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Disappearance of Vitamin C from Adrenals of Guinea Pigs Having Scurvy.

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Szent-Gyorgyi[1] reported that the cortex of sliced beef adrenals turn dark when placed in a neutral 0.4% solution of silver nitrate and that the reducing substance is an hexuronic acid. King and Waugh[2] reported that the crystalline vitamin C which they isolated from lemon juice is identical with the hexuronic acid from adrenals obtained by Szent-Gyorgyi. We reported[3] that the cortex and medulla of sliced adrenals of normal guinea pigs turn dark in a neutral 0.4% solution of silver nitrate, but that the cortex from guinea pigs having scurvy does not darken under similar conditions. This confirms the conclusions of King and Waugh. We had observed the action of the adrenals in 6 normal guinea pigs and 6 with scurvy. We have increased this number to 42. We have investigated also the time required for the disappearance of vitamin C from the adrenals of guinea pigs on a scorbutic diet and the time for its reappearance in the adrenals when orange juice is added to the scorbutic diet. Recently Moore and Ray[4] have also reported that adrenals from guinea pigs having scurvy do not reduce neutral 0.4% silver nitrate, which confirms our first observation.

Twenty-five guinea pigs, weighing 150-180 gm., were fed oats and carrots for 8 days. On the eighth day, 2 were killed and the reducing power of their adrenals was noted. The remaining animals were fed the scorbutic diet of Sherman, LaMer, and Campbell.[5] Ten animals were killed at various intervals from 2 to 18 days of the diet period. The adrenals were removed, one from each animal was sliced and placed in a neutral 0.4% silver nitrate solution for 15 minutes. The pieces were exposed to the light from a 115 watt Mazda bulb at a distance of 8 inches. Both the cortex and the medulla reduced the silver nitrate; however, the medulla reduces more

[1] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] King, C. G., and Waugh, W. A., *Science*, 1932, **75**, 357.

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

[4] Moore, T., and Ray, S. N., *Nature*, Dec. 31, 1932, 997.

[5] Sherman, H. C., LaMer, H. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.

slowly than the cortex. The cut surfaces of the adrenals of normal guinea pigs turned very dark brown, almost black. On the second day of the diet period, there was a noticeable decrease in the intensity of reduction. The intensity of reduction decreased progressively until on the sixth day it was scarcely noticeable. The color of the cut surface changes progressively from a very dark brown, almost black, to a reddish orange on the fifth day. We do not know whether this change in appearance is due to the action of a very small amount of vitamin C or whether it is due to the precipitation of the proteins by silver nitrate. After the sixth day, if any reduction takes place, the extent is so small that it is scarcely evident. After the sixth day, it was noticed that the capsule of the adrenals showed a more marked change in appearance than did the cut surface.

The animals continued to gain weight until the eleventh day. Autopsies showed no hemorrhagic areas about the joints and sternum, with one exception. Beginning on the eighteenth day, each animal was given 3 cc. of orange juice daily by dropper. On the twenty-second day, one animal was killed and its adrenals examined for reducing power. The sliced adrenal turned black in the silver nitrate solution. The intensity of reduction after orange juice feeding was greater than the reduction in the case of the guinea pigs when fed a normal diet.

A comparative study was made of the intensity of reduction in the adrenals with the age of the animals. Five adult guinea pigs, weighing 380-420 gm., were fed a diet of oats and carrots for 2 weeks. They were killed and their adrenals removed; one from each was sliced and placed in the silver nitrate solution according to the usual method. The cortex, medulla and capsule turned black. Animals weighing 150-190 gm., under similar conditions, give a very dark brown but not a definite black.

Photographs were taken for a permanent record.

It has generally been considered that the guinea pig can store only a small amount of vitamin C, since it develops scurvy. We conclude from our study that the amount of vitamin C stored is even smaller than is generally thought and that the supply is rapidly lost either by excretion or by metabolism. The supply is lost more rapidly than is indicated by the development of gross symptoms of scurvy since the decrease is noticeable on the third day on a scorbutic diet and since by the sixth day it has either fallen to a low constant level or is entirely absent. Since considerable time elapses between the disappearance of vitamin C from the adrenal and the

development of the gross symptoms, it seems to us that the gross symptoms of scurvy are secondary changes arising as a result of the altered metabolism of the vitamin C deficient animal. The reduction test is a convenient qualitative method for testing for scurvy and for anti-scorbutic substances. We have found that vitamin C is rapidly returned to the adrenals when orange juice is fed to animals with scurvy. This indicates that the cortex of the adrenals has not changed in scurvy enough to interfere with storage. The gross symptoms disappear as a result of the restoration of normal metabolism incident to vitamin C replacement.

Quantitative studies are in progress for determining the amount of vitamin C in the adrenals under varying conditions.

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Spontaneous Duodenal Ulcers in Dogs with Chronic Mild Icterus and Hepatitis.

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We reported a method[1] for the plastic reconstruction of the common bile duct in which a viable tube was made from a flap of the pyloric mucosa. The tube was connected to either the gall bladder or the common duct, the gastric orifice of the tube being constructed to prevent regurgitation.

Seven dogs were kept to observe chronic effects. Of the 7, five (Dogs 1, 5, 7, 8, 9) have developed duodenal or gastric ulcers after 3 months. After 3 months all dogs showed an elevated icteric index, even though bile could be detected easily in the feces or gastric contents. On autopsy all dogs except one, which is still alive at 21 months, had varying degrees of biliary cirrhosis and hepatitis. Four of the 7 (Dogs 3, 4, 5, 9) developed ascites.

Very brief protocols of the 7 dogs will be given. Dog 1 died at 9 months from a perforated gastric ulcer and 2 duodenal ulcers. Dog 7 died at 5½ months with a duodenal ulcer. Dog 8 died at 3 months from a perforated duodenal ulcer. Dog 4 developed ascites (5 liters) and died without an ulcer at 7 months. At this

[1] Schrager, Ivy and Morgan, *Surg. Gynec. and Obstet.*, 1932, **54**, 613.