without reference to the protein or water content of the plasma. The rate of tubular excretion was determined by subtracting the filtration rate from the total rate of excretion (UV).

As has been shown with all substances excreted by tubular activity, the phenol red/inulin clearance ratio is depressed as the phenol red plasma concentration increases in the frog. These values range from 5 to 10 with a phenol red plasma concentration of about 1 percent, and fall below 1.0 when the concentration is higher than 40 mg. percent, thereby emphasizing the unavailability of the plasma protein-bound fraction for filtration. No attempt was made to obtain a maximal phenol red clearance because of the difficulties involved in obtaining accurate phenol red plasma determinations of less than 1.0 mg. percent. The maximal phenol red clearance probably lies above 185 ml. per kg. per hr. (the average inulin clearance in this experiment was 27 ml. per kg. per hr.). At phenol red plasma concentrations of 2.0 mg. percent the average phenol red clearance is 80 ml. per kg. per hr. and the average phenol red/inulin clearance ratio 4.2.

Calculations of the rate of tubular excretion show that a tubular secretory maximum is reached with a total plasma phenol red concentration of about 7.0 mg. percent. The maximum amount of phenol red which the frog renal tubules can transport from peritubular fluid to lumen is roughly 0.50 mg. per kg. body weight per hr. or 13 mg. per kg. per day.

Summary:

1. The process of tubular excretion is a large factor in the renal elimination of phenol red in the frog.
2. The rate of tubular excretion reaches an apparent maximum at a total phenol red plasma concentration of 7.0 mg. percent.
3. The maximum amount of phenol red which the frog renal tubules can excrete is approximately 0.50 mg. per kg. body weight per hr.

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THE MORPHOLOGY OF THE DOGFISH RENAL TUBULE

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In connection with a study of the process of acidification of the urine, and to lay a groundwork for other studies of a similar nature, an examination of the structure and configuration of the kidney tubule of *Squalus acanthias* has been undertaken. While this is still incom-

plete, it is indicated that in this animal the tubules do not follow the classical description of the elasmobranch tubule.

Both dorsal and ventral approaches to the living kidney were used. In each the animal was tied in a trough with its mouth immersed in cold circulating sea water. Complete immobilization was obtained by tying the animal firmly by the tail, with neither narcosis nor destruction of nerve tissue. With a ventral approach the abdominal wall was slit with scissors along the midline from the pelvic to the pectoral girdles, precautions to prevent bleeding being unnecessary. The heavy peritoneum overlying the kidney was carefully removed by dissection. This was thinner and more easily removed in the female. The advantage of this approach lay in the small amount of tissue destruction and the lack of difficulty with bleeding, but no glomeruli were visible on the ventral surface.

The dorsal approach was more difficult and time consuming, but revealed glomeruli with active circulation. The greatest problem was the production of complete haemostasis. The method finally evolved and which was very satisfactory was as follows. From the level of the anterior end of the base of the pelvic fins two longitudinal incisions were made through the skin to the level of the posterior end of the base of the anterior dorsal fin. One was barely to the side of the mid-dorsal line, and the other just ventral to the lateral line. These incisions, together with transverse connections at their ends, were made with an electrocautery. A large block of muscle was then removed with scissors, following longitudinally the fasciae which extend from the longitudinal skin incisions toward the vertebral column. Immediately after the hurried removal of the muscle, any damaged vessels were cauterized. The entire incision was then cleaned by blunt dissection, cauterizing any broken vessels, until the fasciae were free of clinging muscle fibers. If these were not completely removed, they continued to ooze blood. The bottom of the large cavity thus prepared was formed by a very heavy and dense fascia which immediately overlay the dorsal surface of the kidney. This layer was removed by blunt dissection, accompanied by any necessary cauterization. The kidney could be exposed and ready for observation in about 13 minutes. While the loss of blood seemed considerable, no deleterious effect on circulation could be observed. Animals thus prepared and kept under observation well over an hour behaved normally when returned to the ocean to test their vitality.

The number of glomeruli visible on the dorsal surface was not large, but there were usually between three and ten available for experimentation in the region exposed by this method. These usually lay near the middle of the kidney. Extending from each glomerulus in an antero-lateral direction there was always a clear streak which was so transparent that no structure could be observed by reflected light. The mesial half of the kidney was mainly occupied by wide tubules having a very shiny internal margin, so the lumen was sharply delineated. Mixed with these large tubules were finer ones of two kinds, each of whose outside diameter was about one half that of the wider ones. In one type the lumen was easily seen, as in the larger tubule, while the other possessed somewhat opaque walls and its

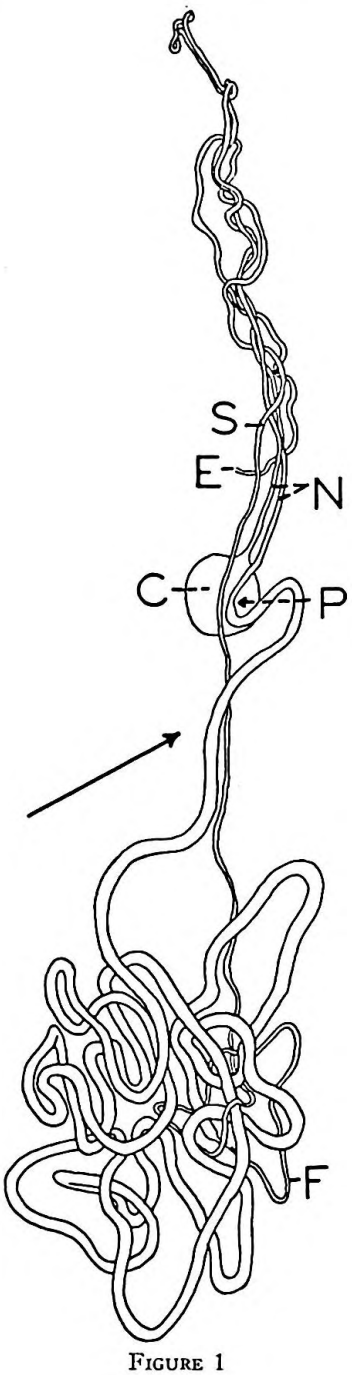

FIGURE 1

lumen was invisible. Modifications of direction of lighting did not change the invisibility of the internal border.

If, by means of a Richards' micromanipulator, diluted India ink was injected into the capsule, the course of the tubule could be followed in a general way (figure 1). It always ran from the capsule in a slightly winding antero-lateral direction through the clear streak mentioned above, immediately returning to the neighborhood of the glomerulus. Here there was usually, but not always, a turn and an increase of caliber which marked the beginning of the large proximal tubule. The latter was the region of large diameter and sharply visible lumen. If the injection was sufficiently prolonged, and if the tubule did not become plugged with ink, the injection passed through the finer tubules in the neighborhood of the loops of the proximal tubule. In what order it passed through the two types of finer tubules was not observed during the progress of any injection. On continued successful injection, the ink returned to the neighborhood of

FIG. 1. Diagram of a single tubule as seen from the dorsal surface of the left kidney. It is simplified by the omission of many convolutions of the large proximal tubule. The first double loop is a drawing of an actual tubule. The large arrow parallels the longitudinal axis of the body, pointing anteriorly. The capsule (C) gives rise to a double loop (N). This, in the neighborhood of the glomerulus, typically widens and makes a bend (P) to start the proximal segment. Mixed with the wide loops of the proximal are finer ones (F), some of which bear a brush border while others have a smooth surface and striated cytoplasm. The latter connect with a second set of convolutions (S) around the first double loop. These have striated cells. This portion dips ventrally (E) and joins the collecting duct.

the glomerulus and passed through a series of coils encircling the first double loop, returning still another time to a point near the glomerulus from which it dipped into the kidney and was no longer visible. The tubule was so very long and tortuous that such a complete injection could be made in only a small fraction of attempts.

A correlation between these observations on the progress of the injection and the histological picture of the different regions is fraught with great difficulty and the chance of misinterpretation. The presence of ink within all cut loops of a single tubule within the sections was of great aid. An idea of the complexity of the tubule was given by the fact that in one section of kidney a single injected tubule might appear over fifty times. The work is as yet incomplete but certain facts have been definitely established.

1. *The "first striated segment" ("special segment").* The conventional picture of the elasmobranch tubule (see Marshall '34) is one in which a ciliated neck leads from the capsule, and changes into a region of tubule characterized by striations (Haller's second segment). These striae are described as running from base to apex of the cuboidal cells (Bargmann '37, Borcea '05, Haller '02). These two segments of the tubule, according to Bargland and Borcea, together form a packed convolution, the loops of which lie in a spacious blood sinus. The region with striated cells leads into the proximal segment.

This conventional description does not apply to the tubules on the dorsal surface of the dogfish kidney, because no striated cells precede the proximal tubule. When India ink was injected into the capsule in such amounts as to fill only the portion of the tubule preceding the proximal segment, two different types of tubule were found to contain ink in the sections, but neither type had striated cells. The only major difference between the two regions of the tubule was that the cells nearer the capsule had long cilia, while the remainder were free of these structures (figure 2, A, B). A minor difference was that the region possessing the second type of cells was quite variable in diameter, sometimes having extremely flat cells and a very wide lumen. Such differences were described by Bargmann in the neck segment of several elasmobranchs.

In this richly vascular area there were striated cells meeting the description in the literature (figure 2, C). However, in sections following such abbreviated injections these were completely free of ink and lay beside the injected loops. If, on the other hand, the injection was complete and the ink was observed to return to this region after first passing through the proximal tubule, sections showed that the loops characterized by striated cells were fully injected.

2. *The proximal tubule.* There appeared to be two different sizes of proximal tubule, accepting as a criterion of this region the presence of a brush border on the free surface of the cells (Figure 2, D, F). While measurements of cross-sections indicated considerable variation in the diameter of the wider region, the average outside diameter of the finer proximal tubules was less than one-half that of the larger, and there was no overlapping of the two classes. The

brush border was interrupted by the presence of cilia much more frequently in the finer tubules than in the larger. Cilia were occasionally present in a cross-section of the wider tubules, but usually present

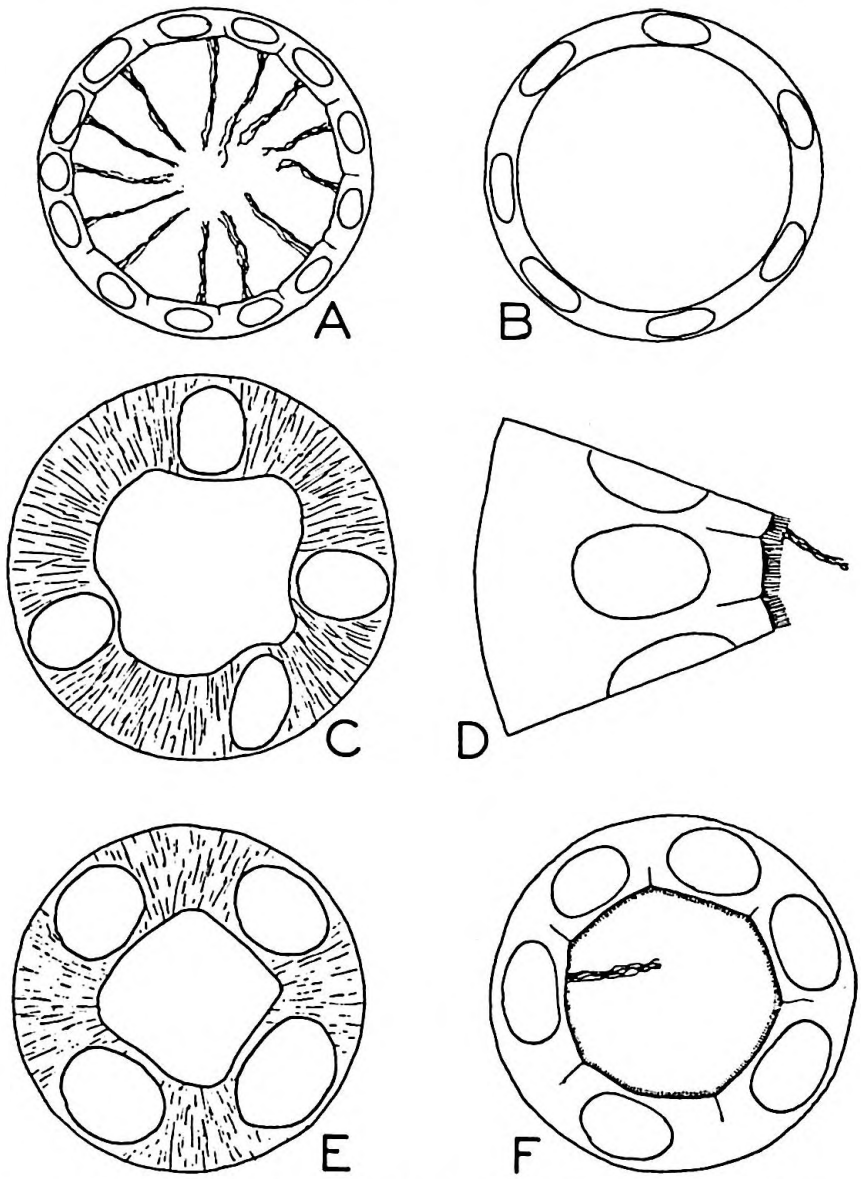

FIG. 2. Types of cells found in the tubule. While the figures are purely diagrammatic, the outside diameters, height of cells, width of lumen and width of brush border are based on averages of a large number of measurements of cross-sections with a Zeiss filter micrometer. Types A, B, and C are found anterolateral to the glomerulus, types D, E and F postero-mesial to the glomerulus. A. Neck, with ciliated cells. B. Cells which appear similar but are without cilia. C. Striated cells. D. Portion of cross-section of a wide proximal tubule showing the occasional cilia. E. Striated cells. F. Fine proximal tubule showing the frequent cilia.

in the finer. Since short injections filled only the wider proximals, and longer injections filled both types, the finer proximals must follow the wider. In several cases the origin of the proximal segment was followed through serial sections and the first part found to be definitely of the wider type.

3. *The postero-mesial striated segment.* The second type of fine tubule mixed with the large proximal loops appeared to have essentially the same structure as the striated loops antero-lateral to the glomerulus, but to be slightly smaller in outside diameter with somewhat higher cells, and appeared to have a somewhat smaller amount of cytoplasm. The striations in the cells were not so clear as in the more lateral loops. For example, it was necessary to turn to high power of magnification to distinguish between these striated cells and the cells of the fine proximal segment, while in those loops antero-lateral to the glomerulus the striations could be seen under low power. However, even though seen with more difficulty, the striations in these cells seem to be quite the same in their distribution as those of the more lateral parts of the kidney.

4. *The order within the tubule.* We have not been able, on the material available at present, to identify in sections the junction between the large and fine proximals, or the connections of the ends of the striated segments. Examination of serial sections of tubules injected with ink to a varying extent of their length, led to the feeling that the order in the tubule was as follows:

1. Capsule
2. Neck segment with ciliated cells (Figure 2, A)
3. Neck segment with non-ciliated cells and variable diameter (Figure 2, B)
4. Large proximal segment (Figure 2, D)
5. Fine proximal segment (Figure 2, F)
6. Striated segment, with slightly higher cells, located in a region postero-mesial to the glomerulus, mixed with loops of the proximal segments (Figure 2, E)
7. Striated segment, with slightly lower cells, located antero-lateral to the glomerulus, in close spacial relationship to the neck (Figure 2, C)
8. Collecting ducts.

If this interpretation of the order of the parts of the tubule be correct, several important points arise. First, it must be determined whether this order is found only in the tubules whose glomeruli are on the dorsal surface. Second, a wider examination must be made to ascertain whether this is a species difference. Third, it must be decided whether the non-ciliated region preceding the proximal segment should be called neck or special segment. Fourth, since the striated cells appear to be fundamentally the same as the distal tubule of other classes of vertebrates, should they be regarded merely as a distal segment and the concept of a special segment dropped as far as this species is concerned? If the striated cells precede the proximal segment, as in the classical description of elasmobranch kidneys, the tubule would indeed be different from anything found in the other

classes. But if they follow the proximal segment, and if they resemble the distal tubule cells of the other groups, there is little fundamental difference between this kidney tubule and that of the amphibia.

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THE SITE OF ACIDIFICATION OF URINE WITHIN THE RENAL TUBULE OF THE DOGFISH

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The site of acidification of the glomerular filtrate during its passage along the renal tubule has been determined previously in only two animals. In both *Necturus* and the frog the shift from alkaline to acid takes place in a short portion of the distal tubule (Montgomery and Pierce '37). It has now been found that the situation in the spiny dogfish (*Squalus acanthias*) is quite different, with the possibility of a different mechanism being involved.

The site of acidification in the dogfish was determined by the injection of phenol red into the tubules of the living kidney. Injections were made into 63 tubules in 31 animals, both male and female. The indicator was introduced through the capsule or directly into the different parts of the tubule. In some cases the phenol red was added to the filtrate previously present in the tubule; in other experiments the pre-existing fluid was pushed away by a preliminary injection of mineral oil. The injected indicator was prepared either as a neutral solution or was alkalinized. The alkaline solutions were selected to give four different grades of buffering action; enough $NaHCO_3$ was added to the phenol red solution to bring its pH to above 8.0; or the powdered dye was dissolved in a saturated solution of $NaHCO_3$; or the dye was dissolved in 1M Na_2HPO_4, or in a saturated solution of Na_2HPO_4.

The part of the tubule preceding the proximal segment is incapable of changing phenol red from its alkaline to its acid color. A yellow color is not produced even if the indicator solution is free of buffer. In the longest test made phenol red, somewhat alkalinized with $NaHCO_3$, failed to turn yellow in one half hour after its introduction into this part of the tubule by way of the capsule. Mixing with fluid from the proximal tubule was prevented by injecting oil in advance of the phenol red.

The proximal segment of the tubule originates in the neighborhood of the glomerulus; it is here that the shift of phenol red to its acid color first occurs. In 17 experiments this junction was under observation continuously, and in each the point of color change was sharp. The fluid in the segment preceding the proximal remained a bluish