THE MORPHOLOGY OF THE SEMINIFEROUS TUBULES OF MAMMALIA

PRELIMINARY NOTE

G. CARL HUBER AND GEORGE MORRIS CURTIS

Department of Histology and Embryology, University of Michigan

FIVE FIGURES

At the Cleveland meeting of the American Association of Anatomists, (1912–1913), Curtis[1] reported on wax plate reconstructions of the seminiferous tubules of the white mouse, presenting figures of a model of an entire tubule having an actual length of somewhat over 13 cm. The tubule completely reconstructed presents the form of an arch, the two ends of the arch lying in close proximity, each terminating in a tubulus rectus attached to the rete testis. In the course of the very thorough study which must of necessity be given to the series of sections in the preparation of drawings on which the reconstruction is based, it became evident that the testis in question contained no seminiferous tubules terminating in blind ends, and that anastomosis between tubules was very limited. Only one branching tubule was disclosed and on graphic reconstruction of this tubule it was found that each of the three branches terminated at the rete testis in tubuli recti. In the entire series of sections of the testis in question there were found but thirty-three tubuli recti, from which it appears that this testis contains but sixteen tubules, one of which is branched and terminates, as above stated, in three tubuli recti. These results, which are so at variance with the usual conception of the form and course of the mammalian seminiferous tubule, made it desirable to extend these observations to other forms, to determine whether the findings in the mouse testis would admit

[1] George M. Curtis, Reconstruction of a seminiferous tubule of the albino mouse.

of generalization or pertained only to the form studied. The time involved in making the necessary drawings and wax reconstructions of the very long and coiled seminiferous tubules of adult mammalia is so great that it occurred to us that it might be possible to ascertain the main facts concerning the form and length of the mammalian seminiferous tubules by the less time consuming though perhaps more difficult process of maceration and teasing. For nearly all the known facts pertaining to the form, length and course of the mammalian seminiferous tubule we are indebted to the observations of the earlier investigators made on macerated and teased material. Successful teasing is dependent on the thoroughness and the uniformity of maceration of the tissue to be teased. Huber[2] has shown that by injecting a concentrated solution of hydrochloric acid into fresh tissues and then placing the injected tissue in a similar acid solution, a much more thorough and uniform maceration could be obtained than is the case when the tissues are placed directly into the macerating fluid as is the usual procedure.

The method as applied to the study of the testis tubules is as follows: A cannula was inserted into the lower abdonimal aorta and the femoral vessels clamped just beneath the inguinal ligament. A 75 per cent solution of hydrochloric acid was then injected as rapidly as possible and under a pressure of about twenty to twenty-five pounds, the pressure being maintained until the parts appeared well injected, or until there is a rupture, which is not unusual. A few moments after the injection is completed the testes were removed and placed in a seventy-five per cent solution of hydrochloric acid in which they remain for from three to four hours. It is advisable to inject several animals at the same sitting and remove portions of the material from the macerating fluid at different intervals. The optimum degree of maceration can only be approximated, the extent of injection and other factors not readily controlled influencing the time required for thorough and uniform maceration. When the desired degree of maceration is thought to have been reached, the tissues

[2] G. Carl Huber, A method for isolating the renal tubules of mammalia. Anat. Rec., vol. 5, 1911.

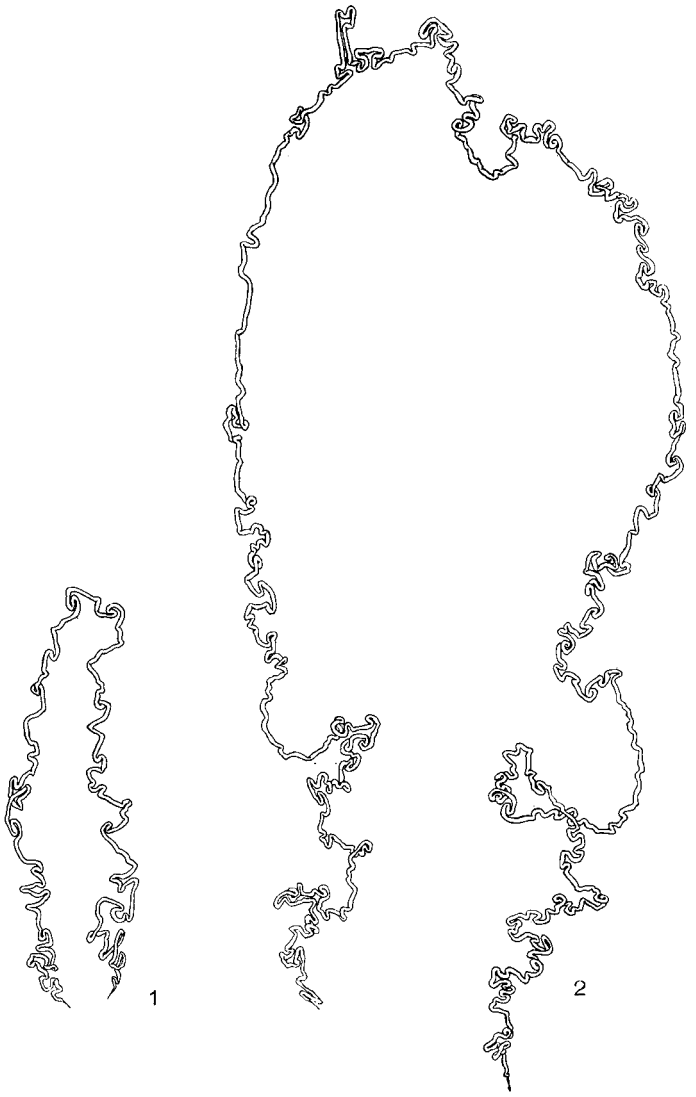
are transferred to distilled water, in which they remain for from twenty-four to forty-eight hours. It is best to transfer the tissues from the acid to distilled water gradually and slowly, this by pouring off in part the acid and then filling the dish with distilled water and repeating the process until all of the acid has been removed. The distilled water is changed at frequent intervals during the first few hours. After a thorough washing in the distilled water the tissues are transferred to Mayer's hemalum solution in which they remain from twenty-four to forty-eight hours and are then transferred to tap water. The hemalum solution not only stains the tubules so that they may be followed the more readily while teasing but also hardens the tissue. Before the final teasing is undertaken the stained tissue pieces are transferred for several hours to a 0.5 per cent solution of ammonium hydrate. This develops the stain to a rich purple blue, clears the tissue and slightly softens the tubules so that they become quite pliable. The final teasing is carried on under the stereoscopic binocular. With thoroughly macerated, well stained and sufficiently ammoniated tissue pieces at one's disposal it is not so difficult to tease out complete seminiferous tubules if the teasing is carried out in shallow Petri dishes with sufficient quantity of distilled water to enable the teased portions of the tubule to float about freely. We have experienced, however, great difficulty in attempting to make permanent mounts of such preparation. The method which has given us the best results, after many others were discarded as unsatisfactory, is the following: The larger pieces of testis tissue are separated in relatively large quantities of distilled water into masses comprising single tubules or tubule complexes (see below). The smaller pieces thus obtained are then transferred to a large slide, the edges of which have been built up by means of melted soft paraffin until a well is formed holding a layer of distilled water having a depth of from 3 mm. to 5 mm. The tubule may now be teased out completely, all of the coils separated so as to admit of moderate extension. The teased tubule or tubule complex is now arranged as desired in the final mount. The water in the paraffin well is then very carefully drawn off by means of a fine pipette or strips of filter

paper until the teased tubule rests upon the slide. The paraffin case is then removed, the slide cleaned and placed on the warm oven to hasten the evaporation of the water adhering to the tubule. If care be taken a stage is reached in which the tubule will adhere to the slide sufficient to admit of mounting, without showing distortions consequent to complete drying. The tubule may now be mounted under a cover glass coated with a layer of glycerine, lowered very slowly from one edge. The method is not simple, requires time and patience, but with it results may be obtained, as here reported. In macerated material details of cell structure cannot be made out, the method is, therefore, not applicable for ascertaining the length of the spermatogenetic wave. The space relations of the coils of a given tubule are of necessity destroyed after complete teasing. The length, general course and relations of tubules, their relation to the rete testis, branching and anastomosis are factors which can be determined in teased preparations.

In this preliminary note we shall deal with observations made on isolated seminiferous tubules of adult rabbits. In a more complete publication, in which the literature bearing on this subject will be given consideration, one of us (Curtis) will report on reconstructions and teased preparations of the seminiferous tubules of several mammals. This work so far as completed, it may here be stated, confirm the results here recorded.

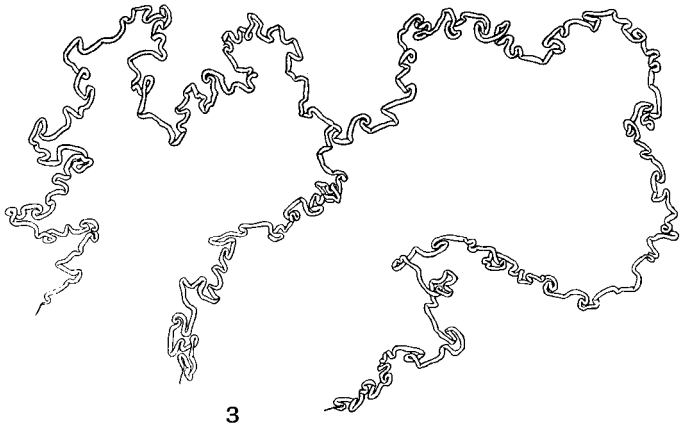
The seminiferous tubules of the rabbit appear to be arranged in the form of lobules having irregular pyramidal shapes, their bases bordering the tunica albuginea. These lobules may readily be seen in cross or sagittal sections of fixed material. The lobules are separated by more or less well defined strands of connective tissue, continuous with the mediastinum on the one side and the tunical albuginea on the other. On attempting to separate these lobules in the preliminary teasing of the larger masses of macerated testis tissue, it became evident that what appeared as a lobule could not be completely separated without tearing tubular structures, that apparent lobules were connected with adjacent lobules by tubules which passed from one to another. In well macerated tissue it is relatively easy to separate these so called lobules in the region of the mediastinum, especially if

care be taken to break the tubuli recti, which form the apices of the lobules, at their point of connection with the rete testis. If the lobules be now separated from the region of the mediastinum toward the periphery of the testis it will be found that certain ones do not reach the periphery but become connected through a bridge of coiled tubules to an adjacent lobule which in turn may be traced toward the rete testis, ending in a tubulus rectus; others with similar general course may extend toward the periphery of the gland and reach the region of the tunica albuginea It is possible to separate from tissue masses taken from any portion of the testis such coiled tubule masses arranged in the form of an arch or inverted U, the ends of the pillars of the arch terminating in tubuli recti attached to the rete testis. Such arch shaped coils of tubules, consisting apparently of two lobules united at the periphery, when completely teased out show a single tubule, both ends of which terminate in a tubulus rectus attached to the rete testis. They vary greatly in length and in the degree of coiling and folding of the constituent tubules. Numerous such tubules, completely teased and mounted, and taken from the testes of several rabbits, have been observed. They form the simplest type of the seminiferous tubule of the rabbit and are in every way comparable to the tubule of the mouse testis reconstructed in full by Curtis. In figures 1 and 2 are shown two seminiferous tubules of this simpler and more prevalent type, completely teased and mounted. These and the other tubules figured were sketched with the aid of the camera lucida at a magnification of 25 diameters, reduced to the present size in the reproduction. Each tubule, as may be observed, begins and ends in a tubulus rectus. The tubule shown in figure 1, one of the shortest teased, presents an actual length of 9.1 cm., while the tubule shown in figure 2, one of the longest simple tubules teased out completely, presents an actual length of 30.2 cm. The measurements here given were obtained by measuring the length of the tubule as presented in the enlarged drawing by means of a map-measurer, the length thus obtained being then divided by 25, the magnification used in making the drawing.

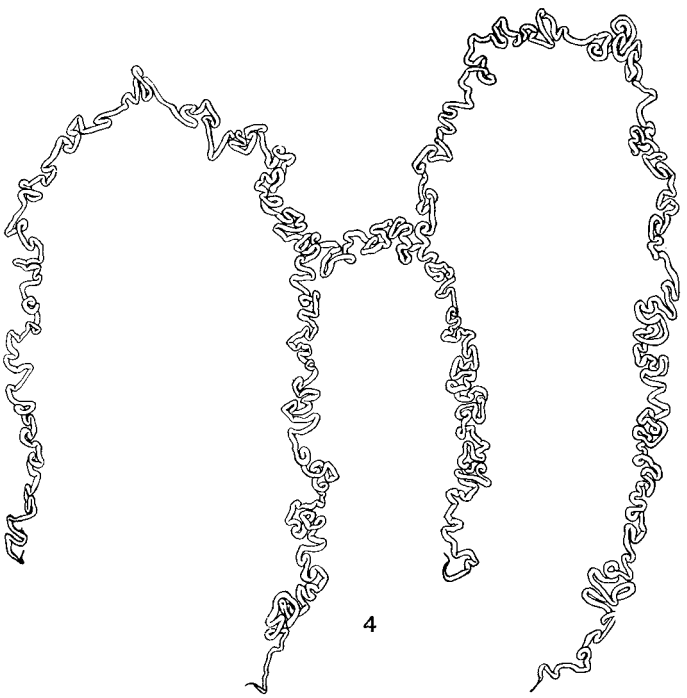


Figs. 1 and 2 Seminiferous tubules of adult rabbit, arranged in the form of an arch, with ends attached to rete testis. × 2.5.

In presenting these measurements we are aware of slight sources of error, due largely to the fact that it is difficult to portray accurately the exact length of a coil which has its direction up and down in the field of vision. Furthermore, there is evidence of slight shrinkage when the tissues are injected and placed in hydrochloric acid, perhaps compensated during washing in distilled water. The ammoniated water causes a slight swelling of the tissue, and this we are forced to disregard in making the measurement. The measurements given appear to us approximately accurate, we believe more so than those given by earlier observers. Our observations on teased preparations show that the seminiferous tubules of the adult rabbit do not present blind ends nor do they show longer or shorter diverticuli nor nodular enlargements. In stained tissue the observer is able to trace the course and outline of a given tubule much more clearly than is the case in unstained tissue. We have often noted what we are inclined to believe might readily be regarded as diverticuli or nodular enlargements were our observations confined to unstained tissue. The tubules often present very sharp turns, the two arms being parallel and in close relation. Such a sharp turn presented to view in such a way that one arm overlaps the other, may in unstained tissue readily simulate a diverticulum. When such a sharp turn is extended, the convex border appears to project as a nodular enlargement, which disappears when the parts are allowed to approximate their normal relations. A study of the numerous teased preparations has led to the conclusion that no seminiferous tubules of the adult rabbit, whether of the type of a simple arch, figures 1 and 2, or of a more complex type with branchings and anastomoses as in figures 3, 4 and 5, can be regarded as teased completely unless all of the free tubular ends can be traced to a termination in a tubulus rectus. This has been our criterion in determining whether the more extensive tubular complexes about to be described are to be regarded as teased completely. In the preliminary teasing of the larger masses one frequently meets with tubular complexes in which more than two so called lobules are joined together. On complete teasing of such portions one meets here and there, and practically at all



3



4

Fig. 3 Seminiferous tubule complex of adult rabbit, showing junction of three tubules, each attached to rete testis. × 2.5.

Fig. 4 Seminiferous tubule complex of adult rabbit showing junction of two arched tubules. × 2.5.

levels, though most frequently toward the periphery, distinct Y-shaped or T-shaped branching, through which several arch systems are linked together. The extent to which this branching and anastomosis may take place is difficult to determine since the connections between the lobules are very readily broken and loops which reach the tunica albuginea are very easily torn during the manipulation necessary for the removal of the tunica and the teasing required to separate the so-called lobules. Many broken ends are encountered in attempting to isolate completely tubule complexes with a number of anastomoses, broken ends very often not evident until the teasing is practically complete. We present here a number of types of tubule complexes with anastomoses of tubules regarded as teased completely, in that in each case each tubule was traced to its termination in a tubulus rectus, ending in the rete testis. The figures are so clear that only a brief word of explanation is deemed necessary. In figure 3 is shown a tubule complex in which three so-called lobules are joined by means of a T-shaped division. The region of the division presents no structural peculiarity, each of the three tubules show functional activity in the region of joining. This statement, however, is based on observations made on sections, rather than on teased preparations, though even in the latter the structure presented in the immediate vicinity of the division is very similar to the other parts of the tubules. As may be seen in the figure, each of the three tubules ends in a tubulus rectus, which in the unteased mass was in close proximity with the other two, and joined the rete testis. The actual length of the tubule complex, measured as above described, we find to be 26.7 cm. In figure 4, are shown two arched tubules, in general arrangement very similar to those shown in figures 1 and 2, but linked by means of a short transverse bridge. Configurations of this type are not frequently met with owing perhaps to the fact that the narrow transverse bridge is readily broken during manipulations necessary to separating the mass from the testis tissue. The mass from which this tubule complex was teased presented four so-called lobules folded together like a closed book, the transverse bridge being hidden and embedded in one of the lobules

and not evident until the teasing was practically completed. Each of the four tubular ends terminates in a tubulus rectus, which join the rete testis in close proximity. At the right of the figure the junction of three tubules is favorably placed, at the left of the figure a like junction is obscured. While still floating freely in the water it was possible to move this tubule complex about, cause tension here and there and determine with certainty the existence of the anastomoses. So with other tubule complexes completely teased. After the water has been withdrawn, so that the tubule complex rests upon the slide, the slightest tension usualy results in a tear. Experience, therefore, leads one to be content with the position assumed by the tubule complex after the withdrawal of the water, even though the resulting figure may not be as clear as is desired. The actual length of this tubule complex, which was removed from the middle third of the testis, in which region are found the largest tubules, proves to be 38.5 cm.

In figure 5, is shown a prevalent type of tubule complex, a type, however, which is very difficult to tease out completely. Many masses recognized as presenting similar tubule complexes have been teased, only to find here and there a broken tubular ending, indicating that only a portion of the complex had been separated from the testis tissue. As stated above, the evidence seems conclusive that only tubules ending in tubuli recti can be regarded as completely teased. A tubule broken at a sharp turn often simulates very closely one terminating in a blind end, especially if the basement membrane seems to pass around the broken end. Closer study, and especially turning over the supposed blind end, reveals a broken surface, admitting of a correct interpretation. In figure 5 are shown seven tubules linked together. The mass from which this complex was teased, when partly separated and unrolled, presented seven slender so-called lobules united at the periphery. After complete teasing it could be readily determined that all of the seven tubules were linked together through Y-shaped divisions of the tubule. In making a permanent mount of this tubule complex a slight shifting of tubule segments from the position originally given them

Fig. 5 Seminiferous tubule complex showing junction of seven tubules, each ending in a tubulus rectus and attached to the rete testis. × 2.5.

has led to the obscuring of certain of the junctions. In an attempt to straighten somewhat the second tubule from the left in this figure, this was torn from its attachment at the T-shaped division. This tear was disregarded in making the figure. The last tubule to the right does not show in the mounted preparation its termination in a tubulus rectus, which is folded under the tubule, yet was clearly evident after the completion of teasing. The other six tubules show clearly their termination in tubuli recti. This tubule complex was removed from the lower third of the testis and presents an actual length of 30.4 cm. We are convinced that the tubule complex shown in figure 5, does not show the extent of linking of tubules which may take place in the adult rabbit testis. In one preparation at least twelve tubules seemed joined together, although in this preparation certain of the tubules could be traced only for a short distance before a broken end was reached; others which could be completely teased terminated in tubuli recti. The difficulty met in separating the parts belonging to a single tubule complex, during the preliminary teasing of the larger masses of testis tissue, masses which are not sufficiently transparent to admit of transmitting even strong artificial light, leaves it largely to chance

as to whether one obtains a complete tubule complex for the final teasing. Three of the six testes prepared for teasing proved to be well macerated. Preparations made of each of these presented the same general features. One was over macerated, teasing very readily, but the tubules were too soft to admit of the manipulation necessary for complete isolation. One rabbit, as was noted after completion of the hydrochloric acid injection, presented a very small cryptorchid on the left side, with the right testis slightly larger than one-half the size of a normal testis of rabbit of similar weight. The left testis was disregarded as it was found to be infantile, with very small tightly coiled tubules. The right testis was not very successfully macerated. From it, however, there was isolated a tubule complex, which though not complete, in that it was not possible to trace all of the constituent tubules to their termination in the rete testis through tubuli recti, presents clearly an extended anastomosis, such as has not been seen in what we regard as normal testes. In two regions of this tubule complex, toward the periphery, tubules were joined together so as to form two folded rings, to different segments of which were attached tubules extending to the rete testis. This tubule complex will be considered more fully by Curtis in a later publication. The question arises as to whether this somewhat unique arrangement may be regarded as characteristic of the incompletely developed state of the gland or merely a chance finding. It is regretted that the maceration of this gland was such that a more extended observation was not permitted. Judging from the teased preparation the gland in question seemed functional. Concerning the life history of this rabbit we have no data. The observations of Bremer[3] on the human seminiferous tubule, based largely on reconstructions of embryonic material, will be considered in the light of our work in a later publication.

[3] John Lewis Bremer, Morphology of the tubules of the human testis and epididymis. Amer. Jour. Anat., vol. 11, 1910–1911.

As a result of our observations on the seminiferous tubules of the adult rabbit we feel warranted in presenting the following conclusions:

1. The seminiferous tubules of the adult rabbit present no blind ends, diverticuli, or nodular enlargements.

2. In their simpler form they are arranged in the form of an arch, the tubule beginning and ending in a tubulus rectus, each attached to the rete testis, both ends of the tubule having thus a functional connection with the rete.

3. The more extensive tubular complexes may be regarded as composed of a series of linked arches, joined through Y-shaped or T-shaped divisions of the tubules, the regions of the divisions showing no structural peculiarity, all the tubules ending in tubuli recti attached to the rete testis.

4. The extent of the linking of the tubules is difficult to determine. Observations show that from three to twelve tubules may thus be linked in one tubule complex.

5. The lobules evident in sections of the rabbit testis, or on macroscopic inspection, do not represent each a complete tubule, if a tubule be regarded as one beginning and ending in the rete testis, but represent a coil complex of a portion of a tubule as it passes from the mediastinum toward the periphery or from the periphery toward the mediastinum.