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.

6929

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

R. H. HAMILTON, JR. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota Medical School.

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.

---

[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.

Four guinea pigs that had been fed on the basal scorbutic diet plus 1.5 cc. of lemon juice daily were killed respectively on the 50th, 56th, 56th, and 57th days of the diet, and a piece of sliced adrenal from each was immersed in the silver nitrate solution for 30 minutes. At the end of this time there was in every case either no change in the color of the gland, or at most only a faint graying. In one case one whole gland and half of the other were ground with sand for a moment, water was mixed in, and the solid particles were centrifuged out. After the addition of silver nitrate solution to the extract there was no change in the color of the mixture in 30 minutes.

Under identical treatment with neutral 0.4% silver nitrate, the cut adrenal of a guinea pig that had been fed on oats, carrots, and alfalfa hay stained quite black within 15 minutes. A water extract of the other gland began to turn dark within 4 minutes after the addition of silver nitrate.

The 4 animals on the basal diet plus lemon juice were examined carefully for signs of scurvy. Their growth curves were normal. They showed no clinical signs of scurvy. Upon autopsy no hemorrhages were found in the muscles or in the wrist joints. The costochondral junctions were clean and sharp. None of the teeth were loose. No microscopic examinations were made.

Apparently it is necessary for the animal to have in its body an excess of vitamin C over and above its immediate requirements before any can be stored in the adrenal glands. If this hypothesis is true, one could not rely upon lack of staining of the adrenals with neutral silver nitrate as a qualitative test for the presence of scurvy sufficiently severe to produce gross signs. However, it might still be possible to use the reaction for assaying foods quantitatively for vitamin C. Animals could be maintained before the assay at such a level that the adrenals just failed to react. Upon feeding graded amounts of the test food to a series of animals, a positive reaction might be expected to appear within a few days in those receiving more vitamin C than is required for maintenance.