwards from the deeper parts of the ovary towards the germ epithelium. By far the greater number of the egg clusters are oval, but spherical-shaped groups or clusters are also met with, generally deeper in the ovary. Each egg cluster consists of a collection of corpuscles, most of which are spherical, and resemble very closely the larger corpuscles of the germ epithelium. If we direct our attention to any one egg cluster, it will be seen that the corpuscles vary in size, just as we described the corpuscles of the germ epithelium. At the lower part of each egg cluster, that is, farthest away from the germ epithelium, we find many large primordial ova, similar to those already described among the corpuscles on the surface of the ovary, and besides these are numerous large spherical nuclei, having round them protoplasm in layers of varying thickness. In each egg cluster it is possible to trace the corpuscles in all stages of development into primordial ova. Every large corpuscle in each egg cluster is potentially a primordial ovum. In contact with each primordial ovum in the egg clusters are small fusiform corpuscles, which may be traced as offshoots from the bundles of similar corpuscles which lie between and separate the egg clusters from each other. It is easy to compare the corpuscles in each egg cluster with the corpuscles of the germ epithelium, and to follow the steps of their development into primordial ova.

In such a young kitten's ovary many of the egg clusters are still in connection superiorly with the corpuscles of the germ epithelium, but in most cases, we find the bundles of young connective tissue which lie between the egg clusters have grown completely round them, separating them not only from each other, but also from the germ epithelium above. While the corpuscles of the germ epithelium are thus being enclosed in the meshes of young connective tissue, the nucleus of each swells out into a spherical body, around which is gradually produced that protoplasm which later constitutes the yolk of the primordial ovum.

When we trace the bundles of young connective tissue downwards, we find they are offshoots of similar tissue of which the central part of the ovary consists. In the deeper parts of the ovary we find a very vascular young connective tissue forming the stroma of the organ, in which are imbedded numerous primordial ova, some in a far advanced stage of development. This stroma consists of minute fusiform corpuscles and blood-vessels. As the vascular bundles or strings of this tissue grow upwards between the egg clusters, delicate offshoots of the same insinuate themselves between the primordial ova and corpuscles in the clusters, and in this manner nourishment is brought within reach of these developing bodies. This interstitial growth begins at the lower part of each egg cluster, and gradually the primordial ova become separated from each other as the connective tissue thickens in between and around them, and they become at last included in separate meshes or primordial follicles. Where the egg clusters have not been completely shut in by bundles of connective tissue, the fusiform corpuscles of the latter may be distinctly followed up as far as the corpuscles of the germ epithelium, and, indeed, seem to disappear among them.

By the growth of the vascular tissue of the stroma among the imbedded corpuscles, the egg clusters in all parts of the ovary are gradually subdivided or broken up into single egg-containing meshes or follicles; and while this process is going on, the primordial ova are rapidly advancing in development. In the more superficial parts of the stroma subjacent to the egg clusters, and in the fibro-vascular zone of the ovary, the above-described process has already taken place. The primordial ova in some of the follicles are of very large size, and in the ovary of a four weeks' old kitten it is of great interest to compare the original germ epithelial corpuscles on the surface of the ovary with these large primordial ova now imbedded deep in the stroma, and we are thus able to observe what an extraordinary change has taken place in them during their development; and, what is of more importance, we recognise the nature of the change. Examined under a magnifying power of 1000 diameters, the ordinary germ epithelial corpuscle on the surface of the ovary appears as if it were of such a size that at least three of them would lie side by side on a threepenny piece without overlapping it; whereas the highly developed ovum imbedded deeply in

the stroma appears as large as a florin or a half-crown piece, and the nucleus or germinal vesicle as large as a threepenny or fourpenny piece. Between these two extremes young ova in all stages of development may be seen in the stroma of such a young ovary. The change which the germ epithelial corpuscle undergoes during its development is the following:—

As soon as the corpuscle is imbedded in the stroma, its nucleus swells up into a round or spherical body, within which generally appears a spot or nucleolus. The nucleus presents a sharply defined limiting membranous wall, and becomes the germinal vesicle of the future ovum. Immediately around the nucleus is gradually produced that protoplasm which afterwards constitutes the yolk of the mature egg. *In all parts of the ovary, wherever we examine such young developing ova, we find fusiform corpuscles, like the fusiform corpuscles of which the stroma is composed, lying in contact with the protoplasm which surrounds the nucleus or germinal vesicle.*

The youngest egg clusters are immediately below the germ epithelium, and many of them are in connection with it. These latter have not, as yet, been completely closed in and separated from the germ epithelial layer by the connective tissue bundles of the stroma.

The clusters of corpuscles in connection at their upper parts with the germ epithelium differ considerably from each other, both in size and form. Some are oval and elongated, others are spherical. Many of them are larger at their lower and middle parts than at the part in connection with the germ epithelial corpuscles. If a vertical section passes down through such clusters as these, such as are swollen out at their lower and middle parts but narrow at their upper parts, we have presented under the microscope the appearance as if bottle-shaped tubes full of round corpuscles extended from the germ epithelium downwards into the stroma of the ovary. These appearances are often seen, and it is important to study them well. It will be remembered that PFLÜGER* described in the young kitten's ovary numerous tubular processes passing downwards from the surface of the ovary into the stroma, in which the germinal vesicles originated, and by the successive divisions of which Graafian follicles containing eggs were formed. I have very carefully examined the ovaries of kittens and puppies, but have failed to find any tubular processes of epithelium passing into the stroma from which Graafian follicles are formed, and it appears to me that PFLÜGER is incorrect in stating that such exist in the ovary of the kitten.

In section, the ovary of a kitten at birth presents a structure very similar to that of the ovary of the two to three weeks' old kitten, but we find in the germ epithelium layer very few primordial ova. Many large spherical nuclei, with a thin film of protoplasm round them, are seen among the germ epithelial corpuscles, and

* PFLÜGER, "Die Eierstöcke der Säugethiere und des Menschen," Leipzig, 1863, p. 4.

the germ epithelial layer itself is much thicker as a stratum all round the ovary. Most of the large egg clusters below it, at this stage of development, are in communication superiorly with the corpuscles of the germ epithelium, and separating these clusters delicate bundles of young connective tissue, made up of minute fusiform corpuscles, may be traced growing up between and around them. In the deeper part or fibro-vascular zone of such a young ovary the stroma is rich in blood-vessels, and numerous young eggs are imbedded in its meshes, but no large eggs, such as we described in the four weeks' old kitten, are found.

THE HUMAN OVARY.

In section, the ovary of a human foetus of about seven months presents a somewhat triangular form (fig. 7) The ovary (*a*) is attached to a stalk or peduncle (*b*) consisting of fibro-vascular tissue, which passes into the ovary at the hilum (*c*), and from the direct prolongations of which the whole stroma is derived. On examining a thin section of such an ovary under low powers of the microscope, direct prolongations from the stalk are seen proceeding in a radiating manner towards the periphery in all directions, and communicating with each other in such a manner that the whole stroma becomes arranged in the form of a mesh-work consisting of fibro-vascular tissue. In the meshes of this stroma are large and small collections of corpuscles. At the periphery of the organ the meshes are large, and the contained groups of corpuscles are correspondingly large; but as we pass deeper into the ovary the meshes with the included groups of corpuscles become smaller, till at last we find small meshes containing but one or two large corpuscles.

The surface of such an ovary is very irregular, presenting numerous small fossæ-like grooves and furrows, seen clearly under a low magnifying power.

Investing the ovary, and passing round it from one lateral border to the other, is a layer of columnar corpuscles. This layer is the germ epithelium (*h*, *h*), and as it invests the ovary it dips down into and lines certain tubular structures and tubiform depressions which, when seen in section, appear as passing down from the germ epithelial layer into the substance of the ovary.

(*a*). *Nature of the Germ Epithelium*.—In a foetus of $7\frac{1}{2}$ months the germ epithelium consists of columnar-shaped corpuscles placed side by side and arranged as an investment to the whole ovary.

The germ epithelial layer rests on a thin irregular stratum of connective tissue which is part of the general stroma of the ovary, and is formed by delicate processes of the same, which at an earlier stage of development grew upwards from the deeper parts of the ovary to surround and inclose in meshes those large groups of corpuscles found immediately under the germ epithelium in its entire extent. This young connective tissue stratum is the forerunner of the tunica albuginea.

On tearing to pieces in a little water a fragment of this ovary and examining the debris under high powers of the microscope, many small portions of the germ epithelium will be found. When the deep surface of the epithelial layer is examined, the corpuscles are seen to be placed close together, and present in the membrane a tessellated appearance (fig. 10). The chief part of each corpuscle is the nucleus. Around each nucleus is a small quantity of protoplasm which acts as a cement substance holding the nuclei together. When the germ epithelial membrane is looked down upon from above, immediately on bringing the corpuscles into view a clear space is seen round the nucleus of each corpuscle (figs. 11 and 12, *h, h*). This space is occupied by clear protoplasm. The corpuscles in this early stage of development appear as little nucleated pieces of protoplasm; the nucleus is always the conspicuous part of each corpuscle, but the protoplasm round it may vary in quantity. In each piece of epithelial membrane examined, the corpuscles are of different sizes. Some of the nuclei are swollen up into large spherical bodies (fig. 12, *l*), and around them is an increased quantity of clear protoplasm. In the larger nuclei a nucleolus is generally seen. The largest corpuscles are undoubtedly primordial ova. Between these and the smallest germ epithelial corpuscles every variety in size is met with.

When seen in profile (figs. 13, 14, 15, *h, h, h*), the germ epithelial corpuscles are columnar, but many of them are assuming an oval and spherical form (figs. 13, 14, 15, *l, l, l*). In the spherical ones the nucleus is clearly defined, and shows distinctly its well-marked membranous wall. Within the nucleus is a nucleolus, and around it is a thin film of protoplasm. In some instances this film is so fine as scarcely to be made out. When a section of a 7½ months' foetal ovary is examined under high powers of the microscope, in many situations among the ordinary germ epithelial corpuscles all round the ovary, we find individuals standing forth prominently and conspicuous by their size and the size of their nuclei (figs. 14, 15, *m, m*) similar to those bodies we described as conspicuous among the corpuscles of the germ epithelium in the kitten's ovary. Such have been termed primordial ova. On comparing these with the smaller round corpuscles, and these latter with the ordinary germ epithelial corpuscles, it is easy to see that they are germ epithelial corpuscles in a far advanced stage of development. In the progress of growth the nucleus of the ordinary germ epithelial corpuscle first swells out and enlarges, becoming oval, then spherical, and around it is gradually produced that protoplasm which assumes such dimensions in the primordial ova. In contact with these primordial ova we often see small fusiform corpuscles (*n, n*), and as in the case of the kitten's ovary, some of them appear to have grown up among the germ epithelial corpuscles from the stratum of young connective tissue (*j, j*) on which the germ epithelium rests.

It is a fact of great interest, that as the germ epithelial corpuscle becomes a

primordial ovum, the nucleus, which at first appears ill-defined and semi-solid, and is surrounded by a comparatively small quantity of protoplasm, now shows a remarkably clear definition, and at last appears as a spherical vesicular body with a fine double-contoured wall, and within it one or two nucleoli are usually seen. The nucleus of each germ epithelial corpuscle becomes the germinal vesicle of the primordial ovum, and the nucleolus corresponds to the germinal spot.

The ordinary germ epithelial corpuscles measure in their longest diameter about $\frac{1}{1800}$ th of an inch, and in their shortest about $\frac{1}{3500}$ th of an inch, but both these measurements vary considerably.

On referring to WALDEYER'S work "*Eierstock und Ei*," plate ii. figs. 9, 11, 13, I find that he represents the germ epithelial corpuscles as little bodies in which the nuclei are comparatively small, while the protoplasm round these is in all cases very extensive. This is not in accordance with my observations. An examination of the ovaries of numerous foetal and newly-born animals clearly shows that in each ordinary germ epithelial corpuscle the nucleus constitutes by far the greater part of the corpuscle, the protoplasm around it being in the form of a fine film. In the primordial ova, however, the enlarged nucleus has around it a correspondingly large quantity of protoplasm, and then these bodies present the appearance as described by WALDEYER.

(b.) *The Relation of the Germ Epithelium to the Peritoneal Epithelium.*—In a section of the ovary of a $7\frac{1}{2}$ months' foetus, the stalk or peduncle (fig. 8, b) is directly continued into the ovary to form the stroma, which we described as arranged in the form of a mesh-work. If we now direct our attention to the epithelium covering this stalk (fig. 9, f), we find it is directly continuous with the peritoneal epithelium, and on the other hand passes without a break into the epithelium which covers the ovary, but a gradual change in its character takes place as it slides into and becomes continuous with the latter.

In the peritoneal epithelium (fig. 9, f), as seen in profile, the nuclei of the cells are oval and flattened from above downwards, and are placed at a considerable distance from each other, and the protoplasm around them is extensive. Tracing this epithelium towards the ovary, as we approach the latter, we observe the nuclei of the epithelial cells to become round and columnar, and gradually to lie closer together until at last they are almost in contact. They then crowd together, and in the form of a thick layer pass on to the surface of the ovary as the corpuscles of the germ epithelium (h). As the corpuscles of the peritoneal epithelium gradually slide into those of the germ epithelium, we find the protoplasm which invests the nuclei of the former gradually becomes less and less in quantity, and the nuclei themselves become gradually larger and more columnar, until at last the nuclei pass on to the surface of the ovary as distinct columnar bodies, each having round it a fine investment of protoplasm. In thus tracing the peritoneal epithelial corpuscles into

germ epithelial corpuscles we see the nuclei undergoing fission, and by this process crowds of germ corpuscles are produced. The whole germ epithelial layer must be regarded as a thick layer of proliferating corpuscles. From the first appearance of the ovary as an organ until its development is completed, there is a constant proliferation of the germ epithelial corpuscles; and, in growth of the ovary, as fast as some corpuscles become imbedded in the stroma, others are produced to take their place.

It is interesting to recall to recollection the statements of SCHENK*, WALDEYER, and others, that in the first instance the whole peritoneal cavity is lined by a layer of columnar corpuscles, and when the Wolffian body appears in connection with REMAK's middle plate, it likewise has an investment of similar corpuscles. The first appearance of the ovary is the thickening of this columnar epithelium on the median side of the Wolffian body (WALDEYER.) At the commencement of this thickening there is seen under it a small outgrowth of young connective tissue, continuous with and proceeding from the interstitial tissue of the Wolffian body. There can be little doubt that the thickening of the columnar epithelium is brought about by a proliferation of its corpuscles, and the proliferation itself is due to the influence on the corpuscles of the vascular young connective tissue subjacent to them. While the columnar corpuscles constantly proliferate to form the germ epithelial corpuscles, from which all the future ova are derived, the rest of the peritoneal chamber becomes lined by a layer of flat epithelium, forming the peritoneal epithelium.

From the very first appearance of the germ epithelium of the ovary as a distinct structure, processes of the subjacent young connective tissue grow upwards among the corpuscles as the first step in that process of imbedding, whereby the corpuscles become surrounded in meshes of vascular stroma, and ultimately form ova. From its earliest condition till late in the development of the ovary, the germ epithelium cannot be stripped off as a layer from the ovary, because delicate processes of the ovarian stroma are constantly growing up between the corpuscles as fast as they are produced.

PFLÜGER states his opinion that the ovum is simply a peritoneal epithelial cell. With this statement I cannot altogether agree. Both the ova and the peritoneal cells are undoubtedly evolved from a common ancestral source—the columnar layer of the great peritoneal cavity; but whilst in one limited region the columnar corpuscles form the germ epithelium and become converted into ova, in the greater part of their distribution they become converted into the endothelium lining the peritoneal cavity. Hence the ovum is no more a modified peritoneal epithelial cell than is the peritoneal epithelial cell a modified ovum.

From the corpuscles of the germ epithelium all the ova are derived. We

* Beiträge Zur Lehre von den Organ-Anlagen im motorischen Keimblatt.

recognise the germ epithelium by the fact that immediately below it all round the ovary are clusters or groups of corpuscles contained in meshes of the ovarian stroma, but under the peritoneal epithelium covering the stalk no such groups are found.

It is worthy of remark, however, that sometimes under those epithelial corpuscles, which, as it were, form a connecting link between peritoneal and germ epithelial corpuscles, we find small groups of round corpuscles apparently consisting of abortive ova. It is only under and in connection with the true germ epithelium that groups of true primordial ova are formed.

(c.) *The manner of Inclusion of the Primordial Ova and Germ Epithelial Corpuscles in the Stroma of the Ovary.*—In a section of the ovary of a human foetus of $3\frac{1}{2}$ months, one sees large strings of connective tissue corpuscles (fig. 19, *j, j, j*) growing upwards in a radiating manner from the deeper parts of the ovary toward the germ epithelium (*h, h*.) These strings or bundles communicate with each other and form meshes, and in these meshes are round groups of corpuscles (*o, o, o*) which resemble very closely the corpuscles of the germ epithelium (*h, h*) which invests the ovary. Immediately under the germ epithelium such groups of corpuscles may be seen partially imbedded in meshes of the stroma. In the deeper parts of the ovary we find large primordial ova (fig. 20, *m, m*) lying separate from each other, and in contact with the protoplasm which surrounds the germinal vesicle of each are small connective tissue corpuscles (*n, n*) exactly similar to those which make up the strings or bundles of young tissue in other parts of the ovary. These little connective tissue corpuscles are quite different in appearance from the corpuscles of the germ epithelium on the surface of this young ovary, or those imbedded in groups in the stroma.

The stroma of the human foetal ovary of $7\frac{1}{2}$ months consists almost entirely of fusiform connective tissue corpuscles, and in a section of such an ovary we find the whole stroma arranged in the form of a mesh-work, in the meshes of which are large and small groups of corpuscles, just as we described in the case of the ovary of the calf, the kitten, and human foetal ovary of $3\frac{1}{2}$ months. In the bundles of connective tissue corpuscles which (as seen in section) lie between the groups of corpuscles we find blood-vessels, and on close examination it is seen that the walls of such blood-vessels consist of connective tissue corpuscles. Wherever the bundles of connective tissue proceed, in the midst of them are blood-vessels. On tracing the bundles of vascular tissue upwards, we find they arch round large and small groups of corpuscles immediately under the germ epithelium, and completely inclose them in meshes. In some situations under the epithelium the connective tissue bundles have not yet grown round the groups of corpuscles, but may be traced up as far as the germ epithelial corpuscles on either side of the groups. Under the germ epithelial layer the youngest connective tissue is found, and in this situation it

is the forerunner of the tunica albuginea. This youngest connective tissue appears as a transparent jelly-like substance, and in it are numerous fusiform corpuscles or nuclei. Fine homogeneous points of this young tissue may be seen insinuating themselves among the corpuscles of the germ epithelium, and in some preparations of 6 and $7\frac{1}{2}$ months' foetal ovaries in which the germ epithelial layer is partially detached such fine points of the jelly-like young tissue may be seen in considerable numbers.

In many places immediately under the germ epithelium, small groups, consisting of a few germ epithelial corpuscles (fig. 21, *q, q, q*), are found in the act of being surrounded by this same jelly-like tissue; some of the groups are completely surrounded and separated from the germ epithelium layer, while others are partially surrounded, and are still in connection with the germ epithelium superiorly. The youngest connective tissue can always be traced in direct continuity with vascular bundles of tissue which completely surround the large groups of imbedded germ epithelial corpuscles, and is part of the general stroma of the ovary. This imbedding of germ epithelial corpuscles takes place under the germ epithelium all round the ovary.

After being thus included in meshes of the stroma, the germ epithelial corpuscles increase in number and in size, and there results the formation of those large egg clusters which are found under the germ epithelium in all parts of the ovary.

Each imbedded corpuscle undergoes the following change:—The nucleus enlarges, gradually becoming a spherical vesicular body, and the protoplasm which surrounds it is at the same time increased in quantity. As the result of the enlargement of each corpuscle in the group, the whole group as a cluster expands and becomes more or less spherical. As these egg clusters expand, those lying immediately under the germ epithelium push the latter structure before them, and in this manner the surface of the young ovary is made to present a very irregular appearance. Between the prominences or irregularities thus produced are depressions or furrows. These originate as simple depressions between two or more adjacent expanding egg clusters, and they become deepened by the growth and expansion of new egg clusters under the germ epithelium in connection with those already formed.

In describing the appearance of a seven and a half months' human foetal ovary in section, I stated that the germ epithelium passed round the ovary from one lateral border to the other, dipping into and lining certain tube-like structures and tubiform depressions which appear to pass from the surface downwards into the organ. Now, if vertical sections are made through a young ovary such as above described, whose surface presents numerous irregularities, we have the appearance presented as if tube-like structures (fig. 22, *p, p*) passed down from the surface of the ovary into the substance of the organ, and

these are all lined by germ epithelium. Similar tube-like structures are produced when sections are made through the convoluted surface of a brain, and the grey matter might even be compared to the germ epithelium, inasmuch as it lines these sulci. But such structures are not tubes, nor are the similar appearances seen between the irregularities of the surface of a young foetal ovary.

An examination of the young ovary, looking down on its surface from above, soon convinces us that there are no real tubular structures passing from the epithelium into the organ. On bringing the germ epithelium into view, and then slightly depressing the tube of the microscope, one sees large and small round groups of spherical corpuscles separated from each other by the connective tissue of the stroma, as was described by WALDEYER. Some of these groups are still in communication with the germ epithelial corpuscles, as may be seen in vertical sections where the knife has passed through a group of corpuscles not yet completely included in a mesh of the upward growing stroma.

In the germ epithelial layer which dips down into and lines the depressions of the surface of the ovary (fig. 22, *p, p*), we find the corpuscles undergoing changes during their development similar to the changes which the corpuscles of the germ epithelium in other situations undergo. At the bottom and sides of the sulci, among the corpuscles of the germ epithelium, we find large spherical nuclei with a thin investment of protoplasm, and large primordial ova, such as may be found in all parts of the germ epithelial layer, whether it lines depressions or passes over prominences of the surface of the ovary.

Frequently large egg clusters are formed under the germ epithelium which lines the furrows or sulci, and it often happens that the walls of these furrows come in contact; pushed together, as it were, by laterally situated expanding egg clusters. At the bottom of the furrows, where the epithelial walls come in contact, connective tissue passes through among the corpuscles, and in this way large egg clusters become formed immediately below the germ epithelium at the bottom of the sulci. A vertical section passing down through such a sulcus and the group of corpuscles immediately below it, produces the appearance as if a tubular prolongation of germ epithelium was dilated at its lower part into a large sac, full of developing corpuscles of the germ epithelium.

During my investigations on the structure of the ovary and development of the ova, I have never found any real tubular structures, neither in the human ovary, nor in any other mammal that I have examined, such as the cat, dog, calf, sheep, guinea-pig, rabbit, and in no instance have I found Graafian follicles formed out of such structures in the manner described by PFLÜGER, VALENTIN, SPIEGELBERG, WALDEYER, and others.

(*d.*) *Development of the Egg Clusters.*—Each egg cluster is a group or collection of germ epithelial corpuscles enclosed in a mesh or capsule of the ovarian stroma.

The germ epithelial corpuscles on the surface of the ovary constantly produce new elements by the process of fission, and when included in a vascular mesh of stroma the corpuscles increase greatly in number by division, and from a few imbedded corpuscles a large group or cluster may be derived. It appears to me a most interesting and remarkable observation that these corpuscles, after a certain increase in number, expand or swell out into spherical bodies, and a careful examination of the egg clusters has convinced me that this change is brought about by the nucleus in each corpuscle swelling out into a spherical vesicular body, which afterwards becomes the germinal vesicle of the primordial ovum, and in close contact with the wall of the nucleus is gradually produced that protoplasm which afterwards forms the yelk of the ovum.

In each egg cluster we find certain individuals much farther advanced in development than the rest, and these appear exactly like the large primordial ova which we described as found among the corpuscles of the germ epithelium on the surface of the ovary. Each corpuscle in the cluster is potentially a primordial ovum. At the first there is but a small quantity of protoplasm round the nuclei of the corpuscles in each egg cluster, but as the nuclei enlarge and expand, the protoplasm round them is gradually produced in considerable quantity.

This development of the germ epithelial corpuscles into primordial ova takes place in the egg clusters in all parts of the ovary. All the imbedded germ epithelial corpuscles do not reach the stage of primordial ova, many of them abort and disappear, and perhaps furnish a pabulum for the more vigorous and healthy ones.

In the ovary of a puppy at birth, one sees, in a beautiful manner the egg clusters under the germ epithelium in all stages of development. Some of the clusters appear to consist entirely of large primordial ova, while in others we can trace the growth of the germ epithelial corpuscles into ova, and immediately under the germ epithelium are little groups of corpuscles in the act of being included in meshes of the stroma.

In following the further development of the ovary and ova, we notice that around each egg cluster is a well-marked capsule or mesh of vascular connective tissue. This connective tissue consists almost entirely of fusiform corpuscles and young blood-vessels. Just as at first, when delicate processes of the young stroma grew upwards among the ordinary germ epithelial corpuscles to include them in meshes, so now delicate processes of the young connective tissue with blood-vessels proceed from the walls of these meshes, and insinuate themselves in among the developing corpuscles in each egg cluster. As these processes thicken, the primordial ova gradually become separated from each other, and at last each is included in its own mesh or capsule of the stroma.

These single egg-containing meshes or capsules are the primordial follicles.

This formation of young Graafian follicles takes place in the egg clusters in all parts of the ovary, and at the same time new egg clusters are being formed under the germ epithelium in the manner described. In the ovary of a 7½ months' human foetus we find many newly-formed egg clusters immediately under the germ epithelium, but below these, earlier formed egg clusters are in various stages of alteration into single egg-containing follicles, while deeper still we find a great number of young follicles all produced from the first formed clusters in the way we have described (fig. 24).

In the first formed Graafian follicles, which are situated most deeply in the stroma of the ovary, the young ova are of large size, and we are at once struck by the fact that the germinal vesicles in all are about the same size, although the protoplasm around them may vary considerably in quantity.

In each young Graafian follicle the ovum fits tightly; it occupies the whole cavity of the follicle; there is no space between it and the wall (fig. 24, *m, m, m*). The mesh of stroma closely embraces the protoplasm of the ovum, and in almost every case we find fusiform connective tissue corpuscles (*n, n*) in the wall of the mesh lying in close contact with and indenting the yelk of the young ovum. Wherever we examine the primordial follicles, we see such fusiform corpuscles of the stroma lying in contact with and indenting the yelk of the contained ova. I called attention to the circumstance that among the germ epithelial corpuscles on the surface of the ovary primordial ova were found, having in contact with their protoplasm small fusiform corpuscles, which in some instances could be traced growing as offshoots from delicate bundles of similar bodies which formed part of the ovarian stroma.

(*e.*) *The Development of the Membrana Granulosa.*—The stroma of the human foetal ovary is remarkable for the great number of connective tissue corpuscles it contains. Wherever we examine the stroma and its processes in all parts of the ovary, we find in it well-formed connective tissue corpuscles. In the middle parts of the ovary, where the stroma is well developed, the connective tissue corpuscles show very distinctly a central oval nucleus with nucleolus. Around the nucleus is a small quantity of protoplasm, drawn out at either end in a fusiform manner. Besides these we see naked nuclei and many small round bodies; in these latter the nucleus is in a state of division into two or more parts. These small round bodies appear to be swollen out connective tissue corpuscles. In a well-formed connective tissue corpuscle the nucleus is comparable to the germinal vesicle of the ovum, and like it, at a certain stage of its development, it shows a sharply-defined double contoured wall. In all parts of the ovary we find the connective tissue corpuscles dividing.

In various parts of the stroma we find delicate fibres developing into connective tissue corpuscles. The central part of such fibres becomes swollen out, and in this swollen out part a distinct oval nucleus appears; sometimes

these fibres are direct prolongations of the protoplasm surrounding the nuclei of well-formed connective tissue corpuscles. The nucleus of a connective tissue corpuscle at first often appears as a solid or semisolid body, but it may become distinctly vesicular, like the nucleus or germinal vesicle of the ovum.

It will be observed that, in my description of the germ epithelium and of the development of the ova, I have avoided the use of the word cell, and substituted the term corpuscle, and my reason for not using the term cell in connection with the germ epithelial corpuscles is, that the germ epithelial corpuscles, and the so-called columnar epithelial cells which line the pleuro-peritoneal cavity of the embryo, are nuclei which have a thin film or investment of protoplasm round them. These nuclei are the homologues of the nuclei of the peritoneal epithelial cells. When a corpuscle divides, each half of the nucleus carries with it a small investment of protoplasm. The protoplasm round the nuclei varies considerably in quantity during the development of the germ epithelial corpuscle. The term cell is employed in somewhat different significations by biologists. Some, for example, holding that a cell must have a definite wall, whilst others look upon the wall as of secondary and minor importance, and hold that a cell essentially consists of a nucleated mass of protoplasm. The germ epithelial corpuscles and connective tissue corpuscles do not possess a cell wall. When once a cell wall has formed round a corpuscle, the nucleus and cell contents may divide. The cell wall does not participate in the division, but incloses the products of the division. The cell wall may burst and liberate the contents of the cell.

Every egg cluster is included in a mesh of the stroma. This mesh consists of connective tissue corpuscles and minute blood-vessels whose walls consist of such corpuscles. Delicate processes of this vascular young tissue from the wall of the mesh grow inwards among the corpuscles which are developing into primordial ova. On tearing to pieces small fragments of a 7½ months' foetal ovary, and placing the debris in a little water under the microscope, we find small groups of primordial ova (figs. 25, 26), and single individuals (figs. 27, 28, 29), which have been torn away from the egg cluster, and in connection with some of the largest of these we frequently find fusiform corpuscles (figs. 28, 29, *n, n*) similar to those which lie in the walls of the meshes. In good specimens the fusiform corpuscles are found lying in indentations in the yelk substance (figs. 28, 29) which surrounds the germinal vesicle of the primordial ovum, and sometimes we see primordial ova whose yelk is indented in many places, but the fusiform corpuscles have been displaced from these indentations. No zona pellucida is found round such young primordial ova. The connective tissue corpuscles must therefore be in contact with the yelk of the primordial ova.

Directing our attention to the youngest follicles, we find these vary in size, but in every case the young ovum fills up the whole follicle in such a manner

that its protoplasm presses against and distends the follicular wall. Little fusiform corpuscles in the walls, *and belonging to the stroma of the ovary*, indent the protoplasm of each young ovum as it lies in its follicle. (Fig. 24, *m, m.*)

In an empty follicle from which the young ovum has been removed, these little connective tissue corpuscles appear as minute buds projecting into the cavity of the follicle from its wall.

In the deeper parts of the ovary numerous young egg-containing follicles are seen. In the youngest follicles but two or three fusiform corpuscles are found in contact with the yolk of the contained ovum. As the follicles increase in size and become older, the number of small corpuscles in contact with the contained ova also increases. In some we find seven or eight, and in still older follicles a perfect wreath of minute corpuscles is formed round the yolk of the young ovum. The oldest follicles are found in the deepest parts of the ovary, and in most of them there is a perfect wreath of minute corpuscles (fig. 30, *r*) lining the follicle. Now, from these young connective tissue corpuscles in the wall of the young follicles which lie in contact with and indent the yolk of the primordial ova, the corpuscles of the membrana granulosa are derived.

In describing the growth of fibre-like bodies in the stroma into connective tissue corpuscles, I stated that the middle parts of such fibres become swollen out, and in the swollen out part a distinct nucleus appears. This nucleus, though at first appearing semi-solid, may become distinctly vesicular, and within it a nucleolus is afterwards seen. By a careful examination of a whole series of young Graafian follicles, we trace the development of the corpuscles of the membrana granulosa in the following way:—Around the young ovum in each follicle the connective tissue corpuscles increase in number by division. As a single fusiform corpuscle divides, its nucleus appears to commence the division, and each half of the nucleus carries with it a small quantity of the protoplasm which originally invested the single nucleus. When a little wreath of such corpuscles is formed round the young ovum the nucleus of each corpuscle swells out and becomes a distinctly vesicular little body, presenting a very fine double contoured wall, around which is a small quantity of protoplasm. Within the nucleus generally a minute spot is seen. The protoplasm which surrounds the vesicular nuclei acts as a sort of cement substance, holding them together in the form of a capsular membrane round the young ovum. This capsular membrane is the first appearance of the membrana granulosa.

Only those connective tissue corpuscles which lie in actual contact with the yolk of the ovum in the follicle develop into corpuscles of the membrana granulosa; while those in the wall outside the membrana granulosa remain as connective tissue corpuscles, and follow the usual developmental process into ordinary fibrous tissue, of which the main part of the follicular wall at last consists. We shall afterwards see that in some of the oldest Graafian follicles in the adult

ovary, in man and other mammals, which are about to burst to liberate the contained ovum, a great part of the wall of the follicle outside the membrana granulosa becomes again converted into large connective tissue corpuscles.

When first formed, the membrana granulosa consists of a single layer of minute corpuscles arranged in the form of a capsule round the ovum (fig. 30, *r*). As the young ovum enlarges, which it rapidly does after the formation of the membrana granulosa, it distends its follicle, and the corpuscles of the membrana granulosa increase greatly in number. In this membrane, when first formed, the corpuscles lie in close contact with each other, and when looked down upon from above they present a polygonal form from mutual compression (fig. 32, *r*). By the constant multiplication by division of its corpuscles, the membrana granulosa at last comes to consist of several layers. As a further stage in development, certain of the corpuscles, generally those in the middle parts of the membrana granulosa, break down, and, it appears to me, become dissolved in a fluid which afterwards forms the liquor folliculi. By the breaking down and solution of these corpuscles, a cavity, the follicular space occupied by fluid, is formed. In section, this space appears semilunar in form. After the formation of this space the ovum is not entirely separated from the membrana granulosa, but remains connected with the wall of the follicle by a heap of corpuscles which surrounds it. In good specimens a layer of corpuscles remains in contact with the zona pellucida round the ovum for a long time after the formation of the follicular space. When first formed, the corpuscles of the membrana granulosa in the human foetal ovary of $7\frac{1}{2}$ months measure about $\frac{1}{3000}$ th inch.

For the complete demonstration of the development of the corpuscles of the membrana granulosa, the ovary of an adult rabbit is admirably suited. In a section of such an ovary, we first direct our attention to the structure of the stroma of the organ, and we find it consists entirely of very minute attenuated fusiform corpuscles. At first sight these appear as simple fibres, but each fibre is an elongated nucleus, having round it a minute quantity of protoplasm. The stroma of the ovary of an adult cat has an exactly similar structure. In the adult rabbit's ovary, in a single section, we may find young ova in various stages of development. In a very young ovum (fig. 33, *m*), we notice first the large germinal vesicle, with its germinal spot. Around the germinal vesicle is a small quantity of protoplasm, immediately in contact with which are several small fusiform corpuscles (*n*, *n*). These lie flattened against the ovum round its yolk, and are exactly similar to fusiform corpuscles, of which the stroma (*j*) is composed. Around an ovum slightly farther advanced in growth we find one or two of the fusiform corpuscles (*n*, *n*) have assumed a swollen condition, and in some instances individual corpuscles are in the act of dividing. In the case of an ovum still farther advanced in develop-

ment, the corpuscles round the yolk substance have now assumed a spherical form, and as they increase in number and press against each other, they gradually become columnar (fig. 35, *r*).

As in the case of the human foetal ovary of $7\frac{1}{2}$ months, the corpuscles of the membrana granulosa thus formed consist of vesicular nuclei placed close together, their walls being almost in contact, but a very minute quantity of cement material lying between them. When this young membrana granulosa is looked down upon from above, the corpuscles present a beautiful paved appearance. The nucleus of each corpuscle is polygonal from pressure by its neighbours (fig. 36, *r*). By the constant division of the corpuscles, the membrana granulosa soon consists of several layers (fig. 37, *r*), and a follicular space is formed by the breaking down and solution of some of the corpuscles. In the nearly ripe Graafian follicle of the rabbit's ovary one frequently finds several small follicular spaces in different parts of the thick membrana granulosa; and in a section made through such a follicle, bands or straps of membrana granulosa cells appear to pass from the ovum to the wall of the follicle. These bands were called "Retinacula" by MARTIN BARRY. They are simply appearances produced when the section has passed through the walls or septa which separate several follicular spaces lying near each other.

In the ovary of the adult or old cat (fig. 39), we can trace in a beautiful manner the growth of the corpuscles of the membrana granulosa from the fusiform corpuscles of the stroma, which lie next to the protoplasm or yolk of the imbedded young eggs. In a section, we have first the epithelium on the surface. This consists of small flat polygonal nucleated cells (fig. 40, *h*₁), about $\frac{1}{2000}$ th or $\frac{1}{2500}$ th part of an inch in diameter. This layer of epithelium is all that remains of the germ epithelium, and it can be stripped off from the ovary without difficulty. Below the epithelium, and passing round the ovary, is a stratum of connective tissue, consisting of elongated fusiform corpuscles. In the lower part of this stratum the fibres decussate freely. Immediately below this layer we come upon the remains of the large egg clusters which we described in the young kitten's ovary. A perfect zone, consisting of young eggs, lies immediately under the stratum of tissue which passes horizontally round the ovary, under the epithelium. In this zone the eggs lie very close together, many of them are in actual contact. In each egg we recognise the central germinal vesicle, with its spot or nucleolus—the germinal vesicles are all about the same size. Below this zone of eggs is the general stroma of the ovary, processes of which consisting of elongated fusiform corpuscles and fibres, grow in between and around all the eggs in the egg zone. These processes of stroma then become continuous with the horizontal zone of tissue which lies external to the egg zone, and with it grow round the ovary to form the tunica albuginea. As the processes of the stroma of the ovary grow in between and around the young eggs in the egg

zone, fusiform connective tissue corpuscles (n, n) may be seen lying in contact with the yolk substance of the eggs, and these fusiform corpuscles are exactly similar in appearance to the corpuscles which make up the stroma (j, j) in other parts of the ovary. Around many of the young eggs the corpuscles of the membrana granulosa may be traced as they develop from these fusiform connective tissue corpuscles. The nuclei of these connective tissue corpuscles divide, swell up, and gradually form a wreath of little nuclei round the ovum. Each little vesicular nucleus has around it a small quantity of protoplasm, and in the nucleus a spot is generally found. By a constant division of these corpuscles the membrana granulosa at last consists of a thick layer, just as in the case of the Graafian follicle in the rabbit's ovary.

In the adult human ovary an exactly similar development of membrana granulosa corpuscles can be followed out. As the eggs lie imbedded in the stroma, the nuclei of those elongated fibres of the stroma which are in contact with the yolk substance swell up, and by constant division produce a wreath of little corpuscles round the ovum.

On comparing the epithelium on the surface of these adult ovaries with the fusiform corpuscles which lie round the young eggs imbedded in the stroma of the ovary, from which the corpuscles of the membrana granulosa are produced, we at once see how altogether different they are in appearance, and how impossible it is that there can be any connection between them. The epithelium on the surface of the adult cat's ovary is separated from the deeply imbedded eggs by a thick layer of connective tissue, and while the corpuscles of the epithelium are flat, polygonal bodies, with oval nuclei, the corpuscles in contact with the imbedded young eggs are elongated fusiform bodies, similar to those which make up the stroma in all parts of the ovary. By carefully examining these fusiform bodies as they lie on the surface of the egg, and as they lie round the egg, as in a profile view, it is seen that they are entirely different from the epithelial corpuscles on the surface of the ovary, and they are parts of the ovarian stroma. These observations appear to me to prove very conclusively that WALDEYER's view as to the development of the cells of the membrana granulosa is untenable.

After a single layer of membrana granulosa corpuscles is produced round the ovum, the wall of the follicle outside this capsular layer becomes fibrous and vascular. The wall of a nearly ripe Graafian follicle is very vascular. In the rabbit's ovary, in a very young follicle, the corpuscles around the egg, which give rise to the membrana granulosa corpuscles, are at first minute fusiform bodies, and lie flattened against the ovum, when seen in profile. As they develop into the corpuscles of the membrana granulosa they swell up, and by pressure against each other become columnar. Now, immediately outside this layer of columnar corpuscles the tissue consists of minute fusiform corpuscles (figs. 35, 36, 37, j ,

j, j) and blood-vessels. In a nearly ripe Graafian follicle, just before bursting to liberate the ovum, these minute corpuscles in the wall of the follicle outside the membrana granulosa swell up and enlarge, producing large fusiform corpuscles (fig. 38, *j, j*) very similar to the corpuscles of the membrana granulosa at a certain stage of development. This condition of the wall of the ripe follicle is also well seen in the human ovary. In the wall of the nearly ripe Graafian follicle of the rabbit's ovary, we can trace these large fusiform corpuscles becoming more and more like the ordinary small corpuscles of the stroma, as we examine them in the more external parts of the follicular wall. I have ascertained by careful observation that from the very first appearance of the Graafian follicles to their bursting, no blood-vessels pass into the cavity of the follicle to reach the ovum, and yet this body increases enormously in size in a short space of time. This great increase in size is brought about by the production of protoplasm or yolk round the germinal vesicle, even after the latter has reached a definite size.

The chief function of the membrana granulosa is to nourish the ovum during its development. From the first appearance of the ovum as an ordinary germ epithelial corpuscle, until the development of the embryo and its extrusion from the uterus, it exists as a parasite. On the surface of the ovary at first it is an ordinary germ epithelial corpuscle. In the ovary, during a certain stage of its development, it is simply surrounded by vascular nutritious tissue, of which, however, it forms no part, but is simply resting on it. Here it imbibes nourishment until it is thrown off from the ovary. It then passes into the uterus, where changes due to impregnation occur in it, and processes of its surface become imbedded in the vascular mucous membrane of the uterus, and by the agency of these nourishment is absorbed for the germ till it reaches a certain stage of development, when it is thrown off from the parent. In the uterus, as in the ovary, the ovum is simply surrounded by vascular nutritious tissue of which it forms no part, but simply rests on it, and absorbs nourishment from it for its own development.

In the old follicles, some of the cells of the membrana granulosa show a distinct but fine cell-wall round the protoplasm which invests the nucleus. In some of my preparations of the rabbit's ovary, the division of the nuclei of the corpuscles in the membrana granulosa is excellently seen (fig. 31, *v*.) In these older follicles the protoplasm round the nucleus of the membrana granulosa corpuscle is sometimes very extensive, forming a thick layer which may be found drawn out in a fusiform manner (fig. 38, *u*) at one or more points.

In certain parts of the adult cat's and rabbit's ovary, we find large patches of granular cells, and running in between the cells are processes of connective tissue. These large granular cells have nuclei, but they are indistinctly seen because of the granular nature of the substance which surrounds them. In most cases the

nuclei appear as round or oval bodies with nucleoli. An examination of the patches shows that the granular cells are swollen up individuals of the *membrana granulosa* from old and probably ruptured follicles, and are undergoing a fatty degeneration, while at the same time connective tissue corpuscles from the wall of the follicle are growing in between them. In almost every old ovary we find large yellow patches, consisting of the cells we have now described.

At birth the stroma of the human ovary is well developed in every part, and is arranged in a perfect network throughout the whole organ. The ovary might be compared to a piece of sponge in whose meshes ova are contained. The ova in the human ovary at birth are very numerous. They may be fairly estimated at about 35,000 in each organ. In favourable specimens a section of such an ovary presents under the microscope a splendid object. In it the stroma appears to be actually saturated with young ova (fig. 23, *m*), in all of which we can recognise the germinal vesicle as a distinct spherical body, and sometimes two or more germinal spots are seen in it, and around each germinal vesicle is the yelk substance filling up the entire cavity of the Graafian follicle. In all parts of the stroma the minute connective tissue corpuscles crowd together, and in the deeper parts of the ovary, where the oldest Graafian follicles are situated, the connective tissue corpuscles in the walls of the follicles may be seen in contact with and indenting the yelk substance of the included young ova, and in these cases the development of the corpuscles of the *membrana granulosa* may be clearly followed out. In such a section, under the germ epithelium, the last formed egg clusters have not as yet been completely subdivided by the connective tissue stroma into the ultimate egg containing meshes or Graafian follicles, but this is rapidly approaching completion.

After birth, the inclusion of germ epithelial corpuscles by the ovarian stroma becomes less and less, until at the age of about two years the process has entirely ceased; for at this period of development the tunica albuginea has assumed a special character as a complete investment to the whole ovary under the germ epithelium. Most superficially its fibres are arranged in a stratified manner, and run horizontally round the organ; and in sections the general stroma of the ovary is seen in connection with the tunica albuginea in all parts.

From the earliest appearance of the ovary we have on the one hand a growth of germ epithelial corpuscles, and on the other a growth of vascular connective tissue. The germ epithelial corpuscles, in groups or clusters, become gradually surrounded by the vascular connective tissue, and, as development proceeds, the connective tissue grows into the groups between the corpuscles, and these become at last separated from each other, and by the thickening of the tissue in, between, and around them, they are ultimately included in separate

meshes of the stroma. At the same time the imbedded corpuscles are developing into primordial ova. This intercorpuscular growth of connective tissue takes place in all parts of the ovary wherever groups of germ epithelial corpuscles become imbedded, and it continues until the inclusion of germ epithelial corpuscles from the surface of the ovary has ceased. It is in this way, and in this way only, that we have Graafian follicles formed. As already explained, the Graafian follicles are the ultimate meshes of the stroma, formed by the growth of the connective tissue around the developing primordial ova. As these young ova become surrounded by the vascular tissue, the connective tissue corpuscles in the wall of the follicles in contact with their yolk develop into the corpuscles of the membrana granulosa in the manner described. According to my observations, tubular structures have no existence in the ovary at any period of its development, and I have never detected the formation of Graafian follicles and the corpuscles of the membrana granulosa in any other manner than that I have indicated.

By the continued free growth of the vascular stroma throughout the whole organ, the walls of the Graafian follicles become greatly thickened. The oldest and largest Graafian follicles are situated in the deepest parts of the stroma; this is what we would naturally expect, for they were the first formed, and lie surrounded by a great number of blood-vessels which are branches of the large trunks entering the ovary at the hilum.

Thus, in the ovary of a rabbit of about six weeks, one sees the largest Graafian follicle situated deeply in the organ; on the surface of the ovary is the germ epithelium. Between the deeply situated Graafian follicles and the germ epithelium on the surface of the ovary, the Graafian follicles with the contained ova become gradually less and less in size. The youngest Graafian follicles, the last formed, are found immediately under the layer of tissue known as the tunica albuginea on which the germ epithelium rests.

Although the eggs are so numerous in an ovary at birth, very few of them come to maturity.

In the ovary of a cat about five months old is a thick zone of young eggs immediately under that stratum of tissue which passes round the ovary constituting the tunica albuginea. This egg zone is all that remains of the egg clusters which I described as forming so large a part of the ovary of a kitten of two to four weeks.

Below the egg zone the ovary consists of fibro-vascular tissue. This part forms about two-thirds of the whole organ, and thick processes of it pass upwards among the eggs in the egg zone. These thick processes have developed from the long strings or delicate bundles of fusiform corpuscles, which in the young kitten grew up between the egg clusters separating these latter from each other. Secondary offshoots may now be traced growing from the larger bundles in

between the eggs, and as these processes thicken all traces of single large egg clusters become obliterated, and a complete zone of eggs, passing round the ovary takes their place. In this egg zone a great number of eggs become destroyed. In some cases large vacuoles form in the yelk or protoplasm which surrounds the germinal vesicle, and this latter structure is often pushed aside or pressed against the wall of the follicle. The germinal vesicle itself does not seem to be involved in this process of vacuolation. The vacuoles form in the yelk and not in the germinal vesicle. In the human ovary at birth I have also seen a large number of eggs distorted or destroyed by the formation of large vacuoles in the yelk substance.

I do not attempt to explain the meaning of this formation of vacuoles in the yelk substance of the young eggs. I have observed it in the ovaries of several mammals, and I have also seen it taking place in the protoplasm which surrounds the nuclei of large cancer cells, obtained from cancerous ovaries and other malignant growths. I may also mention, that I have seen vacuoles in the protoplasm around the nuclei of pus and renal epithelial cells.

In the central part of this five months' cat's ovary large Graafian follicles are found in various stages of development.

In the growth of these large Graafian follicles, many young and smaller follicles are destroyed by pressure; and it would appear that many of the imbedded eggs simply atrophy and form a pabulum for the connective tissue corpuscles which surround them.

It may be asked, What becomes of the germ epithelium after its egg-forming character has disappeared?

After birth the corpuscles of the germ epithelium gradually become smaller in size, losing their columnar form, till at the age of six years, in the case of the human ovary, they present the appearance of small flattened corpuscles (fig. 41, h_1). They are very small, measuring in their longest diameter not more than the $\frac{1}{3000}$ th part of an inch. The epithelial membrane composed of such corpuscles can be stripped off the surface of the ovary without difficulty. In the human ovary at twelve years of age, the epithelium has preserved an almost identical appearance to that above described, the epithelial corpuscles still remaining of very small size. In the ovary of an adult, the epithelial corpuscles do not measure more than the $\frac{1}{3000}$ th to the $\frac{1}{2500}$ th part of an inch in diameter.

In the ovary of old rabbits, the germ epithelial corpuscles remain as a well-marked layer of small oval corpuscles, and in the ovary of old cats the corpuscles are flattened from above downwards, and show very beautifully their epithelial character (fig. 40, h_1). The corpuscles show well-marked oval nuclei, and the protoplasm round them is extended out and abuts against the protoplasm around neighbouring nuclei; at the lines of contact there is the appearance of a cell wall.

STRUCTURE OF THE OVUM.

In man and in the cat the mature ovum measures about the $\frac{1}{180}$ th part of an inch in diameter.

The zona pellucida, so named by VON BAER, appears when examined under low powers as a strong, perfectly transparent, homogeneous lamella sharply differentiated from the yelk. The membrane possesses considerable elasticity, and when torn to pieces with a needle, the yelk substance with the germinal vesicle escapes in a stream.

Generally, the external surface is exactly parallel to the internal, but in certain specimens I have seen fine processes of the membrane projecting outwards in a radiating manner between the epithelial corpuscles which invest the ovum.

In the zona pellucida investing the ovum in the rabbit's ovary, when examined under very high powers, fine radiating lines may be seen running through it from its external to its internal surface. I have not seen these lines in the zona pellucida in the cat. They were first described by J. MÜLLER and REMAK in the vitelline membrane of the ova of fish. In an extremely thin section of the zona in the rabbit's ovum I have not been able to see them, but in thick sections they are readily found. I believe these radiated lines do not indicate any special structure of the zona, but are produced by the membrana granulosa cells which lie in contact with each other, the lines of contact being seen through the zona as fine linear marks.

The zona resists the action of acetic acid, and its chemical characters are not accurately known.

It is extremely difficult to say how the zona is formed. By most continental observers it is considered to be a product of the follicular epithelium, but from the circumstance that in many instances in the cat's ovary I have discovered it as a well-marked structure round the ovum when little or no trace of the follicular epithelium existed, I am inclined to the belief that it is formed by a hardening of the external part of the yelk of the ovum. I regard the ovum as a large cell, of which the zona is the cell wall and the germinal vesicle is the nucleus.

The parts of the ovum within the zona pellucida consist of the yelk substance and the germinal vesicle. The yelk substance forms by far the greater part of the mature ovum, and constitutes a large spherical mass in which a number of minute granules are suspended. In the eggs of young cats these granules and small bright bodies are found collected together at the external part of the yelk near the zona pellucida. As already mentioned, the primordial eggs do not possess a cell wall at first, but lie in the young Graafian follicles with their yelk substance in close contact with the fibrous wall of the stroma which

surrounds them. It is not uncommon to find two primordial ova in a single Graafian follicle, and as these eggs develop and lie in close contact with each other they are flattened one against the other, and a distinct zona pellucida may be seen round the yolk of each, although no follicular epithelial cells intervene between the eggs. Follicular epithelial cells, however, line the follicle in which these eggs are contained.

I have failed to detect any membrane between the yolk and the zona pellucida. In the mature eggs, as well as in the primordial ova, large vacuoles are occasionally found.

The germinal vesicle appears as a sharply defined globular body placed somewhat excentrically in the interior of the yolk, and is about a fifth to a sixth of the whole egg in diameter. It possesses a delicate membranous wall clearly defined under high powers of the microscope. The germinal vesicle is a highly refractile body, and is at once conspicuous within the ovum. Within it a few granules and one or two distinct nucleoli are found. In a human ovary at birth, very frequently primordial ova with two germinal vesicles are detected, and I have one specimen of the ovary of a child of two years in which a large single ovum contains four well-formed distinct germinal vesicles. In this case the yolk substance is very extensive, and completely surrounds the four vesicles. The wall of the Graafian follicle is in close contact with the yolk, and small fusiform corpuscles around this substance may be traced developing into the corpuscles of the membrana granulosa, but no zona pellucida is to be seen. In the ovary of an adult woman I have also seen a single ovum with two germinal vesicles, but the egg was comparatively young and no zona pellucida or complete epithelial investment was around it.

The nucleolus or macula germinativa is said to be always present in the primordial egg. The presence of two or three germinal spots in a single germinal vesicle has often been noted. Besides the germinal spot a few minute corpuscles are occasionally seen in the germinal vesicle. By some the germinal spot is thought to be a solid body, and by others it is considered to be of the nature of a vesicle, and I have seen round it an extremely fine linear investment. In the cat's ovum very frequently a minute bright spot or vacuole is noticed in the germinal spot. SCHRÖN described this as a solid body, and termed it the "granule." (KORN).

GENERAL CONCLUSIONS.

The following general conclusions have been arrived at in the course of my investigations :—

The corpuscles of the germ epithelium are derived by direct proliferation from those columnar corpuscles which invest the median side or surface of the Wolffian body, and which are continuous with the layer of columnar corpuscles

that lines the pleuro-peritoneal cavity of the embryo in the early stages of development. The stroma of the ovary in the early stages of development is produced by a direct growth out from the interstitial tissue of the Wolffian body immediately beneath the germ epithelium on the median side of the Wolffian body.

The germ epithelial corpuscles proliferate by fission. In the human foetal ovary of $7\frac{1}{2}$ months they measure $\frac{1}{2500}$ th to $\frac{1}{2000}$ th of an inch in their longest diameter, and about $\frac{1}{3500}$ th of an inch in their shortest diameter. Each germ epithelial corpuscle is a nucleus surrounded by a thin film or investment of clear protoplasm. In the act of becoming primordial ova, the nucleus of each germ epithelial corpuscle swells up into a spherical body, within which is generally seen a nucleolus, and around which is produced clear homogeneous protoplasm which subsequently forms the yolk of the ovum. Germ epithelial corpuscles are seen on the surface of the ovary in all stages of development into primordial ova. In each primordial ovum the spherical germinal vesicle presents a sharply defined limiting membranous wall. Within the germinal vesicle is the nucleolus or germinal spot. All the ova in the ovary are derived from germ epithelial corpuscles.

In all parts of the germ epithelium processes of vascular connective tissue stroma grow in between and around certain of the germ epithelial corpuscles, whereby the latter become more and more imbedded in the stroma of the ovary. Germ epithelial corpuscles are being constantly produced on the surface of the ovary, to take the place of those already imbedded in the stroma. The imbedded corpuscles increase in number by division, and the nucleus of each swells up into a spherical germinal vesicle, around which is gradually produced the yolk of the ovum. In all parts of the young ovary under the germ epithelium, groups of germ epithelial corpuscles become imbedded in meshes of the stroma. As each individual in the group swells up the nucleus or germinal vesicle becomes very distinct as a round or spherical body. From the swelling out of each germ epithelial corpuscle in the group, the whole group expands and becomes more or less spherical. Such groups of developing corpuscles are called egg clusters. Each egg cluster is inclosed in a mesh or capsule of vascular stroma of the ovary. Each imbedded germ epithelial corpuscle is potentially an ovum.

The stroma of the young ovary consists for the most part of fusiform connective tissue corpuscles and blood-vessels. The walls of the young blood-vessels in the young stroma consist of connective tissue corpuscles. The connective tissue corpuscles are direct offshoots from the ovarian stroma, and are found in contact with the yolk or protoplasm of each primordial ovum situated among the germ epithelial corpuscles on the surface of the ovary. Wherever we find primordial ova we see connective tissue corpuscles in con-

tact with the yelk of each. In all parts of the ovary we find the nuclei of connective tissue corpuscles dividing. Sometimes these corpuscles are swollen out into round bodies containing three to four nuclei. In each egg cluster several of the included germ epithelial corpuscles are in a much farther advanced stage of development than their fellows. From the walls of the meshes inclosing the egg clusters, delicate processes of vascular connective tissue grow in, between, and around individual corpuscles in the egg clusters, and by a continued intergrowth of the young stroma in this manner each individual of the group becomes at last enclosed in a separate mesh or capsule. These last formed meshes are the Graafian follicles.

As a rule, each Graafian follicle is occupied by one young ovum. The protoplasm or yelk of each ovum is in close contact with the wall of each Graafian follicle. In contact with the yelk of each young ovum, and indenting it, are connective tissue corpuscles, which form part of the wall of each Graafian follicle. In the formation of the membrana granulosa, these connective tissue corpuscles in the wall of the Graafian follicle, and in contact with the yelk of the contained ovum, increase in number by division, their nuclei swell out into little vesicles, and at last a perfect capsule of such corpuscles is produced round the ovum. This capsule is the membrana granulosa or follicular epithelium of the follicle. At first the membrana granulosa consists of a simple layer of corpuscles lining the follicle. The individual corpuscles of the membrana granulosa in the human ovary measure about $\frac{1}{3000}$ th inch. As the ovum becomes mature, the corpuscles of the membrana granulosa proliferate, and then many layers of small corpuscles are produced between the ovum and the follicular wall. The cells of the membrana granulosa are thus derived from the corpuscles of the connective tissue stroma, and not, as WALDEYER states, from the germ epithelial corpuscles. The follicular space is formed by a breaking down and probable solution of certain of the corpuscles of the thickened follicular epithelium in the middle parts of the same. The discus proligerus consists of follicular epithelial corpuscles, which are in contact with the zona pellucida of the ovum. The zona pellucida or vitelline membrane is formed by a hardening of the outer part of the yelk or protoplasm of the ovum, and is not, as REICHERT, PFLÜGER, and WALDEYER stated, a product of the follicular epithelium. At birth the human ovary contains not less than 35,000 ova, few of which reach maturity.

In the human ovary at birth the germinal vesicles measure $\frac{1}{1500}$ th to $\frac{1}{1200}$ th of an inch. Most of them are about the same size, and present a sharply-defined membranous wall. In some germinal vesicles two or three germinal spots are seen. The tunica albuginea is the thickened stroma growing round the ovary. At the age of $2\frac{1}{2}$ years all formation of ova from the germ epithelium has ceased.

Graafian follicles are not formed from tubular structures in the manner

described by PFLÜGER, SPIEGELBERG, and WALDEYER. The appearance of tubular structures passing into the stroma of the ovary are produced by sections through furrows and depressions between irregular prominences on the surface of the foetal ovary. The irregularities of the surface of the foetal ovary are produced at first by the expansion of egg clusters upwards under the germ epithelium. Where the walls of furrows and depressions come in contact, egg clusters are formed by the imbedding of germ epithelial corpuscles in that situation, just as in other situations. Egg clusters are formed in connection with the germ epithelium lining the furrows and depressions. Among the germ epithelial corpuscles lining the furrows, &c., we find large primordial ova, and corpuscles in all stages of development into the same, just as in other situations among the ordinary germ epithelial corpuscles.

In the human foetus, round and oval-shaped groups of germ epithelial corpuscles are found in connection with the germ epithelium all round the ovary. When vertical sections are made through these they present the appearance as if tubular structures filled with developing germ epithelial corpuscles passed from the germ epithelium downwards into the stroma of the ovary.

The development of the corpuscles of the membrana granulosa, from connective tissue corpuscles of the stroma, can be well followed out in the ovaries of adult rabbits and cats.

At the age of six years the epithelium on the human ovary consists of very small flat hexagonal-shaped corpuscles, measuring $\frac{1}{2500}$ th to $\frac{1}{3200}$ th of an inch. The corpuscles are undergoing division. This layer can be stripped off without difficulty. At the age of twelve the epithelium has little difference in appearance from the above, the small size of the epithelial corpuscles being remarkable. The epithelium is beautifully seen in old cats, and must be regarded as homologous with the peritoneal epithelium. In old cats the epithelium on the surface of the ovary consists of very small distinct cells, measuring from $\frac{1}{1800}$ th to $\frac{1}{2000}$ th inch, with granular oval nuclei.

EXPLANATION OF PLATES.

In these plates the same letters have been employed to mark corresponding structures in the whole series of figures.

- a.* The parenchyma of the ovary.
- b.* The fibro-vascular peduncle or stalk of the ovary.
- c.* The lower border of the ovary at the hilum.
- d.* The layer of epithelium on the stalk of the ovary.
- e.* The layer of epithelium stripped off from the stalk, and seen from a surface view.
- f.* Peritoneal epithelial corpuscles.
- g.* Corpuscles intermediate between peritoneal and germ epithelial corpuscles.
- h.* Germ epithelial corpuscles.
- h₁.* Corpuscles from the surface of adult ovary.
- i.* Fusiform shaped corpuscles among germ epithelial corpuscles.
- j.* Connective-tissue stroma of the ovary.
- k.* Blood-vessels.
- l.* Large spherical germ epithelial corpuscles.
- m.* Primordial ova.
- n.* Fusiform corpuscles in contact with the yolk-substance of the primordial ova.
- o.* Large egg clusters.
- p.* Tubiform depressions of the surface of the ovary.
- q.* Small groups of germ epithelial corpuscles being included in the stroma of the ovary.
- r.* The membrana granulosa.
- s.* The zona pellucida.
- t.* The yolk substance of the nearly mature ovum.
- u.* Individual corpuscles of the membrana granulosa, showing the nucleus surrounded with a considerable quantity of protoplasm.
- v.* Division of the nuclei of the corpuscles of the membrana granulosa.

PLATE XXVII.

- Figure 1. Vertical section through the ovary of a foetal calf, about 9 inches long, showing the parenchyma (*a*) and the fibro-vascular stalk or peduncle (*b*) prolonged into the ovary at the hilum (*c*). On the surface of the peduncle is a layer of epithelium (*d*). (Page 349.)
- Figure 2. Surface view of this layer of epithelium. (Page 349.)
- Figure 3. Profile view of the same layer of epithelium, showing the gradual change of peritoneal epithelial corpuscles (*f*) into germ epithelial corpuscles (*h*). (Page 350.)
- Figure 4. The parenchyma of the foetal calf's ovary, showing the germ epithelial corpuscles (*h*) and the growth of delicate connective-tissue of the stroma (*j*) and blood-vessels (*k*) among the corpuscles below the germ epithelium. (Page 350.)
- Figure 5. A portion of the fibro-vascular zone of the foetal calf's ovary, showing a primordial ovum (*m*), surrounded with a wreath of small corpuscles, outside of which are blood-vessels (*k*) and young connective-tissue. (Page 351.)
- Figure 6. Portion of the ovary of a kitten about three weeks old, showing the germ epithelium (*h*), large spherical germ epithelial corpuscles (*l*), and primordial ova (*m*). In contact with the yolk substance of these primordial ova are fusiform corpuscles (*n*). Bundles of young connective-tissue (*j*) are seen growing upwards, between, and around large egg clusters (*o*), situated immediately below the germ epithelium. (Page 352.)
- Figure 7. Semidiagrammatic drawing of the ovary of a human foetus of 7½ months, showing the parenchyma (*a*), and the fibro-vascular peduncle (*b*), prolonged into the ovary at the hilum (*c*). From the peduncle the whole stroma of the ovary is derived. The germ epithelium (*h*) rests on the youngest connective-tissue, which is part of the stroma, and is the forerunner of the tunica albuginea. (Page 357.)

PLATE XXVIII.

- Figure 8. Section of a human foetal ovary of 7½ months, seen under a low magnifying power, showing the stalk or peduncle (*b*), the parenchyma of the ovary (*a*), and the lower border of the ovary (*c*).

- Figure 9. A portion of the same ovary highly magnified showing the gradual change of peritoneal epithelial corpuscles (*f*) on the peduncle into germ epithelial corpuscles (*h*) at the lower border of the ovary (*c*). (Page 359.)
- Figures 10, 11, 12. Surface view of the germ epithelium from the same ovary. In figure 12, among the ordinary germ epithelial corpuscles (*h*) large spherical germ epithelial corpuscles (*l*) are seen. (Page 358.)
- Figure 13. The ordinary germ epithelial corpuscle (*h*) and the larger spherical corpuscle (*l*). (Page 358.)
- Figures 14, 15, 16. Profile views of the germ epithelium. Among the ordinary columnar corpuscles (*h*) large spherical corpuscles (*l*) and primordial ova (*m*) are seen. Fusiform corpuscles (*n*) are in contact with the yolk of the primordial ova. (Page 358.)
- Figure 17. A large primordial ovum (*m*), with several large spherical corpuscles (*l*) included in a mesh of the stroma (*j*) under the germ epithelium (*h*).
- Figure 18. An egg cluster (*o*), consisting of large spherical corpuscles (*l*) included in a mesh of the stroma (*j*), under the germ epithelium (*h*).

PLATE XXIX.

- Figure 19. A portion of the ovary of a human foetus of $3\frac{1}{2}$ months, showing the germ epithelium (*h*) and groups of large germ epithelial corpuscles or egg clusters (*o*) imbedded in the stroma. The stroma (*j*) consists almost entirely of connective-tissue corpuscles. (Page 361.)
- Figure 20. A portion of the same ovary, showing large primordial ova (*m*) imbedded in the stroma (*j*). Fusiform corpuscles (*n*) of the stroma are seen in contact with the yolk of these primordial ova. (Page 361.)
- Figure 21. A portion of the ovary of a human foetus of $7\frac{1}{2}$ months, showing the manner of inclusion of the germ epithelial corpuscles in groups (*g*) in the stroma of the ovary. (Page 362.)
- Figure 22. A portion of the ovary of a human foetus of $7\frac{1}{2}$ months, showing in section the furrows (*p*) which lie between the prominences on the surface of the ovary. (Page 363.)
- Figure 23. A portion of the ovary of a child at birth, showing the stroma (*j*) crowded with primordial ova (*m*).

PLATE XXX.

- Figure 24. Section through the ovary of a human foetus of $7\frac{1}{2}$ months, showing the germ epithelium (*h*), large spherical corpuscles (*l*), and groups of similar corpuscles or egg clusters (*o*) imbedded in meshes of the stroma (*j*). In the lower part of the figure many primordial ova (*m*) in various stages of development are seen. In contact with each primordial ovum are fusiform, connective-tissue corpuscles (*n*), similar to the fusiform corpuscles of which the stroma consists. Numerous blood-vessels (*k*), whose walls consist of connective-tissue corpuscles, ramify throughout the whole ovary. (Page 365.)
- Figure 25. A small egg cluster consisting of large spherical germ epithelial corpuscles with fusiform connective tissue corpuscles (*n*) intermingled. (Page 366.)
- Figure 26. A small group of spherical germ epithelial corpuscles.
- Figure 27. A primordial ovum. It has no zona pellucida.
- Figures 28, 29. Primordial ova (*m*). In contact with and indenting the yolk substance of each, are fusiform connective-tissue corpuscles from the wall of the young Graafian follicle.
- Figure 30. The membrana granulosa (*r*) when first formed seen in section. Outside the membrana is the ovarian stroma (*j*). From the human ovary at birth.
- Figure 31. The corpuscles of the membrana granulosa from the nearly ripe Graafian follicle of the rabbit's ovary. The letter *v* points to the division of the nuclei of the corpuscles.
- Figure 32. Surface view of the membrana granulosa (*r*) when first formed. From the human ovary at birth.

PLATE XXXI.

- Figures, 33, 34, 35, 36, 37, 38, illustrate the development of the membrana granulosa in the ovary of an adult rabbit. (Page 368.)
- Figure 33. A primordial ovum (*m*). Fusiform connective-tissue corpuscles (*n*) of the stroma (*j*) lie in contact with the yolk substance of the young ovum.
- Figure 34. A young ovum farther advanced in development than the last. Some of the fusiform corpuscles (*n*) in contact with the yolk are swollen out.

- Figure 35. An ovum still farther advanced in development, completely surrounded by a wreath of corpuscles, which have been derived from the fusiform corpuscles of the stroma (*j*). This wreath is the membrana granulosa (*r*) seen in section.
- Figure 36. A Graafian follicle from which the ovum has been removed. The membrana granulosa (*r*) is seen in section.
- Figure 37. A section through a Graafian follicle, and the nearly ripe ovum contained in it. The membrana granulosa (*r*) consists of several layers of corpuscles. The zona pellucida (*s*) is well developed, and the yolk substance (*t*) at its peripheral part contains numerous bright granules and particles.
- Figure 38. A section through the membrana granulosa and wall of a ripe Graafian follicle just before bursting. The membrana granulosa (*r*) consists of several layers of corpuscles. Each corpuscle is a large nucleus surrounded by protoplasm (*u*). In the wall of the follicle outside the membrana granulosa the fusiform corpuscles of the stroma (*j*) are very large. (Page 371.)
- Figure 39. A section through the egg zone of an old cat's ovary. The stroma (*j*) consists almost entirely of fusiform corpuscles. Around the young eggs (*m*) the fusiform corpuscles (*n*) of the stroma may be traced in their development into the corpuscles of the membrana granulosa. (Page 369.)
- Figure 40. The epithelium (*h*₁) from the surface of the same cat's ovary.
- Figure 41. The epithelium (*h*₁) from the surface of the ovary of a child six years of age.
- Figure 42. The same epithelium more highly magnified showing the "grooved" appearance of the corpuscles.
- Figure 43. Germ epithelial corpuscles (*h*) seen in profile. From the ovary of a 7½ months' human fetus.
- Figure 44. A primordial ovum (*m*) surrounded with fusiform corpuscles (*n*). From the stroma of the same ovary.
- Figure 45. An ovum (*t*) from the deeper parts of the same ovary, showing the appearance of the membrana granulosa (*r*) when first formed round the ovum.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 5.

Fig. 4.

Fig. 7.

Fig. 6.

M^r Parlano & Erskine, Lith^s Edin^r

Jas^s Poulin, M.D. del^t