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Some Unexpected Results in an Attempt to Produce Experimental Scurvy.

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Hay has been considered to contain little if any vitamin C (ascorbic acid) since Holst and Frölich[1] reported the production of experimental scurvy in guinea pigs using this substance as an aliment. Others, notably Hess and co-workers[2] have reported success in producing experimental scurvy in guinea pigs with a diet of hay, oats and water. Animals maintained on the latter diet, by the author, did not show any signs of scurvy, even after 3 months.

The major difference between the diet used by the author and that of the workers quoted above was in the variety of hay used. We fed the South Park variety of Rocky Mountain meadow hay, the main constituents of which are grass-like rushes and sedges. The predominant species are *Juncus columbianus, Carex arapahoensis* and *Carex siccata.* This type of hay has long been recognized by stock feeders as of superior nutritional quality because its protein and mineral ash content are higher than those of most other varieties of hay.

A series of experiments was designed to determine which constituent of the diet used contained the antiscorbutic agent. The first experiment was to substitute another grain for the oats. Rolled barley was chosen since it had been used in several scorbutic diets.[3,4] However, the guinea pigs maintained a steady growth on the diet of hay, rolled barley and water and showed no signs of scurvy. Since the change of grains (oats to barley) did not seem to make any appreciable difference, the hay was next studied. It was thought that heating the hay as much as 7 hours at 105°C. might oxidize any ascorbic acid that was present. The hay was placed in an asbestos oven, heated by an underlying gas burner. The oven has air holes at different levels, which allows a change of air during heating. Animals fed the *heated* hay, oats and water showed a rapid rise in weight throughout the experiment. They did not show any evidence of scurvy after one month of this regime. Evidently, even

1 Holst, A., and Frölich, T., *J. Hyg.*, 1907, **7**, 634.

2 Hess, A. F., *Scurvy Past and Present*, J. B. Lippincott Co., Phila., 1920.

3 Harde, E., *C. R. Soc. de Biol.*, 1934, **116**, 153.

4 Mouriquand, G., Weill, L., et Simon, F., *C. R. Soc. de Biol.*, 1934, **116**, 543.

with this amount of heating, the ascorbic acid was not destroyed completely. It was then thought, perhaps moisture was necessary to catalyze the oxidation of the vitamin C. To test this, hay was heated in the usual manner but a large pan of water was interposed between the hay and the source of heat. After 7 hours of heating at 110°C. all the water had not evaporated so that constant moisture had been present. On the diet of *moist heated* hay, oats and water, the animals did not gain in weight quite as rapidly as on the previous diet, but they showed no signs of scurvy after 7 weeks.

Further evidence that ascorbic acid is present in the hay used was determined in the following manner. Animals were placed on the basal ration suggested by Pelkan,[5] which has the advantage of being adequate in all nutritional factors with the exception of vitamin C. On this diet the guinea pigs showed signs of scurvy after the second week, and these signs were quite marked at the end of the third week. Another group of animals was put on the Pelkan diet plus *moist heated* hay, heated for 7 hours at 110°C. While both the last mentioned groups were started at the same time, the animals on the Pelkan diet plus moist heated hay continued to gain weight constantly and rapidly, showing no signs of scurvy; whereas those on the Pelkan diet alone lost weight rapidly after the onset of scorbutic symptoms.

Other suggestive evidence that ascorbic acid is present in the hay tested, is that a distilled water extract of the hay gives a strongly positive reaction with the reagent of Bezssonoff.[6] While it is true that the reagent of Bezssonoff is not specific for ascorbic acid, merely for di-enolic reducers, it is generally assumed that the only di-enolic reducer commonly found in plants and foodstuffs is ascorbic acid.

On the basis of the foregoing biological and chemical tests it seems probable that rather large amounts of ascorbic acid are present in the hay tested. The ascorbic acid present has not only withstood the oxidation inherent in the "curing" process of the hay, but also during the prolonged heating at 110°C. Yet this ascorbic acid may readily be extracted by distilled water.

These findings are diametrically opposed to those of Holst and Frölich, and Hess and co-workers. It appeared probable, therefore, that there might be a difference in the vitamin C content in different varieties of hay. To test this hypothesis 4 other varieties

[5] Pelkan, K. F., *Am. J. Dis. Child.*, 1925, **30**, 174.

[6] Bezssonoff, N., Delire, A., et Van Wien, H., *Bull. Soc. Chim. Biol.*, 1934, **16**, 1133.

of hay were tried. These were the Middle Park, North Park and Gunnison types of hay and alfalfa. It was possible to maintain guinea pigs in a good state of nutrition with any one of these varieties of hay without any signs or symptoms of scurvy.

It appears likely that some varieties of hay contain considerable amounts of vitamin C. Since some workers have used hay for roughage, with their basal diets, in the belief that hay contained little if any ascorbic acid, it was thought these results should be reported.

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Effect of Ascorbic Acid on Resistance of Suprarenalectomized Rats to Histamine.

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The removal of the suprarenal glands in rats is followed by a drop in resistance to certain poisons, to toxins and to bacterial and protozoan infections.* This drop is due essentially to the removal of the cortex, since the natural resistance may be raised almost to the normal by repeated injections of cortin.[2] With the discovery of the presence in large amounts of ascorbic acid in the cortex[3, 4] and its subsequent availability in synthetic crystalline form, an effort was made to determine the rôle of this factor in the resistance of suprarenalectomized animals. Szent-Györgyi had mentioned some years ago that it could not prolong the life of suprarenalectomized animals, nor did it influence the course of Addison's disease (except in modification of pigmentation).

Seventeen suprarenalectomized adult albino rats (3 months of age) were injected daily intraperitoneally with 10 mg. of ascorbic acid dissolved in physiological salt solution and brought to a pH of 7.5 immediately before injection. The injections were continued during a period of 8 days. On the 8th day, the 17 rats together with 14 untreated suprarenalectomized rats, all received

* For a survey of this subject, see the review by Perla and Marmorston.[1]

1 Perla, D., and Marmorston, J., *Arch. Path.*, 1933, **16**, 379.

2 Perla, D., and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 650.

3 Szent-Györgyi, A., *Biochem. J.*, 1928, **22**, 1387.

4 Svirbely, J. L., and Szent-Györgyi, A., *Biochem. J.*, 1932, **26**, 865.