THE DEVELOPMENT OF THE OVARY OF THE GUINEA-PIG, CAVIA CODAYA, IN EMBRYOS OF EIGHTEEN TO THIRTY DAYS OF AGE INCLUSIVE; WITH SOME OBSERVATIONS CONCERNING ITS SUBSEQUENT DEVELOPMENT

by

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B. A., Kalamazoo College, 1927

A THESIS

submitted in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1929

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INTRODUCTION

This investigation has been carried on as a part of the study of the reproductive system of the guinea-pig Cavia cobaya, begun by Dr. Mary T. Harman.

The purpose of this particular phase of the study has been to trace the development of the ovary of the guinea-pig in embryos of eighteen to thirty days of age inclusive, to give some observations concerning the subsequent development of that organ in the embryo, and in particular to note the growth of the gonad, its relation to surrounding organs, its histological structure, differentiation into testis or ovary, and the characteristics of the ovary in the period immediately following differentiation.

Although guinea-pigs are used extensively as experimental animals, the development of their reproductive system has not been studied to any great extent. In this study an attempt has been made to check observations with the results given by other investigators concerning the development of the ovary in different mammals.

The origin of the germ-cells has not been one of the primary phases of this study. Earlier stages than those used here would be necessary for such an investigation.

These observations begin when the genital ridge has already differentiated to the extent that it is recognizable and they continue through the period of the differentiation of the ovary. A few observations concerning the later development of the ovary are given.

ACKNOWLEDGEMENTS

The writer desires to express her gratitude to Dr. Mary T. Harman for suggestions and criticisms given throughout the investigation and for her aid in caring for the guinea-pigs. Dr. H. L. Ibsen of the Animal Husbandry Department has kindly cooperated by providing animals for this study and by suggestions concerning their care. The preserved material used for the later stages was also contributed by him.

MATERIAL AND METHODS

The guinea-pigs used in this study were obtained from the Animal Husbandry Department and given the same care which is given in that Department.

The males were kept in separate cages so that accurately timed embryos could be obtained. The females were put with the males each day and observed so that the time of copulation could be noted. At first only unmated

females were placed in the cages with the males, but after it was found that pregnancy did not always follow copulation, both the mated and unmated females were put with the males regularly. When copulation occurred, the female was left with the male until a number of copulations had taken place. The time of copulation of each animal was recorded. Several females were mated two or three times without becoming pregnant.

The females were killed by means of gas at the desired number of days after insemination and the embryos removed from the uteri. Embryos from eighteen through twenty-seven days of age were stained in toto in borax carmine and the sections restained in Ehrlich's haematoxylin. The ovaries were removed from the older female embryos, sectioned, and stained in Ehrlich's haematoxylin. Bouin's fluid was used as a fixative. Paraffin was used for imbedding. All sections were cut at ten microns and mounted in serial order.

The age of the embryos is given in number of days after copulation of the parents, for practically all the adult females were mated in the morning and killed in the morning so that there was not more than two or three hours difference in computing the age in this way. The length

of the embryos was taken, but variation in the length of embryos of the same age and even in the same litter is so common that age in days was considered more reliable.

Of the forty-six guinea-pigs killed in order to obtain embryos, twenty were not pregnant. Materials used for this paper were from embryos of the ages recorded in the following table:

Age in days	Number of embryos	Age in days	Number of embryos
18	2	28	3
20	2	30	2
21	3	40	2
22	2	43	2
23	4	46	1
24	3	50	1
25	4	55	1
26	3	60	1
27	4	1 post-partum	1

The fifty-, fifty-five-, and sixty-day material was obtained from preserved embryos.

Study was begun with fully differentiated ovaries and proceeded back through earlier stages to the time when the genital ridge is recognized with some difficulty so that there would be no question concerning the recognition of the gonad in earlier stages or of the germ-cells contained therein. Special study was made of the critical period of differentiation of the gonad into testis or ovary to determine what characteristics distinguish the two at this period.

REVIEW OF LITERATURE

There are many differences in the observations of investigators who have studied the development of the ovary in mammals and other vertebrates. Some may be due to the differences in the stages studied and it may be that there are differences in different animals.

It is generally recognized that the genital primordium appears in the ventro-median surface of the Wolffian body as a genital ridge. According to Saimont (1906), the formation and significance of the genital ridge in amniotes were studied by Borsenkow (1883), Schulin (1881), Laulanié (1886), Coert (1888), Janosik (1885), and

Mihalkovics (1885).

Borsenkow, Schulin, and Laulanié considered that the genital ridge is formed from a tissue whose cells may differentiate either into epithelial tissue or into connective tissue.

Coert, in his study of the rabbit, believed that the genital ridge is made up of mesenchymatous tissue which divides before differentiation of the gonad into a peripheral zone and a basal zone with a gradual transition between the two. The peripheral layer furnishes the epithelium and seminiferous tubules of the testis; the cortical and medullary zones of the ovary. The deeper layer gives rise to the rete testis and rete ovarii.

Janosik and Mihalkovics considered that the germinal epithelium plays a very important role in the formation of the sex-glands by giving rise to a primary proliferation which forms the medullary cords in the ovary and the seminiferous tubules in the testis, and a secondary proliferation in the ovary which gives rise to the egg-tubes of Pflüger.

According to Minot (1897) the development of the genital ridge in amniotes may be divided into four stages: (1) the production of mesenchyme from the mesothelium; (2) the development of the medullary cords; (3) the

appearance of the primitive ova; and (4) the differentiation of the sex-glands.

Heinecke (1900) found in the cat at the earliest stage studied (eighteen-day embryo) that the genital ridge is made up of a uniformly thickened epithelium which is sharply distinguished from the neighboring tissues. In the ovary he noted two proliferations from the germinal epithelium which corresponded to those described by Janosik and Stinzhorius. He found no cells in the early stages which corresponded to the primordial germ-cells described by Waldeyer (1870) in amniotes.

In his studies of the development of the ovary and testis in the pig and rabbit, Allen (1904) found primordial germ-cells in the earliest stages studied, but believed they had migrated to the genital region in an earlier stage. In the testis and ovary, respectively, he homologized the tunica albuginea, the interstitial cells, the primordial germ-cells and the rete tubules which are found in both, the seminiferous tubules and medullary cords, and the germinative cells of the seminiferous tubules and the follicle cells.

Felix (1912) stated that in the indifferent stage of the gonad in mammals, the uniform epithelial mass separates into a superficial epithelium and an epithelial

nucleus and that after separation the superficial epithelium has no further participation in the formation of young ova. The epithelial nucleus, he believed, is the active part which forms the tunica albuginea, testis tubules, and rete testis in the male. In the ovary by three concomitant but independent processes there occur an ingrowth of connective tissue and vessels from the hilus toward the periphery, the conversion of most of the genitaloid cells and indifferent cells into young ova, and a new formation of the epithelial nucleus at the periphery. In older fetuses he described "foldings of the surface epithelium" or downwardly growing epithelial cords, but did not attach any importance to them.

Kingsbury (1913), in his study of the origin of the interstitial cells of the ovary of the cat, stated that the egg-tubes of Pflüger, which are called germinal branches by Saimmont (1906) and described as a second proliferation from the epithelium are not "downgrowths" from the epithelium, but represent more exactly cell trails left behind in the advancement of the surface as the ovary increases in size. The surface epithelium retains, throughout, its connection with the epithelial cords which he terms egg-cords.

Margitt (1925, 1930) discussed the development of the gonad in the albino rat, taking into consideration the indifferent stage and the history of the sole germ-cells.

Hinkins (1928) traced the development of the gonad of man from its first appearance as a thickening of the genital ridge, through the indifferent stage, and as a differentiated testis or ovary. He observed that the superficial epithelium, during the indifferent period, proliferates cells in cord-like groups into the stroma, forming the early sex-cords which are transformed into the seminiferous tubules of the testis and the medullary cords of the ovary. The superficial epithelium is the source of the indifferent and genitaloid cells of the incipient ovary, but after formation of the albuginea it has no further part in the delivery of cells into the epithelial nucleus. Hinkins does not mention a secondary proliferation of epithelial cells after differentiation of the ovary.

Swift (1915) recognised two proliferations from the epithelium in the ovary of the chick.

There have been different opinions concerning the origin of the germ-cells. Some workers believe that the germ-cells arise in early embryo-formation taking no part in histogenesis and are set aside to eventually give rise

to the gonads of the individual. Hargitt (1925) in a review of the work of several investigators carried on to determine whether germ-cells are segregated in early ontogeny as a special type of cell, stated that Eigenmann (1891), in his work with the teleost fish, was the only one who had recognized germ-cells in any vertebrate before germ layers were formed.

Many investigators believe they have traced the germ-cells from their origin in extra-regional positions through their migration into the genital ridge. Felix (1912) in mammals, Dustin (1910), Berenberg-Gossler (1914), Jarvis (1908), and Allen (1906) in reptiles, Allen (1911) in ganoids, Swift (1914) and Goldsmith (1928) in the chick are advocates of the extra-regional, and usually endodermal, origin of the primordial germ-cells at least.

Rubaschkin (1908, 1909) believed that he could trace the germ-cells of the rabbit and guinea-pig from the endoderm at some distance on each side of the hind-gut through a well-defined path into the genital ridge.

Nussbaum (1880) and his followers denied that any cell which has been included in the formation of soma can be differentiated and subsequently form a germ-cell.

Other investigators believe that all the germ-cells, or at least the definitive germ-cells, are formed by a metamorphosis of the epithelial cells. Waldeyer (1870) was the first to suggest that germ-cells are really differentiated epithelial cells. Felix (1912) in mammals, Gatenby (1918, 1923, 1924) in amphibians and birds, Hargitt (1925) in the albino rat, Simkins (1928) in man, Sun (1923) in post-pubertal oögenesis of the guinea-pig, and others agree that epithelial cells can differentiate into germ-cells.

Minot (1897) doubted whether the large cells appearing early in amniotes and designated as germ-cells could be considered as primordial germ-cells since they were correlated with cell-divisions.

Lainsont (1906) did not find the primordial germ-cells in any of the early stages of the gonad.

OBSERVATIONS

In the early development of the guinea-pig embryo, the primordium of the gonad appears as a region of thickened epithelium, the genital ridge, medio-ventral to the Wolffian body near the angle between the Wolffian body and the root of the mesentery. In the first stage observed (Fig. 1), this ridge is sharply demarcated from

the subjacent epithelium and has a thickness of two or three cells, although the cells are not arranged in definite rows. As the epithelial cells increase in number by mitoses which are very common in the early stages, the genital ridge increases in thickness and becomes somewhat separated from the Wolffian body by lateral and median furrows. The furrows, at first shallow, become deeper, until in the last part of the indifferent period the gonad is connected to the Wolffian body by a narrow mesentery only. The gonad, in the meantime, increases in breadth and length.

The epithelial cells form a syncytium which is broken infrequently in the very early stages by large cells with inclosed cytoplasm. The epithelial nuclei lie with their long axes perpendicular to the surface of the genital ridge while the mesenchymatous cells beneath have their long axes parallel with the surface of the ridge (Figs. 1 and 2).

In the earliest stage studied, only a few of the mesonephric tubules connect with the Wolffian duct, but the mesonephros develops rapidly in the few days following until the metanephros appears in its definitive position in the twenty-five-day embryo, after which it begins to degenerate.

After the early period when the genital ridge becomes a little more than a thickening of the epithelium and begins to project into the coelom, the large primordial germ-cells which were almost lacking earlier become more numerous. At first they are all located near or in the surface epithelium (Figs. 3 and 4), but in later stages they are found in the deeper part of the gonad as well (Figs. 5, 9 and 10). These cells are larger than the epithelial cells and have a limited cytoplasm. Their nuclei are larger, more rounded and clearer than the nuclei making up the body of the gonad.

By the time the gonad has thickness equal to that of the Wolffian body, one can distinguish in it a peripheral layer of cells definitely one cell deep and a deeper part composed of masses of cells similar to those in the peripheral layer, or superficial epithelium (Fig. 5). This division continues in later stages, although at times it is almost lost. The masses of cells assume a cord-like appearance (Fig. 6) and extend as proliferations from the surface epithelium toward the subjacent stroma, forming an irregular boundary between the gonad and mesenchyme. These primitive sex-cords become the seminiferous cords or tubules of the testis and the medullary cords of the ovary. Connective tissue cells appear between the cords in narrow

thread-like strands, [illegible] more abundant in later stages especially after sexual differentiation of the gonad (Fig. 10). The presence of sex-cords gives the appearance of loosely connected masses of cells not entirely separate from the surface epithelium.

The first indications of differentiation of the gonad into testis or ovary are found in gonads of some twenty-six-day embryos in which the sex-cords become more distinct than formerly and in which a very indistinct region subjacent to the surface epithelium suggests the formation of a tunica-albuginea, while other gonads from embryos of the same age retain the appearance of those of the preceding stage.

Differentiation of the gonad into testis or ovary takes place in a relatively short time, in embryos between twenty-six and twenty-seven days of age. The characteristics which distinguish the testis are (1) a thin superficial epithelium, (2) a broad subjacent layer of flattened cells, the tunica albuginea, which is of uniform thickness and sharply separates the superficial epithelium from (3) the seminiferous tubules (Fig. 7). The seminiferous tubules appear as long, twisted, anastomosing cords which have the essential characteristics of the fully developed tubules of later stages. The cells composing them are

somewhat prismatic and have their long axes perpendicular to the direction of the cord.

The ovary of the same age does not show a definite differentiation but rather appears similar to the preceding stage (Figs. 8 and 9). The superficial epithelium is thicker than in the testis but is not so sharply delimited. The nuclei in the extreme peripheral portion are arranged in a layer and are closer together than in the remaining portion of the superficial epithelium. Sex-cords, or medullary cords, are present but become more and more indistinct and usually their connections with the superficial epithelium are broken. Primordial germ-cells appear in all parts of the ovary and are much more abundant here than in the testis of the same age. They are more numerous in the peripheral portion in all the stages of the differentiated ovary.

In the differentiated ovary the medullary cords begin to break up and become less distinct. Strands of connective tissue cells ramify between the cords and spread out beneath the surface epithelium giving a suggestion of an incipient tunica albuginea (Fig. 10) and making the superficial epithelium a distinct layer again. In some gonads of the same age the connective tissue cells are not prominent (Fig. 11).

Secondary proliferations of cells from the superficial epithelium begin in the differentiated ovary and penetrate the tunica albuginea which becomes more distinct temporarily in the thirty-day embryo (Fig. 12). The tunica albuginea of the ovary goes into the formation of the cortical portion in the older gonad. The ovary then is made up of two distinct regions, a cortical portion and a medullary portion as in the forty-day embryo. The cortex is composed of the thin superficial epithelium and cords of the second epithelial proliferation which are separated by connective tissue. The cortical cords, which are still continuous with the epithelial cells, are made up of groups of germ-cells in mitosis. The large number of germ-cells gives a lighter color to the cortical region on account of their lightly staining cytoplasm.

The medullary portion is made up of the medullary cords which are composed of indifferent cells and connective tissue cells, with a few germ-cells scattered among the cords. The cell-nuclei of this region are more closely crowded together than in the cortex, giving a darker color. One portion merges gradually into the other on account of the irregular character of the cords.

In the deeper part of the cortex of the forty-six-day embryo, indifferent cells begin to surround the germ-cells, forming incipient follicles. Such follicles increase in number and degree of development in later embryonic life, throughout the cortex, but those which are more fully developed are usually found in the deeper part of the cortex. The follicles occur in groups or cords separated by connective tissue.

DISCUSSION

The discussion of this investigation includes an explanation of the methods used in obtaining material and a discussion of the data obtained from this study.

Methods

The oestrus cycle of the guinea-pig, as determined by Stockard and Papanicolaou (1917), is normally of fifteen to seventeen days duration. The period of sexual activity lasts about twenty-four hours and Dr. H. L. Ibsen, in conversation, stated that he has found in working with great numbers of guinea-pigs that the female will receive the male during two or three hours of this period only and soon after parturition. Lams (1913) and Stockard and Papanicolaou (1917) also found that copulation will take place soon (two to four hours, according to Lams) after

parturition if the female is placed with the male.

In the period of heat, the vagina is open, but contrary to the condition in most mammals the vaginal orifice closes after the period of sexual activity and remains closed until near the time when this period recurs (Kelly and Papanicolaou, 1927), so that during this interval the female not only refuses to accept the male but is physically incapable of having copulation take place.

Since the female guinea-pig rarely shows an external flow from the vagina, there is no definite way of knowing when the animal is in heat. The only expression of this condition observed is her willingness to receive the male and this is only indicated when the male is present and a copulation takes place. Because there are no external signs of the period of sexual activity upon which one can rely with any degree of certainty, the method found most satisfactory was to try out all the females individually each day with the chance that some of them might be in heat at that time.

Ovulation, according to Lams (1913) and Stockard and Papanicolaou (1917), seems to occur spontaneously during every heat period without exception and is not dependent upon copulation. Fertilization, however, does not

follow every time copulation has occurred for of the forty-six guinea-pigs killed in this investigation twenty were not pregnant although each animal had been mated once, twice, and in some cases, three times.

The length of the embryos was found to vary in embryos of the same age and even in the same litter. Such variation is discussed and accounted for by Ibsen (1923) as resulting from crowding, nutrition, and body weight of the mother. Bergitt (1925), who found similar variation in the size of rat embryos, suggested that different sizes in the same uterus may be due to variation in time of fertilization of the different eggs. Stotsenburg (1915) found similar variations in size of albino rat embryos in a study of their weight.

The period of gestation of the guinea-pig is sixty-five to sixty-seven days normally. The young at parturition are so far developed that they can be taken from the mother almost at once. The size of the litters varies, with an average of two or three young to a litter. Four were the most found in any litter in this study.

Development of the Ovary

The data from this study show that the development of the ovary of the guinea-pig may be divided into two periods

(1) the indifferent period, from eighteen to twenty-six days of gestation, in which the gonad may be distinguished from the surrounding tissues but has not differentiated to the extent that it may be recognized as an ovary or a testis; and (2) the period of significant changes in the gonad which begin in the twenty-six-day stage and result in the differentiation of the gonad into a testis or an ovary with their respective characteristics.

The Indifferent Period. The gestation period of the cat which is sixty-three days (Marshall, 1910) is almost the same as the gestation period of the guinea-pig. Although the degree of development of both animals at parturition is different. Sainmont's (Sainmont, 1906) description of the development of the ovary of the cat shows similar critical periods which correspond in days and, to a certain degree, in character to those which have been found in this study of the guinea-pig. In the first stage studied (eighteen-day embryo), Sainmont found the first indication of a genital ridge in the medio-ventral portion of the Wolffian body. The ridge consists of epithelium, slightly thickened, due to the fact that the epithelial cells instead of being cubical become cylindrical, although they form only a single layer distinguishable from the neighboring stroma.

In the guinea-pig embryo of eighteen-days, a similar thickened epithelial ridge is found. It has a thickness of two or three cells whose axes are perpendicular to the surface. The ridge is definitely demarcated from the subjacent mesenchyme which is not compact and whose cells lie parallel with the surface (Fig. 1). The epithelial cells of the genital ridge merge gradually into the coelomic epithelium, and in the ridge itself form a syncytium, a condition which Sainmont said does not exist in the early period of the gonad in the cat.

The appearance of the genital ridge as a region of thickened epithelium conforms with the findings of Waldeyer (1870), Coert (1906), Minot (1897), Allen (1904), Burgitt (1925), Simkins (1928), and others. The occurrence of mitosis in many of the epithelial cells indicates that rapid multiplication of cells is taking place with the result that this region is becoming thickened. This condition continues without much change during the next few days (Fig. 2). The ridge does not become much thicker and in practically all respects retains the same character as when first observed. This corresponds closely to the condition found by Sainmont in the early development of the gonad in the cat.

Hargitt (1923), Minot (1897), and others have suggested that the mesenchyme subjacent to the genital ridge is also derived from the epithelium of this region. In the early gonad when the genital ridge is merely thickened epithelium, the genital region and mesenchyme are distinct, the former being more compact than the latter. The nuclei of the ridge lie perpendicular to the surface while the nuclei of the mesenchyme are parallel with the surface. Not until the twenty-one-day stage does the one region appear to merge gradually into the other without a sharp boundary. Sainmont (1906) and Allen (1904) mentioned a basement membrane separating the genital ridge from the mesenchyme. This was not observed in the guinea-pig.

Such a distinction is lost in the twenty-one-day stage (Fig. 3) for here the genital region merges gradually into the mesenchyme with no distinct boundary between. At this same age in the cat embryo, Sainmont described the genital ridge as having an uneven winding inner boundary which is broken by epithelial buds penetrating the stroma beneath. The same condition was reported by Allen (1904) in the pig where cells were proliferated by the epithelium which formed chains penetrating the basement membrane.

A striking feature of this stage is the occurrence of cells with large rounded nuclei and lightly staining, well-defined cytoplasm. These cells correspond to the primordial germ-cells described by Waldeyer (1870), Felix (1912), Berenberg-Gossler (1914), Swift (1914), Simkins (1928), Allen (1904), and many others who have studied the gonad of mammals and other vertebrates. Saimmont (1906) did not find any such cells in the early stages in the development of the ovary of the cat. The origin of these cells in the guinea-pig was not taken up in this study for they are present in the genital ridge in the earliest stages observed. In the earliest stages only a very few large cells which look like the primordial germ-cells of the later stages are found. Other large cells are found, but since they are all in mitosis, it seems probable that they are ordinary epithelial cells which are normally larger because of division. Minot (1897) gave such an explanation for his belief that primordial germ-cells are not present in the early stages in amniotes. Allen (1904), in the pig and rabbit, found these cells in the earliest stages studied. In the guinea-pig, Rubaschkin (1909), according to Allen (1904), found the primordial germ-cells in the endoderm at some distance from the genital region, and believed that they migrated into this region.

When they are first observed in this study, the germ-cells have the distinctive characteristics mentioned and there is no evidence either that they have migrated into this region or that they are transformed epithelial cells. After the very early stages they are found in increasing numbers, near the surface of the gonad at first and then in the deeper portion. Since germ-cells in mitosis were not observed, it was not determined how these cells increased in number from one day to the next. Growth of the peripheral portion of the gonad might readily tend to cause some of the germ-cells to lose their proximity to the surface and become imbedded in the deeper part of the gonad (Fig. 5). Such an explanation is given also by Hargitt (1926).

At the same time that the germ-cells are increasing in number and the gonad is growing in breadth, depth, and extent, other changes are taking place in the gonad. Groups of cells appear to be proliferated from the surface epithelium which has become distinct from the inner epithelial cells, and penetrate the subjacent region. These cords of cells are significant for they give rise during the period of sex-differentiation to the seminiferous tubules of the testis and the medullary cords of the ovary.

Janosik (1885), and Mihalkovics (1885) considered that the germinal epithelium of the genital ridge plays a most important role in the formation of the sex-glands by giving rise to this primary proliferation which forms the medullary cords and seminiferous tubules. Saimmont (1906), Simkins (1928), Allen (1904), and Hargitt (1928) described a primary proliferation from the epithelium of the genital ridge in mammals, and Swift (1914) in the chick. According to these investigators the primary proliferations or sex-cords are concerned with differentiation of the gonad into a testis or an ovary. The sex-cords are characteristic of the indifferent period, during which time they make up the larger part of the gonad. They become more distinct from day to day and appear as anastomosing masses of cells (Fig. 6). By the time the sex-cords are fairly recognizable, the gonad has become divided into two distinct regions, an outer superficial epithelium and an inner epithelial mass. The inner portion is composed of the sex-cords. Such an appearance is retained during the remainder of the indifferent period (Figs. 5 and 6).

Felix (1912) stated that after the separation of the superficial epithelium from the inner portion, the epithelial nucleus is the active portion which forms the definitive parts of the testis and ovary. Simkins (1928)

found that the superficial epithelium is the source of the indifferent and genitaloid cells of the incipient ovary.

During this period, in embryos of eighteen to twenty-five days of age inclusive, there is no indication of sex-differentiation. All gonads of the same age have essentially the same characteristics. The significant features of this period, which correspond for the most part to the observations of investigators in similar studies, are (1) the rapid growth of the gonad from a thickened epithelium to a definite organ suspended from the Wolffian body by a narrow mesentery; (2) the appearance of primordial germ-cells in the early stages; (3) the proliferation of epithelial cells which form sex-cords, and (4) the division of the gonad into a superficial epithelium and an inner epithelial mass.

Sex-differentiation. The critical period of differentiation of the gonad into testis or ovary is of short duration for the differentiation takes place suddenly. The testis assumes definite characteristics which distinguish it from the ovary. The ovary does not become differentiated as soon as the testis. In the guinea-pig this transformation takes place sometimes between the twenty-sixth and twenty-seventh day of development. In the twenty-six-day embryo there are slight indications of differentiation, but not great enough to distinguish posi-

tively the testis from the ovary. In the twenty-seven-day embryo, differentiation has taken place to such an extent that there is no question about the sex of the animal.

The characteristics which distinguish the testis are (1) a thin superficial epithelium, (2) a broad tunica albuginea of uniform breadth, and (3) seminiferous cords or tubules (Fig. 7). The cells of the tunica albuginea are flattened and lie with their axes parallel with the surface epithelium. The cells surrounding the seminiferous cords have their axes perpendicular to the direction of the cord and appear distinct from the indifferent cells and connective tissue cells separating the cords. Slight indications of a tunica albuginea are apparent a day earlier but it is not a distinct uniform layer. The sex-cords of the inner epithelial mass have become transformed into the seminiferous cords. At this early stage the testis has the essential characteristics of the definitive testis.

The ovary retains the characteristics of the latter part of the indifferent stage (Figs. 8 and 9)and is thus recognized as an ovary only because the testis is distinctly differentiated. The sex-cords which become the medullary cords of the ovary are not very distinct. The superficial epithelium is thicker than that in the testis and not clearly demarcated. In some places it still

retains a connection with the medullary cords, but usually these connections are broken. Strands of connective tissue appear among the cords in a few places but are of infrequent occurrence. Primordial germ-cells are numerous and scattered throughout the ovary, but are infrequent in the testis of the same age.

Sex-differentiation of the gonad in the cat probably takes place at practically the same age because Saimmont (1906) stated that differentiation was taking place in the twenty-six-day embryo and had completely occurred before the twenty-nine-day stage. Hargitt (1926), Allen (1904), Felix (1912), Simkins (1928), and others have described this period in mammals and have remarked about the extremely quick differentiation that occurs and the fact that the testis is differentiated before the ovary acquires its definitive characteristics. Differentiation is concerned with the sex-cords mainly, and with the formation of the tunica albuginea in the male.

In the ovary, after the critical period of differentiation, the medullary cords become more and more indistinct. The masses of cells forming the cords are somewhat separated by connective tissue but not to such an extent that they retain their identity (Fig. 10). In some ovaries of the twenty-eight-day stage, the connective tissue cells ramify as far as the superficial epithelium under which

they spread out. This makes it appear like an incipient albuginea (Fig. 10). In other ovaries of the same age, the connective tissue cells are not prominant (Fig. 11). Allen (1904), Sainmont (1906), and Simkins (1928) noted the occurrence of a tunica albuginea in the ovary of the mammals studied at approximately this same stage of development. Sainmont believed that the tunica albuginea is only temporary in the cat and contributes to the formation of the cortex. Simkins suggested that it is probable that after the formation of the tunica albuginea in the ovary no more cells can be delivered by the superficial epithelium into the inner part of the gonad.

In the guinea-pig the tunica albuginea which is more distinct in the ovary of the thirty-day embryo (Fig. 12) is apparently a temporary formation and may, as Sainmont suggested in the case of the cat, enter into the formation of the cortex, for it does not appear in the forty-day embryo or thereafter. At no time in the stages observed does it form a distinct broad layer as it does in the testis. It is broken at intervals by a secondary proliferation of cells from the superficial epithelium which begins soon after the period of sex-differentiation.

Such secondary epithelial proliferations have been described by Sainmont (1906), Hargitt (1925), Kingsbury (1913), Felix (1912) and others. Felix described these as "foldings of the surface epithelium" or downwardly growing epithelial cords, and attached no importance to them. Kingsbury believed that these so-called egg-tubes of Pflüger are not "downgrowths" from the epithelium but represent cell-trails left behind in the advancement of the surface of the ovary as the latter increases in size and that these egg-tubes remain connected with the surface epithelium.

In the forty-day embryo the ovary is made up of two distinct parts, a cortical portion and a medullary portion. The latter which stains intensely consists of the medullary cords which appear simply as masses of cells separated by connective tissue. Very few germ-cells are present in this region. The cortex contains cords of germ-cells which are continuous with the surface epithelium and are separated by connective tissue. The germ-cells are present in great numbers, grouped in clusters, and are practically all in mitosis. Because of the many germ-cells with their lightly staining cytoplasm, the cortex is much lighter in color in section than the medullary portion. The

boundary between the two is indistinct as a result of the irregular character of the cords in both portions. These cords are probably the same as the egg-tubes of Pflüger described by other writers as the secondary proliferations from the surface epithelium.

The principal change which occurs in the ovary during the remaining period of embryonic development is the formation of follicles. This is first observed in the forty-six-day embryo in which connective tissue cells and indifferent cells surround some of the germ-cells in the deeper part of the cortex. In later stages the incipient follicles are more numerous and new layers of follicular cells are added to the germ-cell or ovum. The follicles which are more fully developed are usually found in the deeper part of the cortex and occur in groups separated by connective tissue. It has not been determined whether or not these ova are descendants of the primordial germ-cells of the early stages.

The period of the development of the ovary after sex-differentiation is characterized by the formation of (1) a temporary tunica albuginea, (2) a secondary epithelial proliferation which probably forms the cortical cords or egg-tubes of Pflüger, (3) the differentiation of the ovary into a medullary and a cortical region, and (4) the

formation of follicles after mitoses of the germ-cells.

The development of the ovary of the guinea-pig does not differ in its more important phases from the development of the ovary of other mammals described by different writers. The fundamental processes are similar and the characteristics of the two principal periods do not differ in essential details from those noted in other mammals.

SUMMARY

In summarizing, the foregoing study shows that:

1. The development of the ovary may be divided into an indifferent period and a period of sex-differentiation.

2. The indifferent period, in embryos of eighteen to twenty-five days of age inclusive, is characterized by the rapid growth of the gonad, the appearance of primordial germ-cells, a primary proliferation of epithelial cells which form sex-cords, and the division of the gonad into a superficial epithelium and an inner epithelial mass.

3. The period of sex-differentiation begins the twenty-sixth day and continues throughout the remaining period of embryonic development. It is characterized first by the differentiation of the gonad into a testis or an ovary and secondly, in the ovary, by the formation

of a temporary tunica albuginea, a secondary proliferation of cells from the superficial epithelium, differentiation into a medullary and a cortical portion, and the formation of follicles.

4. Differentiation of the gonad into a testis or an ovary takes place in a very short time and the testis is differentiated before the ovary shows any indication of its definitive characteristics.

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EXPLANATION OF PLATE

All drawings were made with the aid of the camera lucida at table level. For all except figures 8 and 9, a 4 mm. objective and 10 ocular were used. Figures 8 and 9 were drawn with 16 mm. objective and 10 ocular. The reproduction was made at one-half reduction from the original.

Fig. 1. Section through genital ridge of 18-day embryo. m, mesenchyme; g. c., germ-cell; g. r., genital ridge.

Fig. 2. Section through genital ridge of 20-day embryo. g. r., genital ridge; g. c., germ-cell.

Fig. 3. Section through gonad of 21-day embryo. g. c., germ-cell; g. r., genital ridge; s. e., superficial epithelium.

Fig. 4. Section through gonad of 22-day embryo. g. c., germ-cell.

Fig. 5. Section through gonad of 23-day embryo. g. c., germ-cell.

Fig. 6. Section through gonad of 25-day embryo. s. e., superficial epithelium; g. c., germ-cell; s. c., sex-cord.

Fig. 7. Section through testes of 27-day embryo. s. e., superficial epithelium; t. a., tunica albuginea; s. t., seminiprous tubule.

Fig. 8. Section through ovary of 27-day embryo. g. c., germ-cell.

Fig. 9. The same as Fig. 8. g. c., germ-cell; s. e., superficial epithelium.

Fig. 10. Section through ovary of 28-day embryo. s. e., superficial epithelium; g. c., germ-cell.

Fig. 11. Section through ovary of 28-day embryo. s. e., superficial epithelium.

Fig. 12. Section through ovary of 30-day embryo. s. e., superficial epithelium; t. a., tunica albuginea; g. c., germ-cell; m. c. medullary cord.


m.
g.c.
g.r.
Fig. 1
g.r.
g.c.
Fig. 2
g.c.
g.r.
g.c.
s.e.
g.c.
Fig. 3
g.c.
Fig. 4
g.c.
Fig. 5
g.c.
s.e.
Fig. 10
s.e.
s.c.
g.c.
Fig. 6
g.c.
Fig. 8
g.c.
s.e.
Fig. 9
s.e.
Fig. 11
s.e.
t.a.
s.t.
Fig. 7
s.e.
t.a.
g.c.
m.c.
Fig. 12

Date Due
