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Innervation of Kidney of Toadfish.*

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While an intimate relation of nerve fibers to the epithelium of the renal tubules has been reported most strikingly by Smirnow,[1] the finer nervous mechanism of the kidney is still in doubt. The extrinsic nervous control of urinary secretion has been variously interpreted (Bieter,[2] Kuntz[3]), it being particularly difficult to separate results arising from vasomotor changes from strictly secretory phenomena. In the opinion of several investigators (for most recent summary of literature see Marshall and Grafflin[4]), at least the proximal convoluted segment of the renal tubule has important secretory functions. The aglomerular kidney of the toadfish *(Opsanus tau)* has afforded unusually favorable material for studying the secretory processes that may be carried on by the tubular epithelium since there are no glomeruli to complicate the process. The terminal portion consists only of a blind tubule with the histological characteristics of the proximal convoluted tubule of higher animals (Edwards[5]). Bieter[6] showed that in this animal the secretory pressure of urine as registered in the ureters can be greater than the blood pressure in the dorsal aorta. This phenomena together with the action of diuretics on the urinary output (Bieter[7]) and on the cytological structure of the tubular epithelium (Defrise[8]), and other related problems, naturally bring up the question of the nerve supply to these aglomerular tubules.

Dr. Bieter, having a supply of living toadfish for additional studies on the action of various drugs on kidney function, sug-

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1 Smirnow, A. E., von, *Anat. Anz.*, 1901, **19**, 347.

2 Bieter, R. N., *Am. J. Physiol.*, 1929, **91**, 436.

3 Kuntz, A., Autonomic Nervous System, 1929 (Lea and Febiger, Philadelphia), 271.

4 Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.*, 1932, **1**, 161.

5 Edwards, J. G., *Anat. Rec.*, 1929, **44**, 15.

6 Bieter, R. N., *Am. J. Physiol.*, 1931, **97**, 66.

7 Bieter, R. N., *J. Pharm. and Exp. Ther.*, 1931, **43**, 399.

8 Defrise, A., *Anat. Rec.*, 1932, **54**, 185.

gested this study and generously supplied an abundance of fresh material.

From a study of similar work on other animals, a variety of techniques were selected, including intra-vitam methylene blue in a buffer solution so that the pH could be changed as described by Hirt,[9] modified Bielschowsky stain (Oertel,[10] Rogers[11]), gold chloride, silver-pyridine (Ranson[12]), and various stains for connective tissue and muscle fibers.

The application of methylene blue and gold chloride to teased material was impractical because of the large amount of intertubular lymphoid tissue, so these methods were applied to small blocks of tissue which were afterward embedded in paraffin. Most of the methods gave very unreliable results due to the tendency for the argyrophil fibers of the reticular tissue to take the stain, in spite of the valuable suggestions of Beech and Davenport[13] with reference to the Bielschowsky methods. Some of these connective tissue fibers are very fine and closely resemble nerve fibers. The most specific staining of nerve fibers was obtained by Ranson's silver pyridine method.

The kidneys are in close contact with large spinal nerves and the sympathetic plexus. From the latter, numerous small nerves, composed of non-myelinated fibers, follow the blood vessels into the kidney. Numerous non-medullated nerve fibers also course in the wall of the ureters and terminate among the muscle fibers. Such fibers could be followed along the branches of the ureter as far as there is a muscle coat and considerable connective tissue, but not to the terminal portion where the wall is reduced to nothing but the epithelium and a basement membrane.

The nerves along the blood vessels and in the associated connective tissue are carried far into the kidney, the individual nerve fibers losing themselves among the muscle fibers of the vascular wall; but no nerves could be identified with certainty on or between the epithelial cells of adjacent urinary tubules where the reaction was very favorable for the staining of nerve fibers.

Hirt[9] comments on the difficulties encountered in bringing out the nerve terminals in kidney tubules of the frog and concluded that they were not nearly as numerous as one would expect from

[9] Hirt, A., *Z. f. Anat. u. Entwcklngsges.*, 1930, **91**, 580.

[10] Oertel, H., *J. Path. and Bact.*, 1929, **32**, 558.

[11] Rogers, W. M., *Anat. Rec.*, 1931, **49**, 82.

[12] Ranson, S. W., *Am. J. Anat.*, 1911, **12**, 69; *J. Comp. Neur.*, 1912, **22**, 159.

[13] Beech, R. H., and Davenport, H. A., *Stain Techn.*, 1933, **8**, 13.

the work of Smirnow.[1] While this failure to find nerve fibers in the kidney epithelium may be due to inadequate technique, the fact that the very finest nerve fibers to smooth muscle within the kidney (in the ureter and blood vessels) could be readily seen in Ranson's silver-pyridine material, while adjacent tubular epithelium showed none, strongly indicates a lack of strictly secretory fibers to the kidney of the toadfish. There are, however, abundant vasomotor nerve fibers and nerve fibers to the musculature of the ureter and its branches.

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Staining of the Adrenals with Neutral Silver Nitrate as a Test for Scurvy.

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Moore and Ray[1] and Miller, Siehrs, and Brazda[2] observed that, whereas immersion of the cut surface of the adrenal gland from a normal guinea pig in neutral silver nitrate solution resulted in a blackening of the surface of the gland, there was no reduction by adrenal glands taken from scorbutic guinea pigs. Siehrs and Miller[3] showed that staining ability of the adrenals disappeared after the animal had been on a scorbutic diet for 6 days, but reappeared even after 18 days on a scorbutic diet if the animal were then fed 3 cc. of orange juice per day until the twenty-second day. They suggest that "the reduction test is a convenient qualitative method for testing for scurvy and for antiscorbutic substances."

The adrenals of 14 guinea pigs dying of scurvy were tested by immersion in neutral 0.4% silver nitrate solution for 30 minutes. None showed any staining. One animal fed on a scorbutic diet for 37 days was then given 1.5 cc. lemon juice daily for 4 days and was killed with ether. Its adrenals showed no staining, even after 30 minutes in silver nitrate.

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[1] Moore, T., and Ray, S. N., *Nature*, 1932, **130**, 997.

[2] Miller, C. O., Siehrs, A. E., and Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 636.

[3] Siehrs, A. E., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 696.