

These methods have been found applicable to the preparation of the specific polysaccharides of pneumococcus types I, II, III, V, VII and VIII.

8812 P

Fixation of Ascorbic Acid by Tissues.

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During the last few years considerable data have been accumulated to show that scorbutic animals suffer a marked depletion of the ascorbic acid content of the tissues particularly in organs such as the adrenal, pituitary, intestine, and liver. This depletion is readily made up when vitamin C rich foods are fed to the animals. The importance of the presence of ascorbic acid has been subsequently linked up with its function as an oxygen carrier, particularly through the studies of Harrison¹ and Euler and Klussmann.² They showed that tissue slices from scorbutic animals have a low oxygen uptake which is restored by the addition of ascorbic acid. Söderström and Törnblom³ further show that in scorbutic animals the oxygen consumption is diminished.

The ability of tissues to fix ascorbic acid was suggested by the report of Harris, Ray and Ward.⁴ They believed from their study of the urinary output of vitamin C that the amount excreted might serve as a gauge of the nutritional state of the individual. This suggests that the tissues must be saturated with ascorbic acid before it will overflow through the kidney. This conception is supported by further observations of Harris and Ray,⁵ Johnson and Zilva,⁶ Ippen,⁷ Euler and Malmberg,⁸ Hou⁹ and others on the urinary

¹ Harrison, D. C., *Biochem. J.*, 1933, **27**, 1501.

² Euler, H. v., and Klussmann, E., *Arkiv. Kemi, Mineral. Geol.*, B 11, No. 4, 1932-33.

³ Söderström, N., and Törnblom, N., *Skand. Arch. Physiol.*, 1933, **66**, 67.

⁴ Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

⁵ Harris, L. J., and Ray, S. N., *Lancet*, 1935, **228**, 71.

⁶ Johnson, S. W., and Zilva, S. S., *Biochem. J.*, 1934, **28**, 1393.

⁷ Ippen, F., *Schweiz. Med. Wochenschr.*, 1935, **65**, 431.

⁸ Euler, H. v. and Malmberg, M., *Svensk. Kem. Tidsskr.*, 1935, **47**, 25.

⁹ Hou, H. C., unpublished data.

excretion of ascorbic acid in man and animals, in various nutritional states and following the administration of ascorbic acid orally or intravenously. Zilva¹⁰ found a marked increase in the ascorbic acid content of the intestine of scorbutic animals after the injection of ascorbic acid. He¹¹ further showed that the antiscorbutic activity of the ascorbic acid and its related compounds appeared to be "connected with their capacity of being retained by the tissues of the animal organism." By means of histological staining with AgNO₃, Giroud and coworkers¹² were able to trace the deposition of ascorbic acid in the intestine, the blood, the liver and kidney, after orange juice was given to scorbutic animals. De Caro¹³ studied the ascorbic acid content of the organs of normal and scorbutic guinea pigs at different periods following the intravenous injection of ascorbic acid and found a transient rise in normal animals. The concentration in the kidney and muscles of the scorbutic animals reached normal or even above normal in 15 minutes; other organs showed an increase although they did not reach a normal value. He further studied the effect of ascorbic acid solutions in contact with tissue emulsions and concluded that the behavior of the normal and scorbutic tissue emulsions was essentially the same. The slight difference obtained was considered to be due to loss in filtration and to the amount of ascorbic acid present in the tissue. De Caro thus believed that in scorbutic animals the ability of the tissue to fix ascorbic acid was decreased.

We have recently investigated this problem in a slightly different manner and obtained results which indicate an increased capacity of the scorbutic tissues to remove ascorbic acid from solution.

Two groups of comparable guinea pigs were used. One group was kept on a vitamin C free diet (Hou¹⁴) and the other on a diet supplemented with plenty of cabbage. After 22 days the animals were killed by decapitation and bleeding. Tissues were removed at once, weighed, cut into slices, teased, put into Ringer's solution containing known (titrated) amounts of ascorbic acid and thoroughly mixed. The solutions were overlaid with liquid paraffin to prevent oxidation of the ascorbic acid by air and incubated at 37°. At the end of one hour and 5 hours, samples of one cubic centimeter of the solu-

¹⁰ Zilva, S. S., *Biochem. J.*, 1935, **29**, 100.

¹¹ Zilva, S. S., *Biochem. J.*, 1935, **29**, 1612.

¹² Giroud, A., Leblond, C. P., and Galelovitch, S., *C. R. Assoc. Anat. Bruxelles*, 1934, Mar., p. 4.

¹³ De Caro, L., *H. S. Zeits. Physiol. Chem.*, 1934, **223**, 229.

¹⁴ Hou, H. C., *Chinese J. Physiol.*, 1935, **9**, 253.

tion were removed for titration with 2,6-dichloro-indophenol. The results obtained were compared with that of a control solution containing no tissue but similarly treated. It was found that the scorbutic tissues were able to take up more ascorbic acid from Ringer solution than the normal tissues. Among the tissues examined, adrenal and muscle took up most, next the skin, then the intestine and kidney. It is interesting to note that the adrenal took up to its full capacity during the first hour of incubation since nothing more was taken up during the next 4 hours of incubation. On the other hand, the intestine continued to take up more during the following four hours than during the first hour. The other tissues took up comparatively little during the second period.

8813 C

Effect of Small Doses of Oestrin upon Ovarian and Vaginal Cycles of Mature Rats.

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Much work has been done to demonstrate the effect of large doses of oestrin upon the ovaries, but scarcely any to show the effects of threshold or subthreshold doses. Bialek-Laprida,¹ injecting rats with 2-25 r.u. (Coward-Burn units) daily for 30 days, showed that the oestrous cycle was interrupted and also that the ovaries contained many atretic follicles at the end of this period. Thirty days after the injections were stopped the ovaries were slightly degenerate, there were few corpora lutea, many atretic follicles and the average weight of the ovaries was decreased. However there were also a few mature follicles present. Sixty days after the injections had ceased the ovaries were normal.

Selye, Collip and Thomson² treated 6 adult rats with 20 gamma of oestrin (200 int.u.) daily and obtained almost continuous vaginal oestrus. At the end of 20 days, however, the ovaries were noticeably atrophic, the average weight being 25 mg. In only one case did they contain recent corpora lutea.

¹ Bialek-Laprida, Z., *Compt. Rend. Soc. Biol.*, 1934, **117**, 456.

² Selye, H., Collip, J. B., and Thomson, D. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1377.