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EIGHTEEN FIGURES

INTRODUCTION

A female which is born co-twin with a normal male in cattle is usually sterile, and is known among stockmen as a 'free-martin.' The internal reproductive organs of such a female are decidedly male-like, and the external genitalia are usually female-like, although they may be modified also in the male direction. Lillie ('17) showed that the sterile free-martin is zygotically a female which is modified in the male direction by the action of sex hormones from the male twin. These hormones circulate in the vascular systems of both foetuses, owing to the establishment of a common circulation by an early fusion of the embryonic membranes and the anastomosis of the extra-embryonic blood-vessels of the two individuals. If no vascular connections between the twins are made, the female is a 'fertile free-martin.'

The effect upon the foetal reproductive glands of the introduction of the hormones from the male embryo into the circulation of the female twin was described by Chapin ('17). The reproductive organs which are present in the indifferent stage at the time when the secretion from the male enters the circulation of the female develop toward the male condition, while those structures which would develop at the time of sex differentiation in the normal female, are absent. That is to say, the tunica albuginea, first set of sex cords,[1] rete testis, and epididymis

[1] The term sex (or sexual) cord is used to include the invaginations of the germinal epithelium, whether they are male or female structures; in the case of the testis there is one set of sexual cords, which are destined to form the func-

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develop, while the distinctly female structures, the cords of Pflüger, and definitive ovarian albuginea fail to develop.

In both the foetal and post-natal gonads of free-martins, the tunica albuginea, sex-cord region, and rete are of constant occurrence, although each structure may vary greatly as to size, degree of differentiation, and degree of transformation in the male direction. Such structures as the tubules of the epididymis and the spermatic cord are in some specimens entirely absent. There may be a correlation between this high degree of variation in the structure of the reproductive glands of the free-martin and the time and degree of anastomosis of the blood-vessels of the two blastodermic vesicles. In other words, there may be considerable variability in the time at which the internal secretion enters the circulation of the female twin; variations in quantity of the hormone and in the intensity of its action are also conceivable.

Owing to these male characteristics of the gonad, several investigators have misinterpreted the true sex of the free-martin. Hart ('10) and Magnusson ('18), who described the microscopical anatomy of the reproductive organs of the post-natal free-martin, both reached the conclusion that it is an abnormal male. It was not until the embryological history of these gonads was known that a correct interpretation was possible. With the embryological data and from an examination of a number of post-natal gonads of the free-martin, the conclusion is reached

tional seminiferous tubules, while in the case of the ovary there are two sets of sexual cords; the first set forms the medullary cords, which are destined to degenerate and which are homologous with the seminiferous tubules; the second set forms the cords of Pflüger.

The term sex-cord region is used to include both the sexual cords and the intercordal tissue (stroma of connective-tissue fibers between which are the interstitial cells).

The term rete is applied to the network of tubules of the rete testis, of the rete ovarii, and of the modified rete of the free-martin gonad.

The term tunica albuginea is used to designate the connective-tissue capsule of the testis, and the layer of connective tissue between the medullary cords and the cords of Pflüger of the ovary. It is the primary tunica albuginea of the ovary and the definitive tunica albuginea of the testis. In the ovary a second layer of connective-tissue fibers develops between the cords of Pflüger and the germinal epithelium; this is the definitive tunica albuginea of the ovary.

that primarily the gonad is female in structure and that secondarily it is transformed into a male gonad by hormonic action.

Since the embryological evidence is alone the key to the correct explanation, it is the purpose of the present account to interpret the microscopic structure of the post-natal gonad on the basis of its development. The facts presented in this paper were gathered in a study of the gonads of seven foetal free-martins (Miss Chapin's material) ranging in length from 7.5 cm. to 28 cm., and from nine post-natal free-martins ranging in age from five days to three years. Data obtained from a study of the gonads of normal males and females of approximately the same sizes and ages are introduced for comparison. For complete lists of the specimens and tabulated summaries of the microscopical findings, tables 1, 2, and 3 may be consulted. A list of the foetal free-martin gonads studied is given in Miss Chapin's tabulated summary (Chapin, '17, p. 478).

We are deeply indebted to Prof. Leon J. Cole, of the University of Wisconsin, for the reproductive glands from the following post-natal free-martins: H-18, H-46, H-36, H-37, H-42, and H-40 of table 1, for the use of the manuscript, "The anatomy of the urino-genital system of the free-martin," prepared by his student, Mr. J. V. Seids, and for other data not contained in the manuscript. Without these the present work would have been impossible. The remaining specimens are from Professor Lillie's collection. Different fixing agents were employed, the more common ones being formalin, Zenker-acetic, strong Fleming's solution, and Bouin's solution. Heidenhain's iron hematoxylin and Mallory's triple stain were the stains most commonly used.

The study of this problem was undertaken at the suggestion of Prof. Frank R. Lillie, and it gives me great pleasure to acknowledge my deep indebtedness to him for instruction and for kindly advice.

GENERAL INTERPRETATION OF THE POST-NATAL GONADS

Diagrammatic reconstructions shown in figure 1 summarize the detailed study presented beyond. The gonads of the free-martin may be divided into three groups, characterized, respectively, as low, medium, and high degrees of transformations in the male direction. These three groups are represented in figure 1 (B, C, and D) as graphic reconstructions and constitute a chain of connected links between an embryonic ovary (A) and a testis (E). For comparison similar graphic reconstructions of ovaries (A' and A'') and a testis (E) are introduced. Diagram A represents an ovary in the indifferent stage of development. It is covered with a superficial layer, the germinal epithelium (ge), from which the first set of sexual cords (sc) arise by invagination. At the anterior end, the rete tubules (r) enter the hilum and project for a short distance posteriorly into the sex-cord region (sc). The ovarian blood-vessels (bv) also enter the gonad at the hilum. Under normal conditions, this gonad (A) differentiates into an ovary, but under the influence of the male sex hormones it differentiates into a gonad which is morphologically a testis.

Diagrams A' and A'' illustrate in two stages the normal differentiation of an ovary from the indifferent stage A. The ovary shown in A' may be regarded as derived from the indifferent stage A by the addition of a second set of sexual cords, the cords of Pflüger (p), which arise as secondary proliferations of the germinal epithelium (ge). Between the first set of sexual cords (sc), which in the ovary are known as medullary cords, and the cords of Pflüger (p) is located the primary tunica albuginea (ta), homologous with the tunica albuginea (ta) of the testis (E). Between the germinal epithelium (ge) and the cords of Pflüger (p) is the definitive ovarian albuginea (oa), which has no homologue in the testis. The rete tubules (r) have penetrated to the posterior end of the medullary cord region, their eccentric position is retained, and connections (tubuli recti) are never established between the medullary cords (sc) and the rete tubules (r). The arrangement of the dots in the stippled area (sc) of the
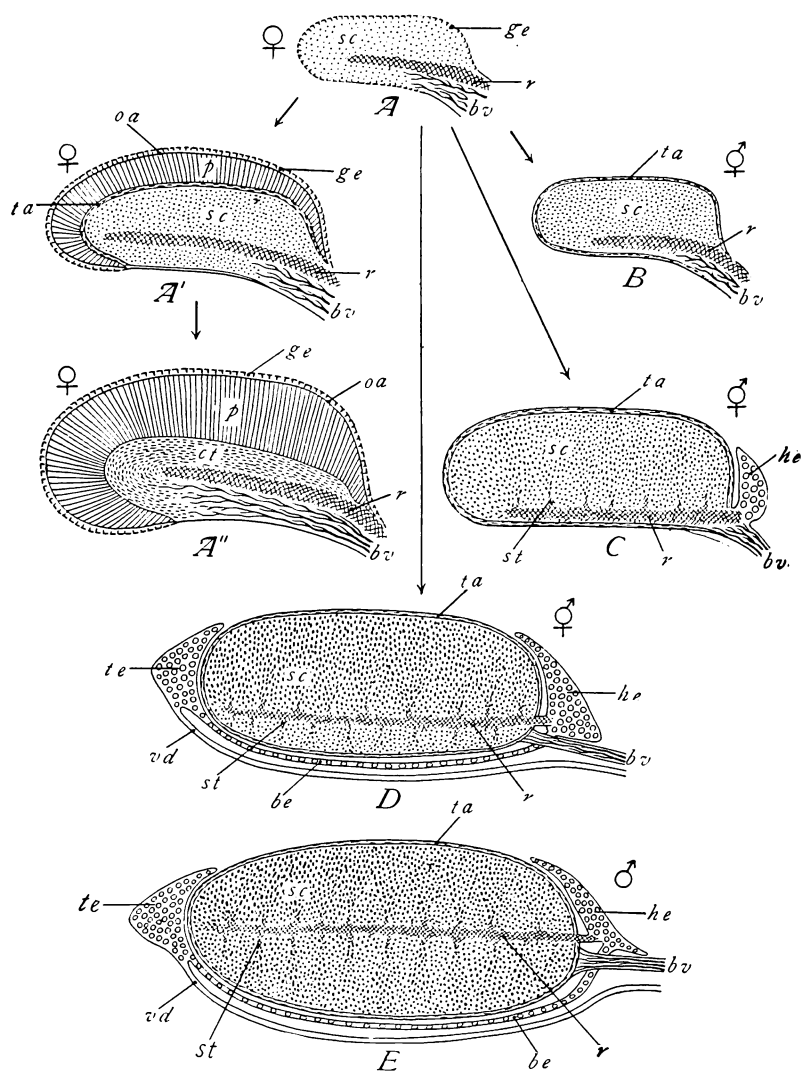
Fig. 1[2] Diagrammatic reconstructions to show a normal testis, and an indifferent stage of an ovary, which under normal conditions differentiates into an ovary (shown in two stages), but under the influence of male sex hormones may form gonads which exhibit three stages of transformation in the male direction.

diagram indicates that the sexual cords bear no definite orientation to the rete (r).

Diagram A″ represents an ovary at about the time of birth. It differs from the ovary A′ chiefly through the increase in size of the cords of Pflüger (p) and the retrogression of the medullary-cord region. In the latter the medullary cords have mostly degenerated; connective-tissue fibers (indicated by the area of short dashes, ct) and blood-vessels have increased. The rete, although distinct, is destined to degenerate in still older stages.

Diagrams B, C, and D, on the other hand, illustrate three steps in the transformation of an ovary of the indifferent stage A into a free-martin gonad which is morphologically a testis. Diagram B represents a low degree of transformation in the male direction. The sex-cord region (sc) is comparatively small and poorly organized. The arrangement of the dots in the stippled area (sc) indicates that the sexual cords are irregularly arranged. The rete (r) lies in its primitive position at the hilus, and has not penetrated far into the sex-cord region (sc). It is important to note that stage B resembles the indifferent stage A in possessing homologous parts; however, stage B differs from stage A in the absence of the germinal epithelium (ge) and the presence of a tunica albuginea (ta). It differs from stage A′ in the absence of the cortex (cords of Pflüger, definitive albuginea, and germinal epithelium), but resembles it in the irregular arrangement of the sexual cords (sc), the absence of connections (tubuli recti) between the sexual cords and the rete tubules, the position of the blood-vessels and rete, and the presence of a tunica albuginea (ta).

The next step in transformation is illustrated by diagram C, and exhibits an increase in size and organization of the sex-cord region (sc); establishment of connections (tubuli recti, st) between the sexual cords (sc) and the rete tubules (r) on all sides of the rete except the side adjacent to the tunica albuginea (ta); and the addition of a head of an epididymis (he), the tubules of which are connected with the rete tubules (r). The sexual cords in this stage of transformation are so arranged that they radiate out from the straight tubules (st), as indicated in the diagram by

the arrangement of the dots in the stippled area (*sc*). Entering the anterior end of the gonad just below the rete is a distinct cord of convoluted blood-vessels, which, aside from its eccentric position, resembles the vascular cord (*bv*) of the normal testis (E).

The highest step in the transformation series is shown in diagram D, which very closely resembles the normal testis (E). By the growth of the sex-cord region, the rete (*r*) has been shifted toward the center of the gonad, so that on all sides the rete tubules make connections (*st*) with the sexual cords (*sc*). Although the rete (*r*) in D is still slightly eccentric in the sex-cord region (*sc*), its position marks a distinct advance toward maleness; the rete normally forms a core in the center of the testis (E). At the anterior end of gonad D, the rete tubules (*r*) make connections with the tubules of the head of the epididymis (*he*). Attached to the posterior end of the gonad is the tail of the epididymis (*te*), connected with the head (*he*) by the body of that organ (*be*). Passing anteriorly from the tail of the epididymis (*te*) is a vas deferens (*vd*). It will be noted that as the rete shifts toward the center of the gonad, the vascular cord (*bv*) also moves in the same direction. In this highly transformed gonad (D) the epididymal structures, vas deferens, and rete are in essentially the same mutual relations as in the normal testis (E).

THE MICROSCOPICAL ANATOMY OF THE POST-NATAL GONADS

The following pages comprise detailed descriptions of the microscopical anatomy of the reproductive glands of nine postnatal free-martins, comparisons with the structure of the gonads of foetal free-martins and of normal gonads, and discussions based on these comparisons. An examination of the microscopical anatomy indicates considerable variation in structure. Some have approximately the typical structure of a testis, while others are much less typically male. In other words, they exhibit a graded series of transformations between an ovary and a testis. Three more or less distinct steps may be recognized, which may be characterized as low, medium, and high degrees of transformations in the male direction. An examination of this series

will furnish additional information concerning how complete the transformation of a zygotically determined female into a male individual by the action of male sex hormones is possible. It will also furnish a positive demonstration of the existence of 'sexual dimorphism' in the sex glands of mammals; that is, for the theory that the primordium of the mammalian gonad has the potentialities for the development of both ovary and testis.

A. Gonads having a low degree of transformation

The reproductive glands belonging to this group are the least modified of any in the series, yet they show the majority of the typical male structures. They are characterized by their undescended or ovarian position, by the absence of an epididymis, and by the small size and low degree of organization of the sex-cord region. The rete is comparatively large and well differentiated.

The histories of the two specimens which belong to this group are detailed as follows:

Case H-36. Born co-twin to potent bull. A Holstein freemartin aged approximately two and a half years when killed, December 15, 1916. External genitalia typically female. Uterus rudimentary. Small gonads located in normal ovarian position. Rudimentary spermatic cord; poorly developed seminal vesicles enter the vasa deferentia. Only the posterior portion of the vasa deferentia developed.

Case H-40 (fig. 2). Born co-twin to normal bull. Three years old when slaughtered, February 3, 1917. External genitalia female, but smaller than normal. Vagina rudimentary; uterus absent. Wolffian ducts well developed, but made no connections with the gonads. Gonads in ovarian position. A plexus of blood-vessels was attached to the anterior border of each gonad. Seminal vesicles enter vasa deferentia (Wolffian ducts).

The tunica albuginea and tunica vasculosa. Owing to poor preservation, the gonad of H-36 will be only briefly described. It is covered with a thick capsule of densely arranged connective-tissue fibers, the tunica albuginea, as in the normal testis. Imme-

diately under the compactly arranged fibers are similar fibers more loosely arranged and enclosing blood-vessels. The position and structure of this vascular zone makes it homologous with the tunica vasculosa of the normal embryonic testis in cattle (fig. 4). Its existence as a distinct layer in the normal foetal testis is lost shortly before birth, through an increase in connective-tissue fibers and its intimate union with the tunica albuginea (table 3 for details). Thus the two layers are merged into one thick layer, known as the tunica albuginea, the inner portion of which contains the blood-vessels. It is thus seen in this free-martin gonad that the embryonic relationships of the tunica albuginea and tunica vasculosa are retained.

Gonad H-40 (fig. 2) the oldest one of the entire series, is undergoing certain pathological changes. Of chief importance is the infiltration of connective-tissue fibers into the sex-cord region, thereby crowding out some of the sexual cords and the interstitial cells. The sex-cord region is approximately reduced to a crescentic area which surrounds the rete region except where the rete comes in contact with the tunica albuginea. The tunica albuginea and the tunica vasculosa are not distinguishable as they are in H-36, but the blood-vessels are scattered throughout the inner portion of the thickened capsule (fig. 2, bv). The connective-tissue fibers of the tunica albuginea are very compactly arranged, and this arrangement is maintained and continued on into the sex-cord region, so that no line of demarkation can be recognized between the sex-cord region and the tunica albuginea. Comparatively, the tunica albuginea of this free-martin gonad has overgrown the usual limits of this structure in free-martin sex glands.

The sex-cord region. Immediately under the tunica vasculosa of H-36 a faint trace of a sex-cord region may be recognized. It is represented by only a few sexual cords which in structure resemble seminiferous tubules. Each tubule has a slightly thickened connective-tissue wall, within which is a syncytium of supporting epithelial cells (Sertoli cells). Germ cells are entirely absent.

In the dense connective-tissue fibers which have penetrated the sex-cord region of gonad H-40, islands of sexual cords and interstitial cells are frequently found, and occasionally isolated sexual cords embedded in the dense fibers. These isolated sexual cords, the islands of sexual cords and interstitial cells, and the
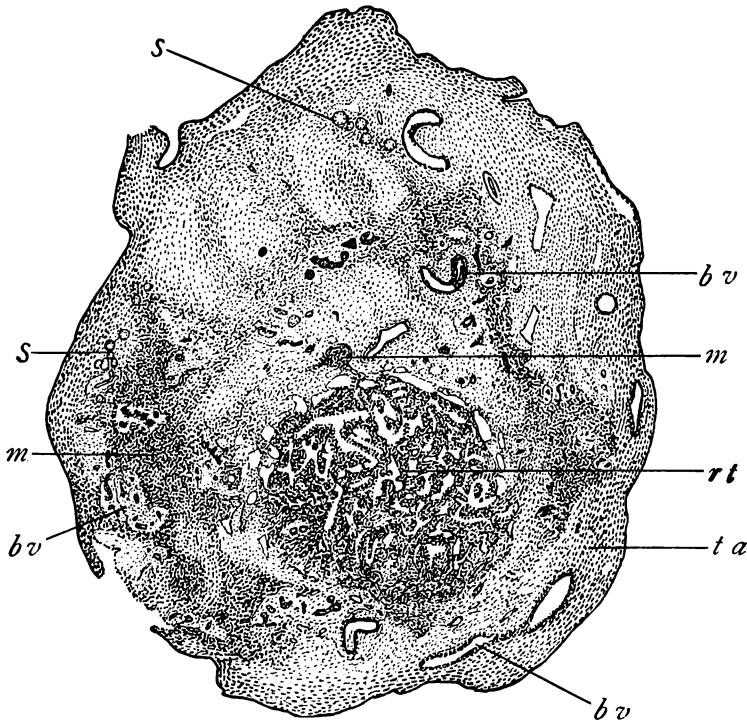

Fig. 2 Transverse section through the middle of free-martin gonad, H-40. bv, blood vessels; m, areas of interstitial cells and sexual cords; s, seminiferous tubules; rt, rete tubules; ta, tunica albuginea. × 14.

crescentic area (mentioned above) constitute the sex-cord region (fig. 2, s, m). The interstitial cells are abundant, but the sexual cords are comparatively few in number and of small size. The sexual cords are characterized by thickened connective-tissue walls, within which is a syncytium of supporting epithelial cells. This syncytium is free from germ cells. Such a syncytium is formed by the coalescence of a number of supporting epithelial

cells, the cytoplasm of which runs together, but the nuclei remain distinct. The structure of this cell aggregation varies considerably in the different sexual cords. In some the nuclei are closely crowded and irregularly arranged; the amount of cytoplasm varies considerably, for in some cords it is clearly seen, while in others it is hardly detectable. Such an irregular arrangement and crowding of the nuclei resemble the condition in the medullary cords of the normal ovary (N 14, table 2) and of very young seminiferous tubules (N 13, table 3). In other sex cords the supporting epithelium is loose and the nuclei are arranged in a single layer which lines the wall of the cord. The long axes of the ovoid nuclei are arranged perpendicular to the wall of the sex cord, forming a palisade arrangement. From the inner ends of the nuclei cytoplasmic strands extend toward the potential lumen. Such sex cords are typical seminiferous tubules and present a structure similar to the condition in the seminiferous tubules in the testis of a young foetus, with the exception that there are no male germ cells in the free-martin tubules (T 16, T 6, table 3), (fig. 2, s). The nuclei of the supporting epithelial cells of both types of sexual cords resemble very closely the nuclei of the Sertoli cells of the normal adult testis, in having a slight amount of chromatin, chiefly distributed in a distinctive nucleolus, and a faintly staining nuclear membrane. A few of the tubules show distinct pathological changes, where the cytoplasm of the supporting epithelial cells has in places disappeared, leaving naked nuclei; in others even the nuclei have disappeared, leaving mere spaces in the dense connective tissue.

The rete tubules. Located at the hilus of both gonads is the well-developed cylindrical mass of rete tubules, which constitutes by far the greater portion of the reproductive gland in case H-36. Figure 2, *rt*, shows the primitive position of the rete in H-40 and the way it is marked off sharply from the sex-cord region by connective tissue, so that no connections (tubuli recti) are established between the rete tubules and the sexual cords. No tubuli recti are developed in H-36. The distance of penetration of the rete into the sex-cord region has not been determined in H-36, but in H-40 it only extends two-thirds of the way back.

Morphologically, the rete is a cord of densely arranged connective-tissue fibers in which is a network of anastomosing channels, the rete tubules. These tubules are lined with a single layer of columnar epithelial cells having elongated nuclei. They resemble the rete testis tubules of the normal testis, with the
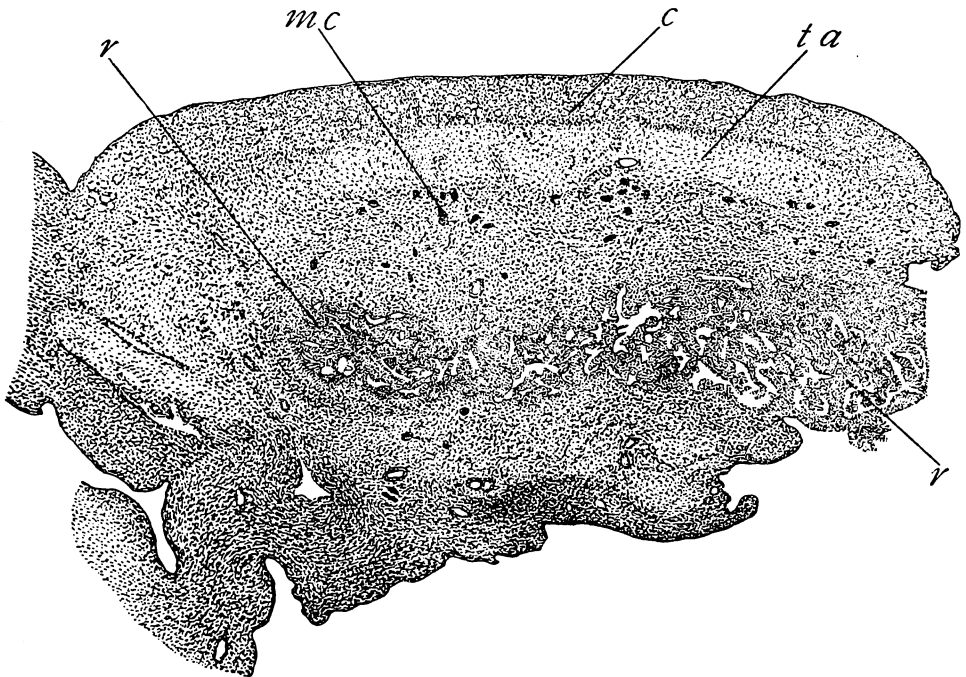

Fig. 3   Longitudinal section through an ovary of a 56-cm. female bos foetus, Bos 8.   c, cortex; mc, medullary cord; r, rete enters anterior end and penetrates to posterior end of medullary-cord region; ta, tunica primary albuginea between medullary-cord region and cortex.   × 21.

difference, however, that the epithelial cells of the free-martin tubules are much less regular in arrangement.

The constancy of the eccentric position of the rete region will be understood from the consideration of the normal development of this region in the ovary and the testis. The rete ovarii and the rete testis both originate from a region anterior to the sex gland, and penetrate into the anterior end of the gonad, the point of entrance in each case being the hilum. The rete ovarii

penetrates to the posterior end of the medullary cord region, but not at as early a stage as the rete testis. It retains its primitive position at the hilus of the ovary during its further development (fig. 3). On the other hand, the rete testis penetrates at an
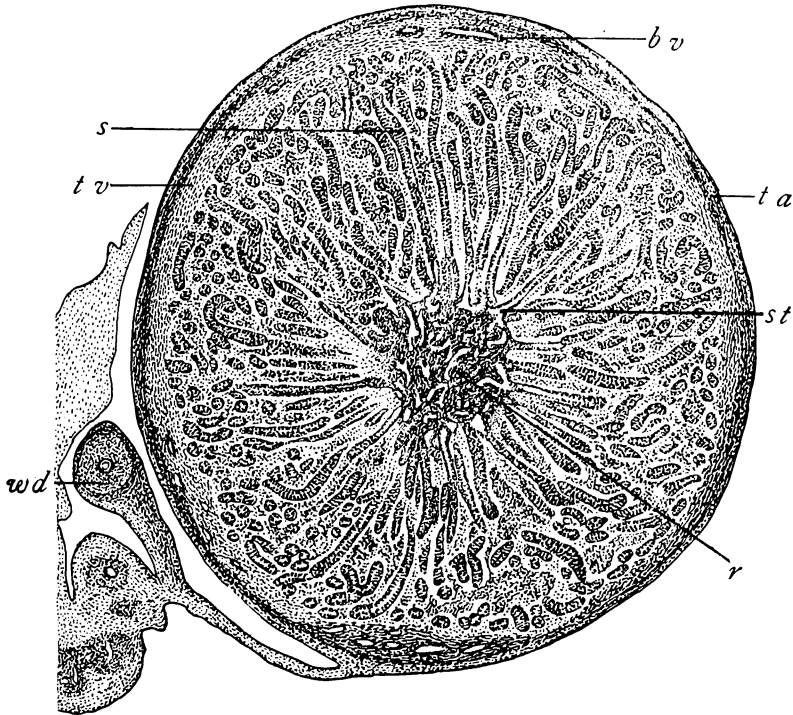

Fig. 4 Transverse section through the middle of the testis of a 20-cm. male bos foetus, N 21. *bv*, blood-vessel in tunica vasculosa; *r*, rete testis (note its central position); *s*, seminiferous tubules (note that the seminiferous tubules are more convoluted near the periphery of the testis); *st*, tubuli recti; *ta*, tunica albuginea; *tv*, tunica vasculosa; *wd*, Wolffian duct. × 33.

early stage to the posterior tip of the testis, to form an axis from which the seminiferous tubules radiate in all directions (fig. 4). During the early development of the testis the rete lies between the mesentery and the sex-cord region, and as the seminiferous tubules begin to grow, they extend around the sides of the rete until they meet, making the rete appear to be in the center of a round gland; that is, seminiferous tubules grow around the

eccentrically placed mass of rete tubules in such a manner as to enclose it.

Obviously, from the above embryological account, the rete mass is primarily located at the hilus of the free-martin gonad, and since this gonad is transforming into a testis it is to be expected that the rete mass should come to lie in the center of a round gland. However, in gonads having a low degree of transformation in the male direction, as in cases H-40 and H-36, the rete region lies at the hilus. This is due to the small size of the sex-cord region. In other words, the sex-cord region in these cases has not grown enough to surround the rete region.

B. Gonads exhibiting a medium degree of transformation

The characteristics of the gonads in this group are: the undescended position which they occupy; the contorted tubules of the epididymis are few or entirely absent (as in H-18); the large size and high degree of organization of the sex-cord region; and the establishment of connections between the rete and the sexual cords, and between the rete and the epididymis.

The histories of the four specimens belonging to this category are as follows:

Case 66. Born co-twin with a normal male calf. Age five days when slaughtered (youngest post-natal free-martin gonad obtained). In place of the uterus were fine ducts; very minute gonads in ovarian position.

Case 42. Born co-twin with normal male. Killed twenty-one days after birth. Uterus reduced in size, vagina short and ending blindly. Gubernaculum absent; gonads in ovarian position.

Case H-18 (fig. 5). Born co-twin to normal bull. Killed October 14, 1913, aged thirty-one days. External genitalia typically female. Vagina and uterus quite rudimentary. Seminal vesicles and parts of Wolffian ducts (solid cords) present. Data incomplete on position of the gonads, but they were probably in the ovarian position. No spermatic cord.

Case H-42. Born co-twin with a normal bull. Aged about eighteen months. External genitalia typically female. Vagina

and uterus quite rudimentary. Vas deferens (lower half) and seminal vesicles present. Gonads rudimentary and lying in ovarian position. No spermatic cord. Owing to the atypical organization of the sex-cord region in this case, it will be considered separately at the end of the discussion of the other three cases.

The general topography of the gonads. These four gonads are very small, oval structures (fig. 5). For comparative sizes and other data, consult tables 1, 2, and 3. They are in reality rudimentary testes, which have failed to descend, and thus retain their embryonic position, i.e., the position normal for ovaries. Like the gonads having a low degree of transformation in the male direction, these possess the majority of the parts typical of a testis. Entering the anterior end and projecting nearly to the posterior end of the reproductive gland in each case, is the rete. It forms an eccentrically placed core, about which are the sexual cords and connective-tissue stroma containing interstitial cells. Surrounding the entire gonad is a capsule of connective-tissue fibers, the tunica albuginea. The anterior end may be further modified by the presence of a well-developed vascular plexus (cases 42 and 66) and of a few very definite epididymal tubules (cases 42 and H-42).

The tunica albuginea and tunica vasculosa. Without exception, the free-martin gonad is enclosed by a capsule of connective-tissue fibers, but the relative thickness of the capsule and the compactness of its fibers vary in the different groups of cases. In the specimens under consideration, the fibers are coarse, compactly arranged in the outer layer, and run parallel with each other as they encircle the gonad (fig. 5). If these fibers are compared with corresponding fibers in the tunica albuginea of a mature foetal testis, the latter are seen to be more closely packed together and the individual fibers finer. The capsule of the mature foetal testis is thirteen times thicker than that of the free-martin gonads of this group.

In some of the free-martin gonads of this group, the inner portion of the connective-tissue capsule is characterized by a loose arrangement of the fibers. In cases 66 and 42 a few blood-vessels make their appearance in this layer, forming a primor-

dium of the tunica vasculosa. This primordium shows a degree of differentiation comparable to the condition of the tunica vasculosa of a normal testis of a 4.8-cm. embryo. Blood-vessels from this vascular zone pass to the stroma of the sex-cord region, supplying it with blood. In case H-18 no tunica vasculosa is apparent, so that the blood-vessels enter the hilus and are distributed in a centrifugal manner to the rete tubules and the sex-cord region (fig. 5).
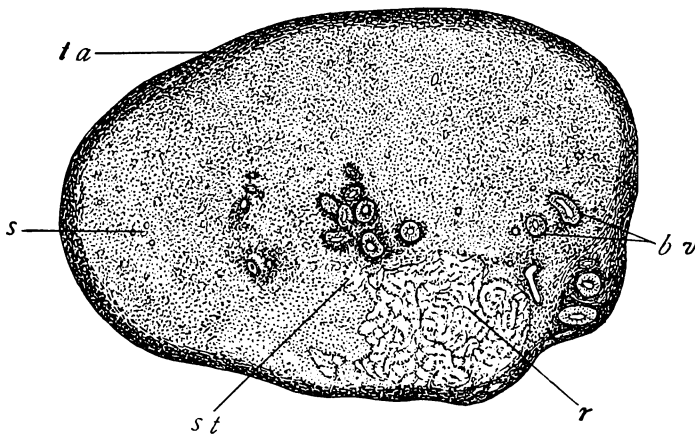

Fig. 5 Transverse section through free-martin gonad, H-18. *bv*, blood-vessels; *r*, rete (note its eccentric position); *s*, seminiferous tubule; *st*, tubuli recti connecting rete tubules and seminiferous tubules; *ta*, tunica albuginea (note absence of tunica vasculosa). × 14.

These two ways for the distribution of the blood in the free-martin gonads will be understood from the normal arrangement of the blood-vessels in the ovary and in the testis, and in the light of the fact that the free-martin gonad is primarily an ovary which is being transformed into a testis. A comparative study of the normal method of distribution in ovaries and testes shows a marked difference. In the testis blood-vessels enter the anterior end, ramify extensively throughout the tunica vasculosa, from which they send branches inwardly, in a centripetal manner, to the seminiferous tubules and rete tubules (fig. 4). In the ovary the blood-vessels are distributed in a centrifugal

manner; the blood-vessels enter the hilus (fig. 3), from which they are distributed outwardly to the medullary cords and cortex.

It is thus seen that the distribution of the blood in a centripetal manner is characteristically a male feature, while the centrifugal method is strictly a female attribute. Since the free-martin gonad is an ovary which is changing into a testis, as has been shown, it is not an unexpected finding that the manner of the distribution of the blood-vessels should also change in the male direction. This is exactly what is found in the free-martin gonads of cases 66 and 42, where the blood is distributed in a centripetal manner to the sex-cord region. On the other hand, in case H-18 the original ovarian method is retained, so that the blood is distributed in a centrifugal manner to the sex-cord region.

The peritoneal surface of the gonad consists of a single layer of cuboidal epithelial cells in case 66, and of a single layer of flattened epithelial cells in cases 42, H-42, and H-18. Instead of the thick germinal epithelium of the normal ovary, these, as well as all other free-martin gonads, are enclosed within a single layer of more or less flattened epithelial cells as in the normal testis.

The sex-cord region. In this group of gonads (except in H-42, which is atypical and will be considered separately) the sex-cord region constitutes the largest portion of the gonad and shows a moderate degree of organization of the sexual cords and intercordal tissue. The latter comprises a stroma of a moderate number of loosely arranged connective-tissue fibers, and between them the so-called interstitial cells. The fibers of the stroma are continuous with the connective-tissue fibers of the tunica albuginea and ramify throughout the sex-cord region, forming a framework between the sexual cords. Histologically, the stroma is composed of fusiform-shaped cells, which are apparently fibroblasts of connective tissue. These cells are further characterized by elongated and very prominent nuclei, but the cytoplasm is scanty and quite inconspicuous. From the ends of the nuclei extend fibrillar processes, which anastomose,

with similar processes attached to other nuclei to form the network of the stroma. In this loose network are the interstitial cells, which, in the different gonads of this group vary greatly as to size, shape, and structure. In gonad H-18 they are relatively few in number, and possess large, round nuclei and a homogeneous cytoplasm containing a few fuchsinophilic granules. In gonads 42 and 66 the interstitial cells are difficult to recognize, owing to the presence of large masses of cells which at ruptured places in the walls of the sexual cords are continuous with the supporting epithelial cells of the sexual cords. The cells within the cords are identical in shape and structure with the cells of these masses. It is not at all improbable that some of these masses of cells lose their specificity as supporting epithelial cells and become interstitial cells as a similar process occurs in degenerating follicles of the ovary of a normal mature foetus, where the granulosa cells transform into masses of cells which resemble interstitial cells.

Two clearly different degrees in the organization of the supporting epithelial cells may be recognized. They are either arranged in definite cords or they occur in unorganized masses of cells. The latter are particularly abundant and distinct in the gonad of case 66, less abundant in case 42, and least in H-18. The close resemblance of these cells to the supporting epithelial cells within the sexual cords and their close association with the sexual cords suggest their origin from the latter. Similar masses of unorganized supporting epithelial cells, in addition to the medullary cords, occur in the normal ovary (N 15, 18 cm.). Whether or not these unorganized masses in the free-martin gonads transform into sexual cords cannot be determined from the data at hand, but it is suggested that they do, since these masses are most numerous and largest in the youngest gonad (case 66). Obviously, there is a tendency for these unorganized masses to disappear with increasing age. However, differentiation is not entirely the result of aging, but is dependent also on the variability in the action of the sex hormones. For example, in the gonads of the oldest free-martin examined, the differentiation of the sex-cord region is much less than in these younger gonads.

The sexual cords are of two types, those resembling medullary cords and those resembling seminiferous tubules. The former resemble the medullary cords of the normal ovary in that they are solid strands (cords) of irregularly arranged and closely packed supporting epithelial cells forming a syncytium, which may be regarded as the primordium of a Sertoli-cell syncytium. In the free-martin, germ cells are absent from the medullary cords. These sexual cords are not only structurally different, but they are smaller than the sexual cords which resemble seminiferous tubules. Cases 66 and 42 show distinct transitional stages in the transformation of medullary cords into seminiferous tubules through increase in size and rearrangement of the nuclei of the syncytium.

In such transitional stages, the nuclei are two and sometimes three layers deep, and less crowded together than in the medullary cords and accompanied by a slight increase in the size of the cords. In the most completely transformed sexual cords (seminiferous tubules), there is a further increase in size accompanied by the arrangement of the nuclei of the supporting epithelium into a single layer at the periphery. The oval nuclei of this layer are arranged with their long axes perpendicular to the wall of the tube. Extending from the inner ends of the nuclei are strands of cytoplasm, all of which meet at the center of the seminiferous tubule (fig. 6, s). Such a center has a potentiality of forming a lumen, as in the normal seminiferous tubule (figs. 7 and 8). If this syncytium of supporting epithelial cells is compared with that of the mature foetal testis (fig. 7) and a young calf testis (fig. 8), a close structural similarity will be seen, with one important deficiency, namely, that germ cells of any stage are absent from the syncytium of the free-martin seminiferous tubule. It may also be noted that in the tubules of the free-martin there is much less regularity in the arrangement of the nuclei of the supporting epithelial cells; in this respect the arrangement is like that of very young foetal testes (table 3).

Each wall of these sexual cords is composed of a basement membrane upon which the supporting epithelial cells rest, and external

to which is a variable number of concentrically arranged lamellae of connective-tissue fibers. As a rule, the number of lamellae increases as the medullary type of cord is transformed into that of a seminiferous tubule type. The wall of a normal seminiferous tubule has a similar composition, with many lamellae.

In order to understand the changes undergone by a medullary cord of the free-martin gonad as it transforms into a seminiferous tubule, it will be necessary to consider briefly the structure
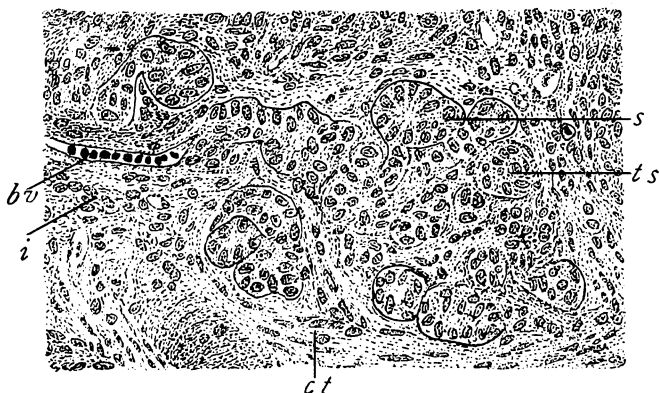

Fig. 6 Portion of a transverse section through the sex-cord region of the free-martin gonad, 66. *bv*, blood-vessel containing corpuscles; *i*, interstitial cells; *s*, sexual cord which resembles a seminiferous tubule (note arrangement of the nuclei of the supporting epithelial syncytium, and strands of cytoplasm extending centrally from the inner ends of the nuclei); *ts*, sexual cord which shows a transitional stage between a medullary cord and a seminiferous tubule (note arrangement of nuclei within syncytium); *ct*, connective tissue. × 380.

of the medullary cords of the normal embryonic ovary, and also to follow the development of the syncytium of supporting epithelial cells in normal embryonic testes and in three post-natal testes.

Examination of an ovary of a normal 8.3-cm. embryo (N 7), shows that the medullary cords consist of solid strands of supporting epithelial cells enclosed within definite walls. The cells are closely crowded and irregularly arranged, and owing to the absence of cell walls they form a syncytium. In a medullary cord of an ovary from an 18-cm. embryo there is some

rearrangement of these cells, as the comparatively large nuclei tend to be crowded into a single layer at the periphery. This leaves an area of the syncytium more or less free of nuclei in the center of the cord, but no lumen is formed. Examination of older embryonic ovaries shows similar conditions. This is the
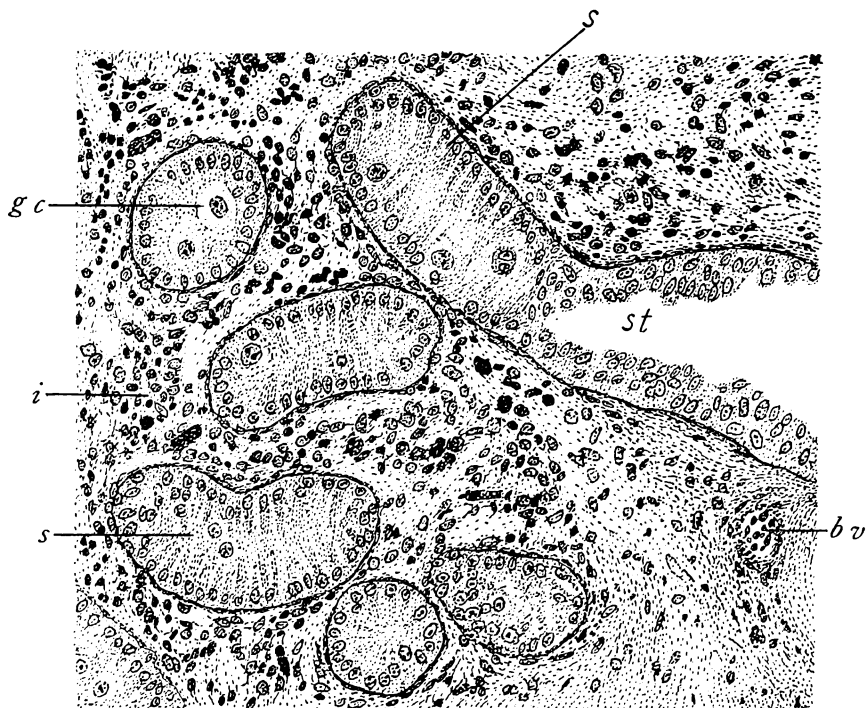

Fig. 7   Portion of a transverse section through the testis of a mature male foetus (89 cm.), Bos 3.   *bv*, blood-vessel; *gc*, primordial germ cell; *s*, seminiferous tubule showing nuclei of the supporting epithelial syncytium arranged into a single layer at the periphery; strands of cytoplasm extending from the inner ends of the oval nuclei toward the center of the tubule; *st*, tubulus rectus (straight tubule) in conjunction with a seminiferous tubule; *i*, inter-tubular tissue. × 307.

normal limit of differentiation, as they are destined to degenerate; no medullary cords are evident in ovaries from birth on.

In the youngest normal testis examined (4.8 cm., N 13) the sexual cords are unorganized, as the walls can only be distinguished with difficulty and the supporting epithelial cells are

irregularly arranged. In testes from a 7-cm. (N 10) and a 12.7-cm. (T 16) embryo some slight degree of organization is recognizable. A definite tubule wall is evident, and the supporting epithelial cells are formed in a syncytium, the nuclei of which are becoming arranged into layers, some having three layers, others two layers. From the inner ends of the nuclei, which
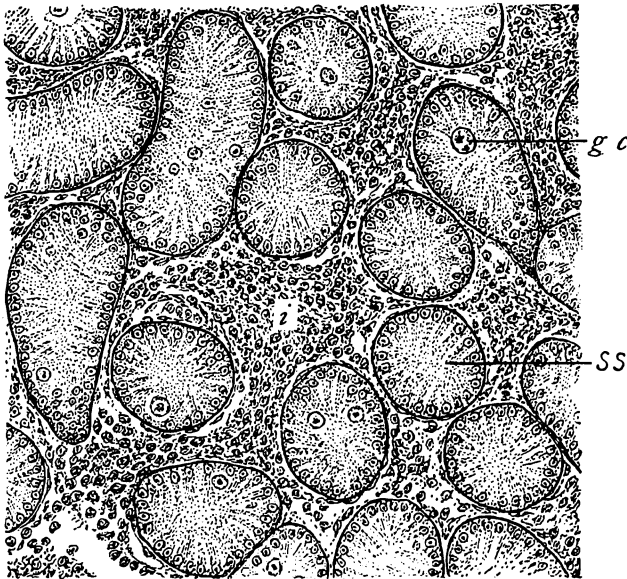
Fig. 8   Portion of a transverse section through a testis of a young male calf (six months?), Bos 12. *gc*, primordial germ cell; *ss*, syncytium of supporting epithelial cells; *i*, interstitial cells. × 307.

show a tendency to be arranged with their long axes perpendicular to the tubule wall, strands of cytoplasm extend toward the center of the tubule. The organization of the syncytium progresses in the older testes, until finally a striking degree of regularity is shown in testes from 31-cm., 61-cm., 89-cm. embryos and in testes from young calves (2 and 12 days, and 6 months). Figures 7 and 8 show the nuclei of the supporting epithelial syncytium arranged in a single distinct layer, closely applied to the wall of the tubule. The long axes of the oval nuclei are

parallel to each other and perpendicular to the tube wall. From the inner end of each nucleus extends a strand of cytoplasm toward the center of the tubule; the point where these strands center has the potentiality of forming a lumen. Within this syncytium are found a few large, clear cells—the primordial germ cells.

Bearing in mind the history of the medullary cords and the normal development of the seminiferous tubules, the development of the seminiferous tubule in the free-martin is comprehensible. The medullary type of sexual cord resembles not only the medullary cords of the normal ovary, but also the youngest sexual cords in the testis, these being homologous structures. As a sexual cord in the free-martin, originally of medullary type, transforms into a seminiferous tubule, it passes through stages which are in essentials similar to those in the normal development of the seminiferous tubule.

The sex-cord region of H-42. The sex-cord region of this gonad presents a striking contrast to the sex-cord region of other gonads of the entire series, in showing a few female elements. This region is smaller and much more poorly organized than in the other cases of this group of moderately transformed gonads. It is made up chiefly of masses of interstitial tissue (connective-tissue stroma and interstitial cells), in which are scattered sexual cords. Of the two types of sexual cords, the larger number here resemble seminiferous tubules, while a few are distinctly female, possessing primary follicles containing germ cells. These sexual cords which resemble seminiferous tubules show different degrees of organization of the syncytium of supporting epithelial cells; in some the nuclei are arranged in a single layer and in others they are irregularly arranged and closely packed. The walls of these sexual cords are thickened, otherwise they agree very closely in structure with the sexual cords of cases 66, 42, and H-18 described above, and therefore will not be considered further.

The sexual cords which appear to be female in structure are located largely near the periphery of the sex-cord region. They are composed of a typical egg cell surrounded by a single layer of more or less flattened follicular epithelial cells. In some more

than one egg is present. Such primary follicles are enclosed by a thickened wall of connective-tissue fibers, which is not unlike the wall of the male sexual cords. Magnusson ('18) described large vesicle-like structures which in six different cases appeared to resemble Graafian follicles, but in none did he find germ cells.

The interstitial cells are of two types, those which resemble the interstitial cells of the normal testis and of other free-martin gonads and those which resemble lutein cells of the corpora lutea in cattle. The latter are of large size, and the cytoplasm is filled with large granules staining yellow with Mallory's 'triple stain.' These lutein-like cells usually occur in localized masses distributed chiefly at the periphery of the sex-cord region. One of these masses is a large, rounded collection enclosed by a connective-tissue capsule, which measures 1 mm. in diameter, and resembles closely a miniature corpus luteum. Magnusson ('18) described three gonads in which similar bodies of lutein-like cells were found. He found even larger bodies, as he states on page 47, " . . . einen verhältnismässig grossen intensiv gelben Körper von 3 mm. Durchmesser ein, der von einer fibrösen Kapsel umgeben war." The origin of these lutein-like cells cannot be decided from the meager evidence furnished by this one case, but it is not improbable that they arise from the follicular (granulosa) cells of atretic follicles.

The occurrence of primary follicles in the sex-cord region raises the question of their origin. There are two possible explanations of their occurrence. In the first place, a few of the cords of Pflüger may have invaginated before the optimum conditions for the action of the male sex hormones were established. This view is supported by the peripheral position of the majority of the primary follicles and masses of lutein cells, which probably arise from the follicles. Secondly, such follicles may have arisen from the 'medullary follicles' of the ovary. The evidence is good for this point of view, since the sex-cord region is comparatively small, and it ought to be larger if any of the cords of Pflüger had invaginated. Allen ('04) and others have described simple follicles containing germ cells in the medullary cords of foetal ovaries. Such follicles with germ cells are also present in

the medullary cords of foetal ovaries in cattle. It may be that the germ cells which are present in the sexual cords of the foetal free-martin gonads persist to form follicles, instead of degenerating as in all of the other free-martin gonads examined. (For a further consideration of the fate of these germ cells, the general discussion may be consulted).

The rete tubules and connections. The rete region in each free-martin gonad of this group is a distinct cord of tubules, which enters the anterior end and penetrates nearly to the posterior end of the gonad. It retains its primitive relation to the sex-cord region by its eccentric position. In other words, although the sex-cord region is large and moderately well organized, it has not grown sufficiently to entirely surround the rete, so that the rete lies next to the tunica albuginea (fig. 5) as in the gonads exhibiting a low degree of transformation. The structure of the rete tubules resembles the condition described previously for gonads having a low degree of transformation.

The relationships of the rete tubules to the sexual cords and to the tubules of the epididymis are important indices of the degree of transformation in the male direction. In the normal ovary no branches of the rete establish connections with the medullary cords (fig. 3) but in the normal testis connections between the seminiferous tubules are formed by lateral branches (tubuli recti) of the rete tubules extending to meet the seminiferous tubules (figs. 4 and 7, *st*). If such branches of the rete tubules make connections with the sexual cords in the free-martin gonad, it is a further indication of the assumption of male characters. This is exactly what is found in this group of free-martin gonads, although the number of tubuli recti is comparatively small. Such connections are shown in figure 5, *st*.

The relation of the rete tubules to the tubules of the epididymis is clearly shown in case 42 (fig. 9, *rt, e*). This figure illustrates a transverse section just anterior to the sex-cord region, and shows rete tubules extending to establish connections with a tubule of the vasa efferentia. There are four of these tubules; they are short, closed at both ends, and lined with ciliated epithelial cells, which rest upon a basement membrane enclosed
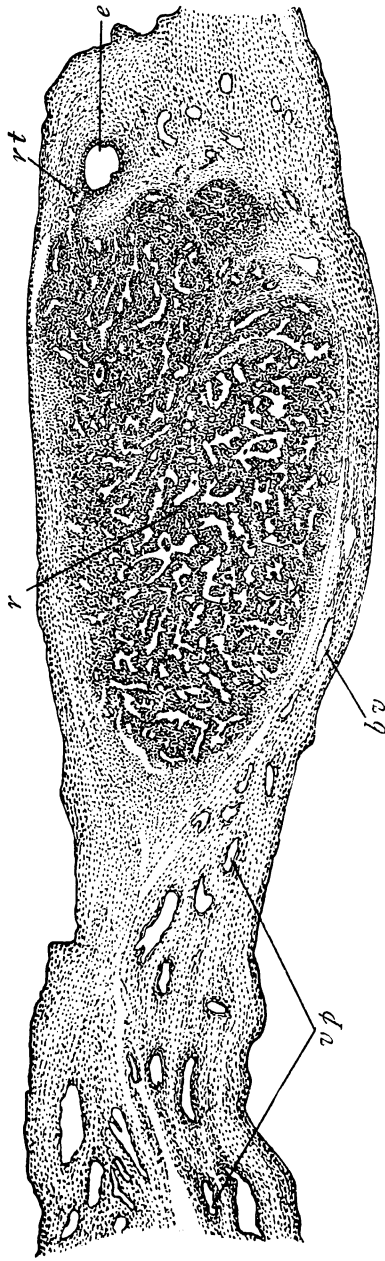
Fig. 9 Transverse section through anterior end of free-martin gonad, 42. *bv*, blood vessel; *e*, epididymal tubule (vas efferens); *r*, rete tubules; *rt*, rete tubules connecting with epididymal tubule; *vp*, vascular plexus. × 32.

by a layer of circular smooth muscle fibers. Similar connections are shown in case H-42, but the number of vasa efferentia tubules is larger (about twenty in transverse section). In neither case can traces of a Wolffian duct connecting with the vasa efferentia be found. These conditions will be understood from a comparison with the normal relationships of these parts in the ovary and testis.

Although the rete tubules at the anterior end of a normal foetal ovary (6.3 cm., N 14) may establish connections with the renal corpuscles of the Wolffian body, these connections are not of much significance, owing to the early degeneration of the Wolffian body in the female. However, in the male these connections are permanent, owing to their persistence as the vasa efferentia of the epididymis. The vasa efferentia are connected with the upper portion of the vas deferens (Wolffian duct), which is known as the ductus epididymis. The vasa efferentia and the ductus epididymis are both much contorted tubules and constitute the head (globus major) of the epididymis, which fits over the anterior end of the testis. From the head of the epididymis the coiled vas deferens passes posteriorly as the body of the epididymis (corpus epididymidis) to the posterior end of the testis where it forms a conical mass, the tail (globus minor) of the epididymis. From the tail of the epididymis, the vas deferens passes anteriorly along the side of the testis to the spermatic cord.

From the above accounts, it will be seen that in the free-martin gonads of this group (42 and H-42) the rete tubules establish connections with the vasa efferentia tubules of the epididymis, and constitute the beginnings of the head of an epididymis, but as the vas deferens is absent, this remains rudimentary.

The spermatic cord. In gonads 42 and 66 a rudiment of a spermatic cord occurs. Broadly attached at the hilus near the anterior end, and at the side of the rete opposite the epididymis, is a flattened cord of blood-vessels—the vascular cord (fig. 9, *vp*). Structurally, it is a plexus of blood-vessels embedded in connective-tissue fibers. The fibromuscular wall of the blood-vessels

includes both circular and longitudinal fibers. In relative position and structure it resembles the vascular cord of the normal spermatic cord. The vascular cord, vas deferens, nerves, and lymphatics are bound together by connective tissue to form the spermatic cord of the normal male. Obviously, in these free-martin gonads (cases 42 and 66), the spermatic cord is rudimentary, owing to the presence of only one constituent part—the vascular cord.

C. Gonads exhibiting a high degree of transformation

This group illustrates the highest degree of transformation in the male direction of all the free-martin gonads examined. That they are well-developed male sex glands is shown by the following characters: the majority of the gonads are descended to a position in the groin; the seminiferous tubules are well differentiated; tubuli recti (straight tubules) connect the rete tubules with the seminiferous tubules; the rete tubules connect with the vasa efferentia; the epididymis is typically male, and a typical spermatic cord is present.

Histories of the three cases to be examined:

Case 44. Born co-twin with a normal male March 12, 1916, of Holstein-Friesian parentage. Slaughtered April 29, 1916. Gonads descended into peritoneal sacs lying between the skin and the abdominal muscles in the region of the groin. No trace of vagina, uterus or tubes. No scrotum. External genitalia typically female. Vasa deferentia open into dorsal wall of urinogenital sinus, from thence to the gonads. Seminal vesicles lateral to bases of vas deferens. Spermatic cord typically male.

Case H-46 (fig. 10). Born co-twin with a normal bull. Slaughtered when five and one-half weeks old. Gonads in ovarian position. Vagina rudimentary, bases of Müllerian ducts fused (uterus), free horns of uterus short. Vasa deferentia extend from rudimentary vagina nearly to gonads—present again along gonad. No seminal vesicles. External genitalia typically female. Distinct rounded vascular cord.

Case H-37. Co-twin of normal bull. Born October, 1915, slaughtered December, 1916. Large gonads descended through

abdominal wall to a position immediately under the skin in the region of the groin.   No udder development, but the teats were large.   No scrotum.   External genitalia female except the clitoris, which is transformed into a penis-like structure posteriorly directed.   Vas deferens led from epididymis, which is normally related to the gonad, to urethra.   Seminal vesicles present, but only the left has a duct which lacks a lumen.   Spermatic cord normal in appearance.   Rudimentary prostate.

The general topography of the gonads.   These three highly transformed gonads are comparatively larger than gonads less transformed, but smaller than normal testes of the same age. The gonad of H-37, the most completely transformed of the entire series, is approximately the same size as a testis of a six-months-old calf (40 x 12 mm.).   The testis of a bull of about the same age as this free-martin measures 125 x 57 mm.   In cases 44 and H-37, the left gonad is much larger than the right gonad (table 1 for details).   Not only has the size increased, but the shape has changed from ovoid to oblong.

In contrast to the other groups, the ovarian position of these gonads is not retained (except H-46), but they have descended into peritoneal sacs, which instead of entering a scrotum, as the normal testes do, are retained between the abdominal muscles and the skin in the region of the groin.   Even this partial descent is a distinct indication of a further transformation in the male direction of these free-martin gonads.

The general morphological relationships of the parts are like that of a normal testis.   Each gonad is enclosed by a thickened capsule, the tunica albuginea.   The rete enters the anterior end, extends toward and in two cases reaches the posterior end of the sex-cord region.   It forms an eccentrically placed core about which are found the seminiferous tubules and intertubular tissue.   Many tubuli recti connect the rete tubules with the seminiferous tubules.   At the anterior end of the gonad the rete tubules establish connections with a well-developed head of the epididymis.   The body and tail of the epididymis bear the typical male relationships to the gonad.   In each case there is a well-developed vascular cord which is attached to the anterior

end of the gonad; this vascular cord is loosely bound with a vas deferens to form a spermatic cord.

The tunica albuginea and tunica vasculosa. A study of the connective tissue capsule of these three gonads indicates that it also may be rather completely transformed in the male direction. The average thickness of the capsule is about 0.8 mm. which is several times thicker than in the capsules from gonads less transformed, and which approaches very closely the normal thickness of 1 mm. in the testis of an adult. As in the other groups, the inner layer of the connective-tissue capsule is modified by the addition of blood-vessels, constituting therefore a vascular zone. This zone varies in the different gonads of this group. In case 44 it is poorly formed, yet at places a distinct layer is present containing blood-vessels which pass in a centripetal direction to the sexual cords. In addition, blood-vessels also enter the hilus to be distributed in a centrifugal manner to the sexual cords. In case H-46 (fig. 10, *tv*) is seen a very definite vascular zone, from which blood-vessels are distributed centripetally to the sex-cord region. The existence of the tunica vasculosa in case H-37 as a distinct layer is lost by its close mergence with the outer layer, the two constituting the tunica albuginea, the inner portion of which contains the blood-vessels. In contrast to the coarse condition of the fibers in less transformed gonads, the fibers are fine in H-37. The fineness of the fibers and the mergence of the two layers into one tunica albuginea, resemble in close detail the structure of the normal testis (table 3).

Upon the tunica albuginea is a single layer of flattened epithelial cells, which constitutes the visceral layer of the tunica vaginalis.

The sex-cord region. The sex-cord regions of these three free-martin gonads have reached the highest degree of differentiation, organization, and largest size of any of the post-natal gonads. The primitive relationships of the sex-cord region to the core of rete tubules is shown by the eccentric position of the latter. The sex-cord region completely surrounds the rete cord, and this is a distinct advance in the male direction where the

rete normally forms a core in the center of the testis (fig. 4). In no case has the sex-cord region advanced to this condition, but a definite step in that direction is seen in case H-37, and toward the posterior end of gonad H-46 where the sex-cord
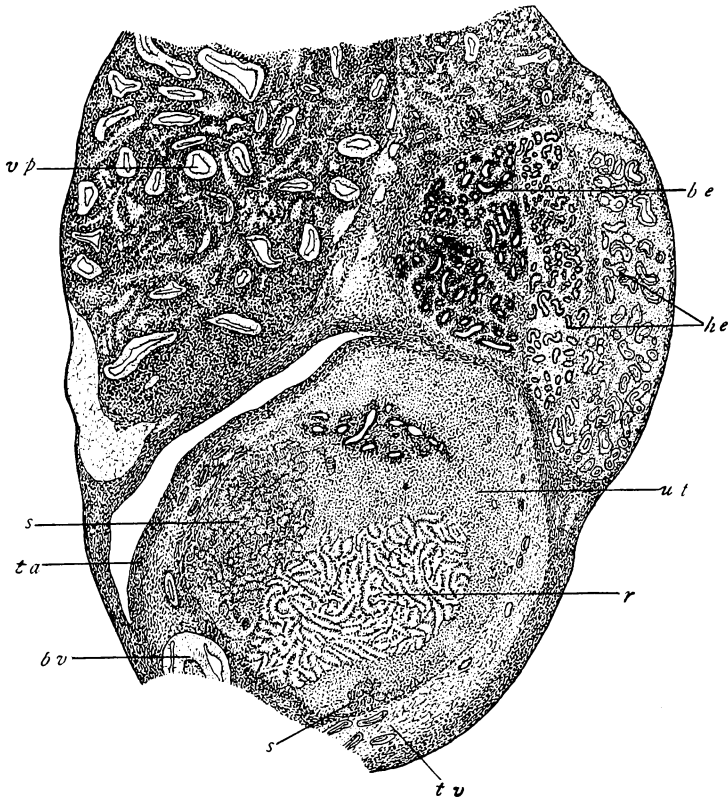
Fig. 10 Transverse section through gonad, vascular plexus and epididymis of free-martin, H-46. *bv*, blood-vessel; *be*, tubules of the body of the epididymis; *he*, tubules of the head of the epididymis; *r*, rete tubules; *s*, seminiferous tubules; *ta*, tunica albuginea; *tv*, tunica vasculosa; *ut*, 'undifferentiated tissue'; *vp*, plexus of blood-vessels. × 11.

region has grown around the rete to a degree such that the rete no longer touches the tunica albuginea, but has shifted toward the center of the gonad. Nevertheless, it is still eccentrically situated with respect to the greater portion of the sex-cord

region. In the other two cases of this group, the sex-cord region, although well developed, does not lie between the rete and the connective-tissue capsule. In other words, the rete still lies in its primitive position at the hilus (fig. 10, r).

As in the other free-martin gonads, the intertubular tissue is composed of a stroma of connective-tissue fibers among which
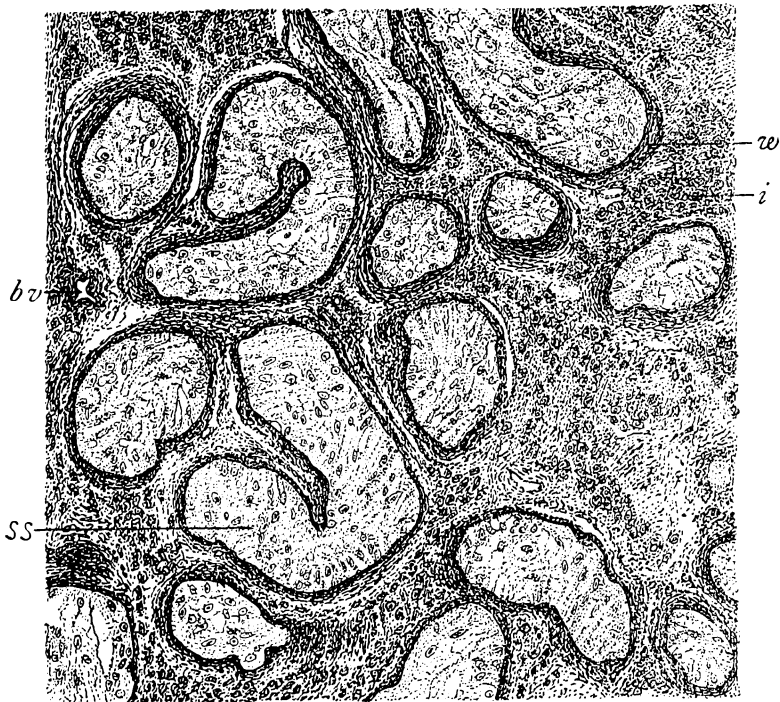
Fig. 11 Portion of the sex-cord region of the free-martin gonad, H-37. bv, blood vessel; ss, Sertoli-cell syncytium (no germ cells) of a seminiferous tubule; i, interstitial cells; w, thickened wall of tubule. × 107.

are interstitial cells. A noticeable difference, and one probably of much significance, is the great abundance of these interstitial cells. The interstitial cells are most abundant in the highest transformed gonads. In case H-37 (fig. 11, i), which is the most completely transformed gonad of all of the post-natal free-martin gonads, these cells are exceedingly abundant and of large size.

Particularly large collections are found around blood-vessels
and at the 'carrefours,' that is, in the intervals between the sec-
tions of three or more seminiferous tubules. These large areas
may be observed with the unaided eye. They are not isolated
collections, but are continuous with the interstitial cells in the
stroma strands between the tubules. In cases 44 and H-46
(fig. 12, i) the interstitial cells are moderately numerous.
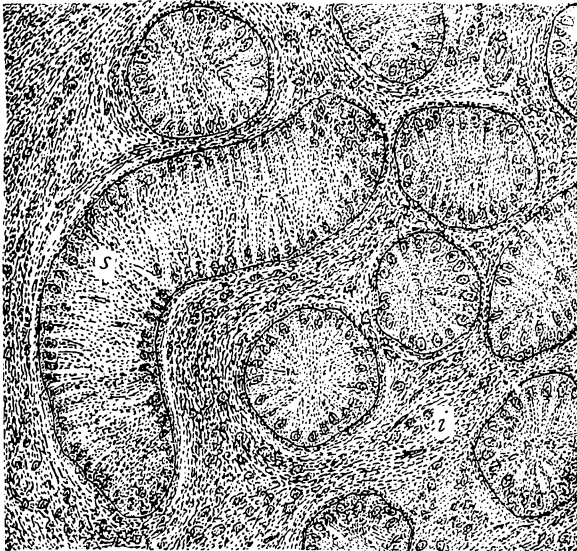
Fig. 12   Portion of the sex-cord region of free-martin gonad, H-46. i, inter-
stitial cells in a connective-tissue stroma; s, seminiferous tubule showing the
nuclei of the supporting epithelial syncytium arranged into a single layer closely
applied to the wall of the tubule. From the inner end of each nucleus extends
a strand of cytoplasm toward the center of the tubule. Note absence of germ
cells. × 307.

A detailed examination of the interstitial cells in case H-37
(fig. 11, i) shows much variation in their structural features.
There is much variation in size, the majority being many times
larger than the interstitial cells of the normal testis of the adult.
In shape, the majority are irregularly polygonal. The eccen-
trically placed nucleus is large, somewhat vesicular, and contains
scattered chromatic granules. The structure of the cytoplasm
also varies in the different types of cells. In a small proportion

Figure 13

it is homogeneous throughout, in a few others it is extensively vacuolated, while in the majority of the cells the cytoplasm contains bundles of rod-like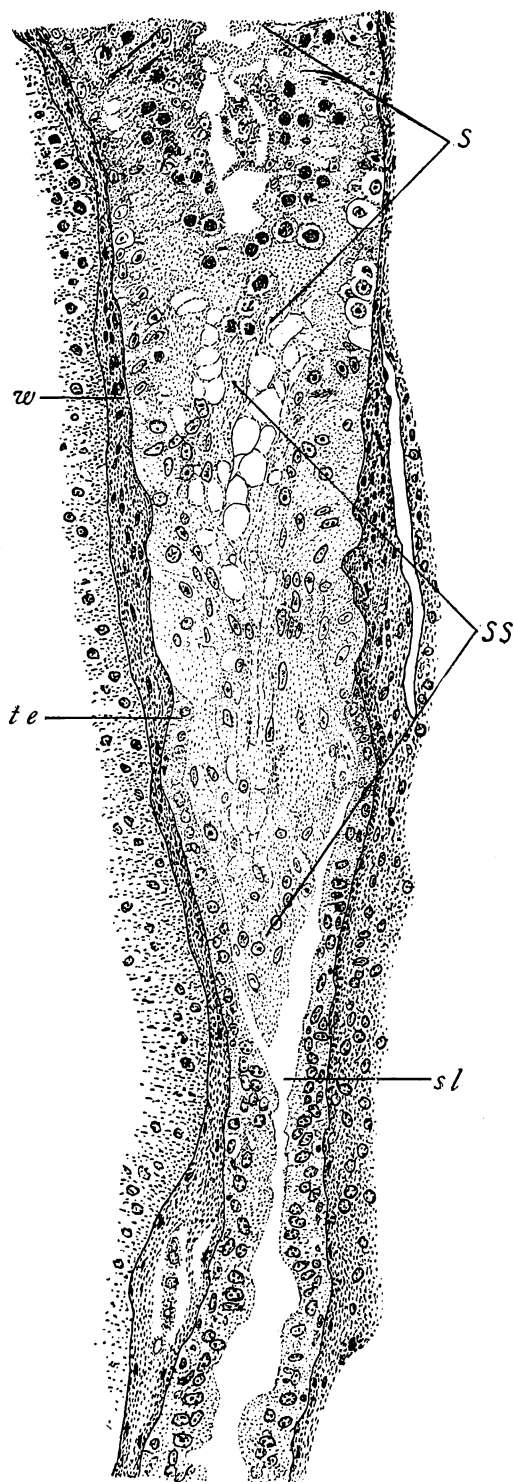 structures. Owing to the poor fixation of the gonads from cases 44 and H-46, a detailed description of the interstitial cells cannot be given.

The organization of the sexual cords has reached its highest degree of transformation in the male direction, so that a majority of the sexual cords are definite convoluted seminiferous tubules. In H-37 the tubules are scattered more or less evenly throughout the sex-cord region, but in H-46 and 44 there are areas which are free from tubules. They appear as areas of apparently undifferentiated tissue (fig. 10, *ut*), but on closer examination it is seen that some organization has occurred. Among the abundant connective-tissue fibers are slender cords of sexual cords, a few of which resemble miniature seminiferous tubules and others which resemble medullary cords, and interstitial cells. Owing to the close resemblance of these undifferentiated areas to the sex-cord region of some of the foetal freemartin gonads, they are regarded as areas which retain the embryonic structure.

The seminiferous tubules appear to be as much coiled as the normal tubules of the testis, but they are not as closely packed together nor as large. The tubules of H-37 (fig. 11), which resemble to a close degree the tubules of the normal testis of the adult, are, however, only half as large in diameter as the latter (adult 0.225 mm.) (fig. 13, *s*). The tubules of cases 44 and H-46 (fig. 12) are about one-fourth the diameter of a normal tubule of the adult testis.

Each tubule is enclosed by a much thickened membranous wall, which is composed of a number of concentric lamellae of connective-tissue fibers. The innermost lamellae are very closely arranged and hyaline-like, giving the appearance of a

Fig. 13 The junction of a seminiferous tubule and a straight tubule (tubulus rectus) from the testis of an adult bull, Bos 2. *s*, seminiferous tubule showing spermatogonia, maturing germ cells and spermatozoa; *sl*, lumen of straight tubule; *ss*, Sertoli-cell syncytium free from germ cells; *te*, abrupt beginning of the epithelium of the straight tubule; *w*, connective-tissue wall of the tubule. × 240.

much thickened basement membrane. The outer lamellae are loosely arranged and blend with the connective-tissue fibers of the stroma.

Within the membranous wall the supporting epithelium is arranged in such a manner that two distinct stages in organization may be recognized. The first stage is illustrated in the gonads from cases 44 and H-46 (fig. 12, s); here the nuclei of the supporting epithelial syncytium are very regularly arranged into a single layer. The long axes of the oval nuclei are perpendicular to the tubular wall, and from the inner ends of the nuclei filmy strands of cytoplasm extend toward the center of the tubule. This syncytium of epithelial cells is identical with the syncytium found in the seminiferous tubules of late foetal testes and young postnatal testes (compare with figures 7 and 8), with one important exception, namely, the complete absence of germ cells in the free-martin syncytium.

The second stage, illustrated by case H-37 (fig. 11, ss), shows more advanced differentiation in the male direction, as the supporting epithelial syncytium has become resolved into a Sertoli-cell syncytium as in the adult normal testis (compare with fig. 13, ss). The cytoplasmic substance of the syncytium stains lightly, and structurally it appears as a loose meshwork of branching strands of cytoplasm. These strands have more or less of a fibrillar structure, the fibrils of which are very delicate and in some cases not very sharply defined. The cytoplasm is continuous throughout the tubules except as interrupted by the scattered nuclei and spaces. The oval nuclei, which lie at various levels in the syncytium, are characterized by their distinctive nucleoli. The nucleolus is a comparatively large, nearly spherical body which takes a deep chromatic stain. Apart from the nucleolus, the chromatic material is very scanty. The Sertoli-cell syncytium is structurally alike in both the free-martin seminiferous tubule and the normal seminiferous tubule of the adult bull, except male sex cells are absent in the former. The structure of the normal syncytium can be very beautifully demonstrated at the zone of junction between a seminiferous tubule and a straight tubule (fig. 13, ss). In this zone the

Sertoli-cell syncytium is free from male germ cells of all stages, so that its normal structure can be easily observed. This is a point of considerable interest, as it is the only region in the seminiferous tubule that furnishes an unobstructed view of the normal syncytium. So far as the writer is aware, this zone has not been used before in describing the normal structure of the syncytium.

The rete tubules and tubuli recti. As in the other free-martin gonads, the rete region is an eccentrically placed core of anastomosing tubules, which enters the anterior end of the gonad and projects posteriorly. The distance that it penetrates posteriorly into the sex-cord region varies; in case 44 it penetrates about half the length of the gonad, in case H-46 it penetrates to the posterior end of the sex-cord region, probably also in case H-37 (the extent was not determined, but owing to the extreme transformation of the gonad, it is probable that it penetrated to the posterior end). The eccentric position of the rete has been considered above under the description of the sex-cord region.

The rete of the free-martin gonad, like that of the normal testis, consists of a network of strands of coarse connective-tissue fibers, in which are anastomosing tubules. The strands of connective tissue are as wide in H-37 as in the normal rete testis, but in cases 44 and H-46 are comparatively narrow. Resting upon the wall of the rete tubule is a lining of columnar epithelial cells, which resemble in a very striking degree the normal condition (compare figures 14 and 15). The lumina of the tubules are wider in more highly transformed gonads, so that the rete region appears less compact than in the less transformed gonads.

In each gonad of this group branches of the rete tubules have established connections with the seminiferous tubules. Figure 16 shows the abrupt transition from the seminiferous tubule into a straight tubule in H-37. The seminiferous tubule is filled with a stringy syncytium of Sertoli cells, while the straight tubule contains a lumen and is lined with low columnar epithelial cells. Comparison with figure 13 shows the transitional zones to be similar. These straight tubules are most numerous in H-37,

the gonad which is most transformed in the male direction. In cases H-46 and 44 they are less numerous than in H-37, but more numerous than in less transformed gonads. Obviously, there exists a graded series in the development of the straight
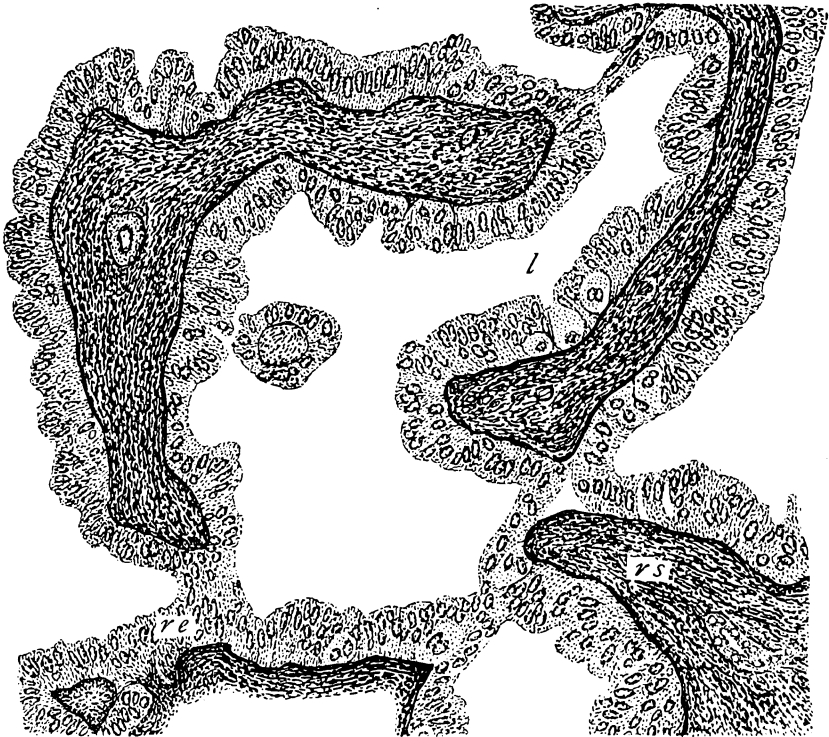
Fig. 14   Rete tubules from testis of a young male calf (six months?), Bos 12. *l*, lumen of tubule; *re*, epithelium of tubule; *rs*, connective tissue stroma between rete tubules.   × 307.

tubules, the moderately transformed gonads having a few, and the most completely transformed gonads having many.

The epididymis. Each of the three gonads of this group possesses a well-developed epididymis, which marks a pronounced step in the direction toward male organization of the free-martin gonad. Although the epididymis bears a relation to the reproductive gland, which is approximately normal, its

parts are misshaped and enlarged. Only a small portion of the
epididymis of H-37 was examined, but Doctor Cole in a com-
munication states that it "bore the normal relation to each
testicle." In each of the gonads examined (H-46 and 44) the
rete continues anteriorly a short distance beyond the sex-cord
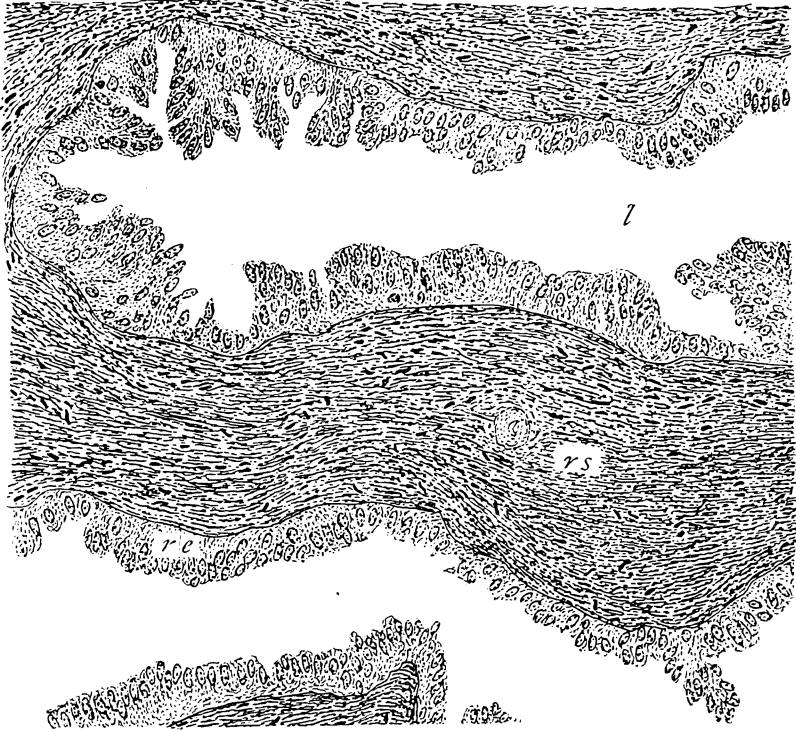
Fig. 15 Rete tubules from gonad of free-martin, H-37. l, lumen of tubule;
re, epithelium of tubule; rs, connective tissue stroma of the rete. $\times$ 307.

region to make connections with an elongated lobe of coiled
epididymal tubules. This elongated lobe constitutes the head
(globus major) of the epididymis. Its length is about twice
the length of the left gonad in case 44, and slightly longer in
case H-46. In the normal testis the head of the epididymis is a
flattened disc which fits over the pointed anterior end. From
the anterior end of this elongated lobe, an attenuated lobe,

reckoned as the body of the epididymis (corpus epididymidis), extends posteriorly and terminates in a conical mass, the tail of the epididymis (globus minor), which fits over the posterior end of the testis. From the tail of the epididymis the coiled vas deferens extends anteriorly. In the normal epididymis the epididymal body is a flattened, narrow band which connects the head and the conical tail of the epididymis.
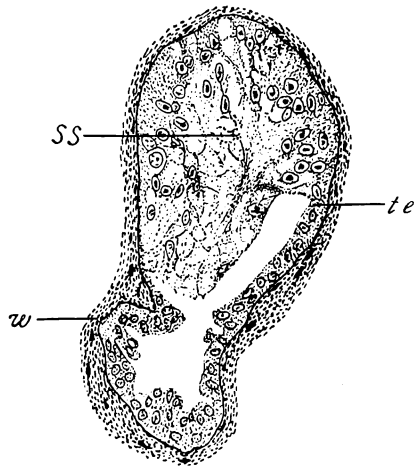
Fig. 16 The junction of a seminiferous tubule and a straight tubule (tubulus rectus) from the gonad of free-martin, H-37. *ss*, Sertoli-cell syncytium; *te*, abrupt beginning of epithelium of straight tubule; *w*, connective-tissue wall of tubule. × 240.

Structurally, the epididymal tubules of the free-martin are well differentiated. The epithelium is usually simple, although in places it may appear two-layered. The cells are of the tall columnar type, except the few basal cells which appear more or less rounded. The former have on their inner surfaces long cilia. The epithelium rests upon a basement membrane, which is surrounded by a layer of smooth muscle fibers, which blend with the surrounding connective-tissue fibers (fig. 17, *mf*, *ee*). If comparison is made with the structure of the normal epididymal tubules (fig. 18, *mf*, *ee*), the resemblance is so close that it is difficult to distinguish the two.
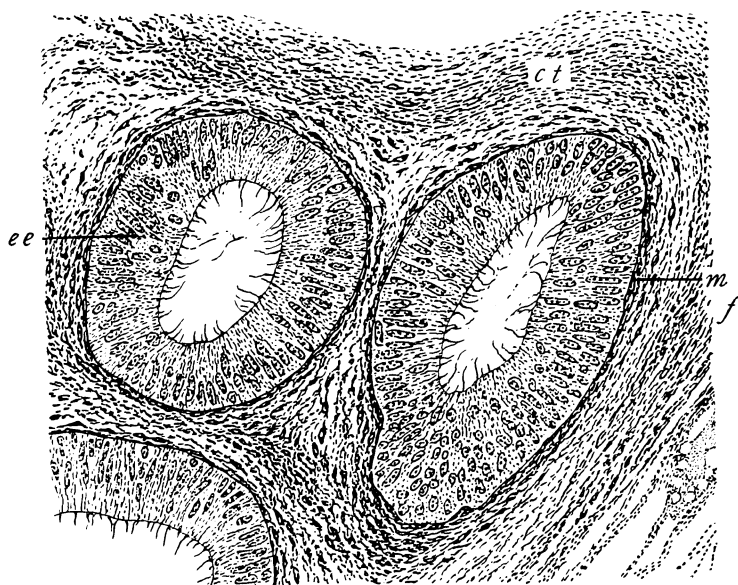
Fig. 17 Tubules from the head of the epididymis of free-martin, H-46. *ct,* connective tissue stroma; *ee,* ciliated, columnar epithelial cells; *mf,* layer of smooth muscle fibers. × 307.

Fig. 18 Tubules from the head of the epididymis of a young male calf (six months?), Bos 12. *ct,* connective tissue stroma; *ee,* ciliated columnar epithelium; *mf,* layer of smooth muscle fibers. × 307.

The spermatic cord. The gonads of specimens 44 and H-37 lie in peritoneal sacs, which are situated between the skin and the abdominal wall in the region of the groin. These peritoneal sacs are diverticula from the body ca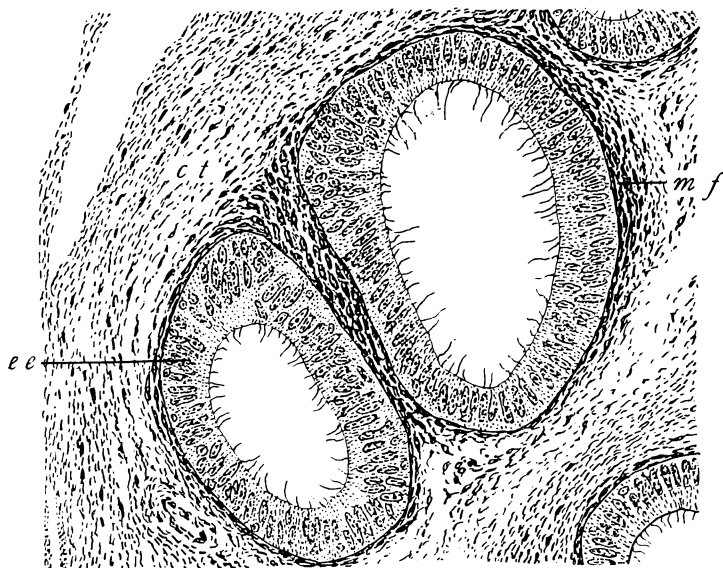vity into which the gonads have descended (both the gonads and spermatic cords are retroperitoneal, and not in the cavities of the peritoneal sacs; the peritoneal sac is merely wrapped around the gonad). In case 44 the cavity of the bulbular portion of the sac, which encloses the gonad, communicates with the rest of the coelom (body cavity) by a narrow canal. Along this channel is a passageway for the vas deferens, spermatic nerves, and blood-vessels. These three structures loosely bound together by connective tissue from the spermatic cord, as in the normal testis. Although the gonad of H-46 is not descended into a peritoneal sac, a more or less typical spermatic cord extends from its anterior end. Its vascular cord is well formed (7 mm. in diameter), and enters the anterior end of the gonad. Running parallel with the vascular cord and loosely bound to it by connective tissue is the vas deferens. In figure 10 the vascular cord (*vp*) is shown as a plexus of blood-vessels, which have thick fibromuscular walls of both circular and longitudinal fibers.

GENERAL DISCUSSION

In the free-martin a gonad with a primary female determination may transform into a structure which is morphologically a testis. The fundamental determining factor in such a transformation is the primary action of the male sex hormones. The sex hormones suppress the development of the ovarian cortex and stimulate the development of the other embryonic rudiments. That is to say, the sex hormones in no case cause the development of any new structure, as the primordium of each male structure developed in the free-martin gonad is present in the ovary at the time of sex differentiation. Whether sex hormones can stimulate the development of any new structure has not been demonstrated. However, if a gonad with a primary male determination were to transform into an ovary by the action of female sex hormones, a new structure, the ovarian

cortex would have to be added, since the male gonad never forms normally any homologue of the ovarian cortex.

Of considerable importance is the demonstration that a mammalian gonad with a primary female determination is capable of a graded series of transformations between an ovary and a testis. The exhibition of such a graded series in the free-martin undoubtedly corresponds to the variability in the time of introduction, the intensity, or the length of duration of action of the male sex hormones. That the time of introduction may vary is indicated by the variability in the anastomosis of the blood-vessels of the two embryos (Lillie, '17). According to this explanation, the gonads most completely transformed in the male direction must have had the most favorable conditions; the sex hormones were introduced early by an early strong anastomosis of the blood-vessels, or were unusually potent, or the duration of their action may have been prolonged. Obviously, if the conditions are less favorable, lower degrees of transformations will occur. It follows, therefore, that the gonads exhibiting a low degree of transformation must have had the least favorable conditions. To produce such gonads, the sex hormones must have had a late introduction, or were of low potency, or the duration of their action was shortened.

Such a graded series of transformations is shown by the relative sizes, shapes, and degrees of differentiation of the chief regions of the gonads and the associated epididymis. It has been shown in this paper that the sex-cord region exhibits definite and progressive steps in the male direction. The sex-cord region in the least transformed gonads is small and poorly organized, while in the most highly transformed gonads it is comparatively large, well organized, and differentiated. The sexual cords themselves exhibit a graded series of transformations between medullary cords and seminiferous tubules. The most highly transformed sexual cords are typical seminiferous tubules in every respect except that the transformation in the male direction has not proceeded to the production of any stage of male sex cells. The only exception to this rule is the brief and unconvincing statement of D. Berry Hart ('10), that in only one

gonad are spermatozoa present. His figure 1, plate II, shows seminiferous tubules, which in addition to the ordinary epithelium (Sertoli cells) show a few rounded cells which resemble in some respects primordial germ cells. No such picture of a seminiferous tubule of a free-martin showing either primordial germ cells or spermatozoa is shown in any of the specimens that I have examined. Certainly, such an important exception should have received more than brief mention.

In some of the foetal free-martin gonads a small proportion of the sexual cords enclose primordial germ cells, which, however, are destined to degenerate before birth. That these germ cells are distinctly female in character is shown by comparing them with the primordial germ cells of the medullary cords of normal foetal ovaries in cattle. As to origin, they are alike since the sexual cords of the free-martin gonad are medullary cords which are transforming into seminiferous tubules. The fate of the primordial germ cells is the same in both the free-martin gonad and the normal foetal ovary. The medullary cords with their germ cells degenerate and disappear before birth in the normal ovary of cattle, the first to disappear being the germ cells. Allen ('04) has described a similar fate of the primordial germ cells found in the medullary cords of rabbit and pig ovaries. It would appear that this phenomenon is the regular event in the development of the mammalian ovary. Since these primordial cells in the sexual cords of the free-martin gonad and in the medullary cords of the normal foetal ovary are identical as to origin, structure, degeneration changes, and disappearance before birth, it seems reasonable to conclude that the primordial germ cells of foetal free-martin gonads are female in type.

As the sex-cord region shows more pronounced male organization, the rete region simultaneously makes progress in the male direction in several particulars. In the first place, its primitive position at the hilus of the gonad is more or less lost by the growth of the sex-cord region around its sides. However, the growth of the sex-cord region is not sufficient even in the most completely transformed gonads to bring the rete into the center of a cylindrical gonad as it is in the normal testis (fig. 1, D and

E), but it lies eccentric to the greater portion of the sex-cord region. Secondly, as a rule, the distance of the penetration of the core of rete tubules into the gonad varies directly with the increase of maleness (fig. 1, B, C, and D). Thirdly, the lumina of the rete tubules increase in size as the gonad increases in maleness. In the fourth place, the rete tubules establish connections with the vasa efferentia and with the seminiferous tubules; only a few such connections are made in the moderately transformed gonads, while in the most highly transformed gonads many are made. Obviously, the rete of the free-martin gonad may be transformed into a typical rete testis.

The connective-tissue capsules exhibit three more or less distinct and progressive stages of development in the male direction. These stages correspond only in a very rough way to the degree of transformation of the gonads, but correspond very closely to the stages passed through in the normal development of the tunica albuginea of the testis. The first stage is characterized by a comparatively thin layer of connective-tissue fibers; in the second stage the capsule possesses two layers, the tunica albuginea and the tunica vasculosa, while in the third stage the tunica vasculosa is merged with the tunica albuginea.

Closely associated with the degree of development of the connective-tissue capsule is the distribution of the blood-vessels. In the highly transformed gonads a vascular cord, which is typically like the normal vascular cord of the testis, penetrates into the anterior end to connect with the tunica vasculosa or with the vascular portion of the tunica albuginea. In less transformed cases a much less typical vascular cord enters the gonad at a point slightly posterior to the anterior end, from which its blood-vessels are distributed in some specimens to the tunica vasculosa, and in others directly outward to the sex-cord region as in the normal ovary.

The epididymis also exhibits a graded series of transformations in the male direction. In the least transformed gonads there are no traces of epididymal tubules; in the moderately transformed gonads there are traces of tubules, while in the most highly transformed gonads the typical epididymal bodies are present.

A question of considerable importance to the problem of sex-differentiation in mammals is to determine the limit of transformation of an ovary in the male direction by the action of sex hormones. The most extreme case is the gonad of H-37. Although the gonad (left) has attained a comparatively large size, it is nearly twenty times smaller than a normal testis of approximately the same age. Structurally, it is a typical testis in every respect except that all stages of the male germ cells are absent. So complete is the transformation that some doubt may arise as to its classification as a free-martin gonad. However, the following facts indicate without a reasonable doubt that the gonads are from a free-martin: 1) The specimen in question was born co-twin to a normal bull. 2) The external genitalia are predominately female, although the clitoris is partially transformed into a perforated penis. Numan ('43) figures in his plate XI (refigured by Lillie, '17, fig. 29) the reproductive system of a free-martin, which shows the external genitalia transformed in a manner almost identical with that of case H-37. 3) The gonad of this specimen forms a member of a graded series of transformations in the male direction. 4) The internal reproductive system, aside from the gonads, is in all essentials of the usual free-martin type, except there is a greater progression in the male direction. 5) The possibility that the gonads are from a cryptorchid male is dismissed by the fact that in all cryptorchids the external genitalia are normal. Furthermore, cryptorchidism in cattle has not been reported. Obviously, then, an ovary under the influence of male sex hormones may transform into a testis which is morphologically complete, but which is functionally inactive so far as the production of germ cells is concerned (the interstitial cells may be physiologically active).

Such a reproductive gland approaches very closely, if not actually reaches the limit of transformation in the male direction by hormonic action, a condition which indicates that the optimum conditions for the transformation of an ovary were fairly closely realized in this case. What are the optimum conditions? The optimum conditions, so far as the primary cause is con-

cerned, have already been considered in the first part of the general discussion as due to an early anastomosis of the blood-vessels between the different-sexed twins, and therefore an early introduction of the male sex hormones or the sex hormones may have been unusually potent or the duration of their action may have been prolonged. These factors are responsible at least for the early changes in the transformation of the gonad. How much of the subsequent events is due to the initial stimulus of the sex hormones and how much is due to other factors is difficult to determine. However, a few suggestions as to the factors responsible for the later changes may be considered. In the first place, the sex hormones may continue to act until birth, providing the vascular connections persist (the foetal membranes are still united at birth, according to Tandler and Keller, '11). This necessarily involves the further assumptions that the interstitial cells of the testis of the male twin are active throughout foetal life or at least periodically active, and that these cells produce sex hormones. Secondly, owing to the absence of the cortex, it is difficult to state how much of the male development may be the result of the absence of normal ovarian secretions. It is known that in the absence of such secretions male characters tend to develop. Pearl and Surface ('15) described a cow with cystic degeneration of the ovaries which led to the development of male secondary sexual characters. Spayed females in birds take on male secondary sexual characters, (Goodale, '16), and the castration of the hen-feathered Sebright male results in the development of the plumage characteristic of the cock-bird (Boring and Morgan, '18, Morgan, '19). In the third place, the interstitial cells of the free-martin gonad may furnish sex hormones which aid in its own differentiation. Certainly, some relation exists between the high degree of maleness and the hypertrophy of the interstitial cells, as in all of the most highly transformed gonads they are abundant. If these cells are to play any part in differentiation of a gonad, they would be expected to occur at least in some of the foetal stages. This is not the case, at least in the early foetal stages, as no traces have been found.

Just how much growth and differentiation of the free-martin gonad occur after birth has not been determined, owing to the difficulty of obtaining the late foetal stages. In the early free-martin embryos examined (oldest embryo was 28 cm. long), the gonad is smaller than normal and the seminal vesicles have not yet appeared. In the normal male the seminal vesicles are well indicated from 16.8 cm. on. In approximately 80 per cent of the post-natal free-martins the seminal vesicles are well-defined structures. In some the prostate appears (H-18) (cf. literature). It would appear, therefore, that there is a delay in growth during the late prenatal stages. With this gap filled, it would be possible to make a more careful analysis of the factors which are responsible for the later steps in the modification of the reproductive system. At birth the possibility of any further action of the male sex hormones terminates. During this period of late foetal development, search should be made for any evidence of interstitial cell activity in the testis of the male twin, and also to determine the degree of anastomosis of the extra-embryonic blood-vessels between the male and female individuals.

A parallelism is seen between the degree of transformation of the gonad and the degree of transformation of other parts of the internal reproductive system, the external genitalia and the phenomenon of descent. In the case of the reproductive-duct system the parallelism is not distinct, but in general there is a tendency for the female parts to disappear and for the male parts to develop as the gonads are transformed in the male direction. The external genitalia are the least liable to undergo modification, but in cases where the normal male limit is closely approached, they may also be transformed. That is, in cases where the reproductive glands are structurally typical testes, and have descended into the groin region, the external genitalia assume male characters (the clitoris in H-37 is partially transformed into a penis). Obviously, the external genitalia, the system of ducts and accessory reproductive glands as well as the gonads exhibit a graded series of transformations in the male direction.

Not only is there this general correlation between the various parts of the free-martin reproductive system, but the correlation is still closer if the parts on one side are compared. Case 44 (table 2) showsala r ge left gonad associated with a large Wolffian duct and a large seminal vesicle, while the right gonad has a smaller duct and a smaller seminal vesicle. Case H-37 shows the left gonad one and a half times larger than the right gonad, and the left seminal vesicle is fairly well developed and possesses a duct which ends blindly, while the right seminal vesicle is poorly developed and its duct entirely absent. Lillie ('17) showed that disturbances in relation of parts in foetal stages are found on the right side, as, for instance, in his case 2 (fig. 20, A) where the gubernaculum on the right side evaginates into the body cavity instead of into the body wall, as the left one does. These lateral variations certainly indicate that physiological factors, other than the action of sex hormones, play a part in correlation. There is some embryological basis for these lateral variations, as is seen from an examination of a number of ovaries, the majority of which are from young calves. In the number examined (20) about 65 per cent show the left ovary to be larger and the follicles more advanced than in the right.

A very interesting situation is presented if the lateral variations of the reproductive glands of the free-martin are compared with similar variations in other vertebrates. According to Simpson ('36), in the human the left side usually has the female type of gonad and disturbances are usually found on the right side (a number of more recent cases support this observation). Among other groups of vertebrates, lateral variations occur normally where the left ovary is larger and the functional one. The monotremes have a larger left ovary and eggs have been found only in the left oviduct. In birds the right ovary degenerates and among certain of the skates (Trygon, Myliobatis and relatives) the left ovary alone functions. Although there may be certain mechanical factors which result in the functioning of one ovary, it is certainly no coincidence that it is always the ovary on the left side which is functional. From the above considerations it would appear that some physiological processes

underlie these asymmetrical disturbances. The blood supply may be greater to the left gonad than to the right, yet this cannot be regarded as the primary factor.

The interrelation between the reproductive glands and other glands of internal secretion is supported by the observations of many, yet none of these observations furnish any definite clue as to the nature of the correlation. The whole developmental history of the free-martin reproductive system is certainly complicated by such correlations as these.

If the intersexual condition in the free-martin gonads is compared with the condition of intersexuality in the gonads of other animals, definite similarities in structure and origin are recognized. Goldschmidt ('12, '14, '16) has shown that both the male and female individuals of Lymantria contain the primordia for each sex. Which sex is to appear depends upon the quantitative relations of both male and female sex factors of the gametes. If the crosses between two species are made in such a way that the male factors have dominance over the female factors, an ovary transforms into a testis. Thus with a variable quantity of the male factors all degrees of transformations are possible. On this point Goldschmidt ('16, p. 713) states: "This is a body [sex gland] looking externally like a testis, but showing in sections every single step between an ovary with nothing but immature eggs through a mixture of ovarial and testis tissue to a real testis." The reverse changes, that is, the transformation of a testis into an ovary, are also possible. It has been shown that the free-martin gonad is primarily an ovary, which would be due, according to the conceptions of Goldschmidt, to the dominance of the female factors over the male. Lillie ('17) pointed out that the intersexual condition in the free-martin gonad may be explained "as due to an acceleration or intensification of the male factors of the female zygote by the male hormones." The degree of transformation would be dependent on the quantity of the hormone. Obviously, in the free-martin gonad the male-sex expression is the result of a variable quantity of internal secretions, instead of a variable quantity of male-sex factors as in the gonads of the intersexual hybrids of the gypsy-

moth. Goldschmidt ('17), in comparing the hormonic inter-
sexuality of the free-martin with the zygotical intersexuality of
Lymantria, attempts to point out that the direction of sexual
differentiation changes at the time when specific sex-determining
hormones can act.   In the free-martin he supposes that specific
sex-determining hormones arise in the interstitial cells, and act
.the moment they enter the blood of the female, while in Lymantria
they arise from every single cell of the body.

Among mammals in general an intersexual condition of the
gonad is somewhat rare.   As a rule, in the cases that have been
examined, the gonad is usually part ovary and part testis (Pick,
'14).   Gudernatsch ('11) points out that hermaphroditism in
the sense of separate ovaries and testes has not been demon-
strated in man or even in other mammals beyond a doubt.
Furthermore, in no case does the testicular part bear any stage
of male germ cells, but the ovarian part may produce germ cells
which are at least structurally normal.   The possibility of the
formation of such intersexual gonads in mammals by the action
of sex hormones is suggested by Lillie ('17).   This possibility
is made still stronger by the study of one of the free-martin gonads
(case H-42).   This gonad is partly ovary and partly testis.   The
ovarian part contains distinct follicles with germ cells which are
apparently normal, while the testicular part contains typical
seminiferous tubules, but the germ cells are absent.   Magnusson
('18), reports free-martin gonads which have a similar structure
with the exception that the primary follicles lack germ cells.
This ovarian part, which is apparently an exceptional condition
in the free-martin gonad, probably arises either as a consequence
of a late introduction or of a temporary action of the male sex
hormones.   In the former the cords of Pflüger invaginate to a
slight extent, while in the latter alternative the ovary was only
partly transformed into a testis.   Since the intersexual condition
of the free-martin gonad (case H-42) resembles the intersexual
conditions of the gonads of other mammals, it appears that they
may have an interpretation in common.   It thus follows that in
all mammals where there is a possibility of embryonic anas-
tomosis of the blood-vessels between individuals of opposite sexes,

the gonads may be intersexual. The rather frequent occurrence of intersexual gonads in the pig may have some such explanation as this. The appearance of intersexual gonads in mammals, which, as a rule, furnish no possibilities of the intermingling of sex hormones through the fusion of foetal membranes, must be interpreted in some other way. In such cases it is not impossible that disturbances in the normal relationship of the maternal blood supply to the foetal blood may account for the intersexual condition of the gonads.

The definite transformation of an ovary into a testis raises the question of the existing morphological homologies between the two reproductive glands. As early as 1870, Waldeyer, in his classic book, "Eierstock und Ei," suggested that the potentialities for the development of both ovary and testis existed in the same individual, but he erred in considering that the male and female reproductive glands arise from different portions of the genital ridge. From a study of the adult gonads of the bat, Van Beneden ('80) formulated the hypothesis that the testis and ovary possess morphological structures in common. He homologized medullary cords with seminiferous tubules, medullary tubes with straight tubules, and the rete ovarii with the rete testis. Such a homology as suggested was not fully justified until the comparative studies on the development of the ovary and testis were made by various investigators. Allen ('04) showed for the rabbit and pig that the first set of sexual cords (medullary cords) of the ovary and of the seminiferous tubules of the testis are homologous structures, which are both formed as tubular invaginations of the epithelium of the genital ridge. Simultaneously with the development of the first set of sexual cords, the rete cords of both ovary and testis arise in the same manner from the epithelium of the anterior end of the genital ridge. A zone of connective tissue, the primary tunica albuginea, which lies between the medullary cords and the cords of Pflüger, is homologous with the tunica albuginea of the testis.

In man (Felix, '11) and in the cat (Kingsbury, '13) the morphological homology of the medullary cords of the ovary and the seminiferous tubules of the testis appears difficult to establish

with certainty, owing to the absence in the ovary of a more or less distinct line of demarkation between an inner epithelial mass (medullary cords) and an outer epithelial mass (cords of Pflüger). Although the separation of the two regions is not obvious in the late foetal stages of the cat's ovary, these regions are distinct in the earlier stages, owing to the growth of a distinct primary tunica albuginea between the two (Sainmont, '05). In the ovary of both the cat and man the sexual cords of the medulla take no part in the formation of ova in the fully developed ovary, and in this respect resemble other mammalian ovaries, where the homology has not been questioned (as in cattle; fig. 3, ta). Furthermore, in both cases occurs the rete ovarii, the undoubted homologue of the rete testis. These facts are certainly sufficient to indicate that the ovarian-medulla-testis homology applies in the cat and man.

Fortunately, in the free-martin, nature has performed a veritable crucial experiment, which demonstrates without question that the ovary possesses structures which have their morphological equivalents in the testis. That they are morphologically equivalent is shown by the transformation of certain ovarian structures into testicular structures under the influence of sex hormones. The following homologies are established: 1) The medullary cords precisely and definitely transform into seminiferous tubules. 2) The rete ovarii transforms into a rete testis chiefly by developing connections with the seminiferous tubules and with the vasa efferentia. 3) The primitive tunica albuginea of the ovary becomes the tunica albuginea of the free-martin gonad. 4) Associated with the embryonic ovary is an epididymal primordium which transforms into a typical epididymis.

If a comparison of the sex-cord region of the free-martin is made with certain well-known changes in the sex-cord regions of mammalian testes, marked structural similarity is seen. In both, the interstitial cells are hypertrophied and each seminiferous tubule has a thickened wall within which is a Sertoli-cell syncytium free from germ cells. Such changes are present in cryptorchid testes (Whitehead, '08, and Hanes, '11), and are experimentally produced in the following ways: exposure to

X-rays (Regaud, '10), feeding a diet deficient in water-soluble vitamine (Allen, '19), eliminating the sympathetic nerve supply (Kuntz, '19), transplantations (Moore, '19), etc.

These cases (at least the experimental ones) all indicate that the atrophy of the male germ cells is accompanied by the hypertrophy of the interstitial cells. In the free-martin gonad there is no atrophy of the male germ cells to accompany the hypertrophy of the interstitial cells. It would therefore appear that the increase of the interstitial cells is not necessarily associated with the phenomenon of the degeneration of the germinal cells. There may be some more fundamental changes in metabolism, which are responsible for the observed modifications. There is some evidence that metabolic changes result in certain structural modifications in the testis, as, for instance, the cyclic changes in hibernating animals (Rasmussen, '17 and '18). It is problematical whether changes in metabolism bring about the degeneration of germ cells or the absence of germ cells brings about changes in metabolism.

A question of considerable interest arises as to the relation of the interstitial cells to the sexual instincts and to the secondary sexual characters in the free-martin. In mammals having cryptorchid testes the typical sexual instincts are manifested and are apparently associated with the functioning of the abundant interstitial cells (Hanes, '11; Whitehead, '08). Since the structure of the sex-cord region of the free-martin gonad resembles in essential details the sex-cord region of a cryptorchid testis, it might be expected that the free-martin would have the usual sexual instincts of a male. This is apparently not the case, at least so far as the literature discloses. According to the accounts of Lundberg ('64), Zschokke ('00), Hunter ('86) and Magnusson ('18), free-martins fail to exhibit sexual instincts. Hart (10) states concerning one of Hunter's free-martins on page 231: "This animal was seven years old; went with the cows and bull; never showed any desire for either." Zschokke states that in the Alps the free-martin is used like the ox as a beast of burden, because it is strong and tame, or it is fattened for the market.

Another characteristic of the cryptorchid male is the normal appearance of the secondary sexual characters.   The free-martin, however, according to the observations of Magnusson ('18), Pusch ('11), Zschokke ('00) and others, resembles a castrated male in body form, development of the horns, etc.   Hunter ('86, p. 53) regarded it more like the ox or spayed heifer than the bull or cow.   Tandler and Keller ('10), who performed elaborate experiments on the influence of castration on the body form of the male and female in cattle, conclude that both male and female converge to a 'common form' (asexual).   They regarded the free-martin as resembling more closely the castrated female in form.   Hart ('10) figures one of Hunter's free-martins which shows very distinctly the head and horns of an ox or spayed female, and the fore and hind quarters distinctly male in type. It would appear from these observations that the interstitial cells in the free-martin gonad fail to play any strong rôle in the production of secondary sexual characters.

Number	Age	Size	Tunica albuginea	Sexual cords	Intertubular tissue	Tubuli recti	Rete tubules	Epididymis	Vascular plexus	Seminal vesicles	External genitalia
66	5 days	About 5 mm. long. 2 mm. wide	Outer layer of compact fibers; inner layer barely indicated and constitutes a tunica vasculosa (few blood vessels)	Masses of supporting epithelial cells; sexual cords—some resemble medullary cords, others resemble seminiferous tubules; and still others showing transitional stages between the two types. Germ cells absent	Few interstitial cells	Few	Eccentrically placed; extends over half way to posterior end	Mere traces of tubules	Flattened cord of blood-vessels enters anterior end of gonads	Not examined	Typically female
42	21 days	13.5 mm. long. 5 x 3 mm. in cross-section	As in case 66	As in case 66	As in case 66	Few	As in 66	Rete established connections with four vasa efferentia tubules (fig. 9)	As in 66, fig. 9	Not examined	Typically female
H-18 Fig. 5	31 days	4 x 6 mm. in cross-section	Outer layer compact, comparatively thick. No tunica vasculosa. Blood-vessels enter hilus, and are distributed outwardly	Masses of supporting epithelial cells very few. Sexual cords as in case 66. No germ cells	As in case 66, except more easily recognized	Few	As in 66	Absent	No definite vascular cord as in 66 and 42. Few blood-vessels enter hilus at anterior end	Two small masses having impervious ducts. (A prostate gland is present)	Typically female
H-46 Fig. 10	5½ wks.	8 x 10 mm.	Capsule increased in thickness. Outer layer of compactly arranged fibers; inner layer loosely arranged, and enclosing blood-vessels, and constitutes a tunica vasculosa	Majority of sexual cords resemble seminiferous tubules of young male calves (figs. 12 and 8). Nuclei of supporting-epithelial syncytium arranged in a single layer at periphery, and from inner ends extend strands of cytoplasm to the center. No germ cells	Connective-tissue stroma abundant. Interstitial cells moderately numerous	Moderately numerous	Penetrates to posterior end of gonad; eccentrically placed (fig. 10). Toward posterior end sex-cord region completely surrounds rete	Rete connects with head of epididymis at anterior end; tail of epididymis attached at posterior end of gonad—connected with head by the body of the epididymis	A vascular cord (7 mm. in diameter) enters anterior end of gonad (fig. 10). Vascular cord and vas deferens loosely united by connective tissue forming spermatic cord	No seminal vesicles	Typically female
44	7 wks.	Left 35 x 25 mm. Right 13 x 15 mm. (Measurements include epididymis)	Outer layer distinct, thick. Tunica vasculosa only well-developed in places	As in H-46	As in H-46	As in H-46	Penetrates about half the length of gonad; eccentrically placed	As in H-46	As in H-46	Two long vesicles	Typically female
H-37	14 mos.	Left 60 x 40 mm. Right 40 x 25 mm.	Tunica vasculosa is intimately merged with the tunica albuginea. In the inner portion of the latter are blood-vessels	Seminiferous tubules typically like normal tubules, except germ cells are absent. A Sertoli-cell syncytium structurally identical with the syncytium of adult testes. Wall thickened	Interstitial cells much hypertrophied	Very numerous. Fig. 16	Extent not examined, but probably extends to posterior end. Sex-cord tissue lies between rete and tunica albuginea	As in H-46	As in H-46	Right vesicle less developed than left. Left duct ends blindly; right duct absent	Clitoris transformed into a penis-like structure, otherwise the external genetalia are female
H-42	18 mos.	Left 12 x 4 mm. Right ? x 4 mm.	Outer layer of more compactly arranged fibers; inner layer of loosely arranged fibers, enclosing blood-vessels, constituting a tunica vasculosa	Two kinds of sexual cords, those which resemble seminiferous tubules, and those which resemble primary follicles. A few egg cells within the follicles	Many intertsitial cells; two types, those resembling the interstitial cells of normal testis, and those resembling lutein cells of a corpus luteum	Few	Extends to posterior end of sex-cord region; eccentric to sex-cord region	Rete makes connections with several epididymal tubules	Not examined. No spermatic cord according to Cole	Vesicle (4 cm. long). Ducts impervious	Typically female
H-36	2.5-3 yrs.	20 x 10 mm.	As in H-42	Very few sexual cords, which resemble seminiferous tubules. No germ cells	No interstitial cells found	Absent	Extent not determined	Absent	Very small plexus of blood-vessels attached at hilus	Two lobulated vesicles, attached to vas deferens	Typically female
H-40	3 yrs.	10 x 4 mm.	Capsule much thickened. Blood-vessels scattered. Fig. 2	Sexual cords few; some resemble small seminiferous tubules, other medullary cords. No sex cells	Interstitial cells moderately abundant	Absent	Extend two-thirds of the length of the sex-cord region	Absent	Plexus of blood-vessels emerge from anterior end of each gonad	Two vesicles communicating with vas deferens	Typically female

Number	Length, age	Size	Tunica albuginea	Medullary cords	Cords of Pflüger	Stroma	Tubuli recti	Rete tubules	Epoöphoron	Vascular plexus
N 14	6.3 cm.		Primary albuginea a distinct layer	First set sex cords numerous; no well-defined walls to cords. Supporting epithelial cells irregularly arranged and closely packed together	Short ingrowths continuous with germinal epithelium	Little connective tissue fibers	None	Enters anterior end at hilus, but does not penetrate far posteriorly. Lumina forming	Rete tubules make connections with tubules of Wolffian body	Blood-vessels enter hilus along with rete
N 7	8.3 cm.		As in N 14	As in N 14. Few primordial germ cells	Short ingrowths continuous with germinal epithelium; little differentiated from it	No interstitial cells	None	As in N 14	Slight degeneration of tubules of Wolffian body	As in N 14
N 40	10.2 cm.		More distinct and wider	As in N 7	As in N 7	Scanty stroma; no interstitial cells	None	Extends only a short distance posteriorly	As in N 7	As in N 14
N 26	14.0 cm.	3.4 mm. long	Primary albuginea as in N 40. Definitive albuginea barely indicated	Cords contain germ cells, forming "medullary follicles"	Cortex relatively thicker. Cords distinctly separated from each other by strands of connective tissue	Few cells in stroma resembling interstitial cells	Absent	Extends about one-half the length of the medullary cord region. Enters anterior end at hilus	Wolffian body tubules growing smaller	Blood-vessels enter hilus as in the above cases
N 23	17.0 cm.	4.39 mm. long	Primary albuginea is wider. Definitive albuginea as in N 26	Medullary primary follicles containing germ cells. Nuclei of supporting epithelial cells more or less irregularly arranged, sometimes forming a single layer with strands of cytoplasm extending centrally	Cords still attached to germinal epithelium. At inner ends of cords are epithelial nests (primary follicles)	No interstitial cells found	Absent	Enters anterior end; extent one-half the length of medullary-cord region. Rete smaller, and extent posteriorly less than in testis from embryo of same age	Wolffian body tubules still smaller and fewer than in N 26	As in N 26
N 15	18 cm.		As in N 23	As in N 23. In addition to the sexual cords, occur masses of unorganized supporting epithelial cells	As in N 23	Stroma abundant	Absent	As in N 23	As in N 23	As in N 26
N 24	20 cm.	4.44 mm. long	Primary albuginea is less distinct owing to its mergence with connective tissue of medulla	Medullary cords fewer than N 15, some degenerating. Connective tissue increased in medullary region	Primary follicles at inner ends of cords more numerous	No interstitial cells observed	None	Extent about one-half the length of the medullary cord region. Smaller than rete of testis from embryo of same age	Wolffian body mostly degenerated. Tubules of epoöphoron and paroöphoron present. Rete connects with epoöphoron	As in N 26
N 25	23 cm.	6.83 mm. long	As in N 24	As in N 24. Some medullary cords having nuclei of supporting epithelial syncytium arranged more or less into layers	As in N 24	As in N 24	None	As in N 24	As in N 24	As in N 24
N 20	29.5 cm.	7 mm.	As in N 24 except the definitive albuginea is thicker	Medullary cords rather numerous, and surrounded by a rather dense stroma of connective tissue	Primary follicles numerous	As in N 24	None. The rete tubules and medullary cords closely approximated	Enters hilus, extends one-half the length of medulla. Diameter less than rete testis of N 19	As in N 24	As in N 24
Bos 8	56 cm.	8 x 4 mm.	Primary albuginea a distinct and wide layer (fig. 3, ta). Very thin definitive albuginea	Few degenerating cords (fig. 3, mc)	Very numerous (fig. 3, p)	No interstitial cells recognized. Stroma abundant	Absent, although some medullary cords lie close to rete tubules	Lies at hilus; projects to posterior end of medullary-cord region	?	A flattened cord of blood-vessels enter hilus at anterior end
Bos 9	106.5 cm. (mature foetus)	12 x 5 mm.	Primary albuginea no longer a distinct layer, but is continuous with the stroma of the medulla and of the cortex. Definitive albuginea thin	Very few medullary cords found. Medulla very vascular	Many follicles enlarged—granulosa cells hypertrophy filling follicular cavity—germ cells degenerating, resulting in large follicular masses of granulosa cells	Occasional cells in medulla stroma which resemble interstitial cells	Absent	Distinct tubules with lumina. Penetrates almost to posterior end of medullary cord region	?	A cord of convoluted blood-vessels enter the anterior end at the hilus, along with the rete tubules
Bos 6	Young calf 6 mos. old ?	Left 25 x 17 mm. Right 25 x 9 mm.	Definitive albuginea very thin and inconspicuous	No medullary cords. Medulla comparatively small and composed of a stroma of dense connective-tissue fibers, between which are numerous blood-vessels	Same as Bos 9, except that the degenerating follicles may become much larger (atretic follicles)	?	Absent	Traces of a few degenerating tubules at hilus	?	As in Bos 9
Bos 1	Adult	30 x 12 mm.	Definitive albuginea, a definite layer of densely arranged connective tissue fibers	No medullary cords	Normal follicles in cortex. One large corpus luteum	?	Absent	Absent	Absent	As in Bos 9

TABLE

Tabular summary of structural conditions of foetal and postnatal testes

SPECIMEN			TESTIS		TESTIS				
Number	Length, Age	Size	Tunica albuginea	Seminiferous tubules	Intertubular tissue	Tubuli recti	Rete tubules	Epididymis	Vascular plexus
N 13	4.8 cm.		Outer layer of compact connective-tissue fibers. Inner layer of loose fibers, which enclose blood-vessels	Solid cords of compactly and irregularly arranged supporting epithelial cells. Cord walls faintly indicated. Primordial germ cells if present, are not evident	Very little stroma of connective tissue.	None found, although rete and seminiferous tubules are approximated	Enter anterior end; penetrate a very short distance posteriorly, Lies at hilus. Lumina barely indicated	Rete tubules establish connections with renal corpuscles of Wolffian body at anterior end of testis	Blood-vessels enter at hilus, thence to tunica vasculosa
N 10	7.0 cm.	3.03 mm. long	Thin. Fibers more compactly arranged in outer layer. Tunica vasculosa as in N 13	Cords more definite, little branching of cords. Supporting epithelial cells formed into a syncytium; nuclei irregularly arranged into two and three layers. Primordial germ cells present	Connective-tissue stroma scanty	Branches of rete tubules make connections with seminiferous tubules	Projects about two-thirds of length of testis. Lies nearly in center of round gland. Several distinct lumina	Large Wolffian body. Connections made as in N 13	Enter along with rete. Distribution as in N 13
T 19	8.0 cm.	3.5 mm. long	As in N 10	Seminiferous tubules increasing in size, and looser in structure. More regularity in arrangement of nuclei of supporting epithelium. Cytoplasmic strands from inner ends of nuclei extend toward potential lumen, are just beginning to develop. Primordial germ cells	As in N 10	More distinct	Extends farther into testis than N 10. Still a little eccentric to the center. Many distinct lumina	As in N 10	As in N 10
T 16	12.75 cm.	4.45 mm. long	As in N 10. Outer layer more compact	Nuclei of supporting epithelial cells mostly arranged into a single layer; in some two layers. Cytoplasmic strands more prominent. Few distinct primordial germ cells	As in N 10	As in T 19	Extends entire length of testis. Center of round testis. Lumina more numerous	Large and well developed	As in N 10
T 6	16.8 cm.	4.86 mm. long	Wider, otherwise as in T 16	Tubules larger, looser. All nuclei of syncytium in a single layer, except a few scattered ones. Distinct cytoplasmic strands extending inwardly from nuclei. Few primordial germ cells	Moderate amount of stroma. Few interstitial cells	Many	Tubules large in diameter; much branched. Extend entire length. A central axis	As in T 16	A distinct vascular plexus (cord-like) enters anterior end of testis
N 21	20.0 cm.	4.04 mm. long	Both layers distinct. Fig. 4	Nuclei of supporting cells as in T 6, except closer together. Tubules more branched at periphery of testis. See fig. 4	Stroma abundant. Interstitial cells many	Many. Fig. 4	Extends to posterior end. Central core shown in fig. 4	As in T 16	Blood-vessels in vascular cord more convoluted and larger
T 4	24.0 cm.	8.67 mm. long	Distinction between outer and inner layers not as well marked at this stage	Same as N 21	As in N 21	As in N 21	As in N 21	As in N 21	Vascular cord structurally is a network of venous spaces and arterial vessels
N 19	31.0 cm.	8.26 mm. long	Two layers still distinct, but connective tissue is increasing in compactness in tunica vasculosa	Nuclei of supporting epithelium lie closer together in the single layer—cytoplasmic strands still more pronounced. Few primordial germ cells	Interstitial cells numerous	As in N 21	As in N 21	Not examined	Not examined
Bos 10	61.0 cm.	25.0 mm. long. 8.0 mm. wide	Connective tissue nearly as compact in tunica vasculosa as in the outer layer	As in N 19	Interstitial cells numerous	As in N 21	As in N 21	Globus major, globus minor, and body of epididymis well developed	As in T 4 except larger in diameter
Bos 3	89.0 cm. (Mature foetus)	36.0 mm. x 11.0 mm.	Two layers merged into one—inner portion of which contains the blood-vessels 1.5 mm. thick	Same as N 19, except there is more regularity in the arrangement of the nuclei of the supporting epithelial cells, and the cytoplasmic strands are more pronounced. Few primordial germ cells. Fig. 7	Interstitial cells moderately numerous	Present. Fig. 7	Central core, extending to posterior end as in N 21	As in Bos 10	As in Bos 10, only larger
H-45	2 days	?	Not examined	As in Bos 3	As in Bos 3	As in Bos 3	Present	Not examined	Not examined
H-26	12 days	?	Not examined	As in Bos 3	As in Bos 3	As in Bos 3	Present	Not examined	Not examined
Bos 12	Young calf 6 mos. ?	40.0 mm. x 12.0 mm.	As in Bos 3, 1.25 mm. thick	As in Bos 3, except the nuclei of the syncytium are more closely approximated. Fig. 8	Interstitial cells numerous. Fig. 8	Numerous connections	As in Bos 3. Fig. 14	Globus major a disc-like cap fitting over anterior end of testis, and connected with rete. Globus minor, a conical mass attached to posterior end of testis. Connecting these two parts is the strap-shaped body of the epididymis. Fig. 18	Vascular cord 5 mm. in diameter enters anterior end, just at one side of the connection of rete and epididymis
Bos 2	Adult	125.0 mm. x 57.0 mm.	As in Bos 3 except thinner 1 mm. thick	The strands of cytoplasm of supporting epithelial cells (Sertoli cells) have become resolved into a net-work of strands, between which are the multiplying male sex cells. Nuclei of Sertoli cells usually lie near periphery of tubules, but in no such regularity as in Bos 12. Fig. 13	Stroma and interstitial cells moderately numerous	Many. Fig. 13	As in Bos 3	As in Bos 12, only much larger	As in Bos 12, except larger; 12 mm. in diameter

SUMMARY

1. In the free-martin an indifferent gonad with a primary female determination transforms in the male direction under the influence of male sex hormones.

2. Three distinct steps may be recognized, which may be characterized as low, medium and high degrees of transformation in the male direction. These three groups constitute a chain of connecting links between an embryonic ovary and a testis.

Such a graded series of transformations is evidenced as follows:

a. The sexual cords exhibit a series of gradations between medullary cords and seminiferous tubules.

b. The interstitial cells increase in number as the gonad transforms in the male direction.

c. The rete transforms in the male direction by developing connections (tubuli recti) between the rete tubules and the seminiferous tubules, and by connections between the rete tubules and the epididymal tubules. Such connections are not made in the gonad exhibiting a low degree of transformation. In the group of moderately transformed gonads a few such connections are made, while in the most highly transformed gonads such connections are numerous.

The distance that the rete penetrates into the sex-cord region varies according to the degree of transformation of the gonad. In the most highly transformed gonad it penetrates to the posterior end of the sex-cord region, as in the normal testis.

The rete becomes less eccentrically located with reference to the sex-cord region in cases of pronounced male organization. It is never centrally placed as in the normal testis.

d. The epididymis is absent from gonads exhibiting a low degree of transformation; only the head of the epididymis is present in gonads showing a moderate degree of transformation, while a typical epididymis is present in the most highly transformed sex glands.

e. The distribution of the blood-vessels correspondingly ranges from a typical ovarian arrangement to a typical male arrangement.

3. The limit of the transformation in the male direction by hormonic action is represented by a testis which is morphologically complete, but functionally inactive so far as the production of germ cells is concerned.

4. The exhibition of a graded series of transformations between an ovary and a testis apparently corresponds to, 1) the variability in the time of the introduction of the male sex-hormones; 2) the potency of the hormones, or, 3) the duration of the hormonic action.   These are the primary and fundamental determining factors.   Other factors may be responsible for subsequent events in the transformation, as, 1) absence of normal ovarian secretions and, 2) secretions from the interstitial cells of the free-martin gonad.

5. The degree of transformation of the gonads is correlated in a rough way with the degree of transformation of other genital organs (vas deferens, seminal vesicles, uterus, and external genitalia).   These correlations are closer between the genital organs of one side than between opposite sides.

6. The hypertrophy of the interstitial cells does not accompany degeneration of male germ cells, since the latter are absent from free-martin gonads.   The interstitial cells of the free-martin gonad bear no relation to the sexual instincts, and little if any relation to the secondary sexual characters.

7. That the mammalian ovary possesses structures which have their morphological equivalents in the testis is demonstrated by the tranformation of an ovary into a testis in the free-martin.

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