

CXXII. VITAMIN P

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IN a series of communications Szent-Györgyi and his colleagues produced evidence which led them to postulate the existence of a substance, not synthesized by animals subject to scurvy, which regulates vascular permeability. They proposed the name of "vitamin P" for this principle. In the first investigation [Armentano *et al.* 1936; St Rusznyák & Szent-Györgyi, 1936] the evidence was based on clinical experiments. It was found that whilst pure ascorbic acid was not effective in modifying certain pathological conditions characterized by an increased permeability of the walls of the capillaries, extracts of Hungarian red pepper or lemon juice acted beneficially. On fractionating lemon juice an active, practically pure crystalline flavone or flavonol glucoside "citrin" was obtained which was responsible for the therapeutic effect. Satisfactory results were obtained with it in 3 cases of vascular purpura, 4 cases of thrombopoenic purpura, 7 cases of infectious diseases, 1 case of myxoedema and 2 cases of diabetes with markedly lowered capillary resistance.

Then followed a contribution by Bentsáth *et al.* [1936] in which they claimed that the influence of "citrin" on the vascular permeability could be demonstrated experimentally. Guinea-pigs ranging in weight from 280 to 485 g. receiving the Sherman-La Mer-Campbell scorbutic diet could be kept alive on the average for 44 days on a daily dose of 1 mg. of "citrin". This survival period was definitely longer than that of the negative control animals, which averaged 28.5 days. Moreover, at autopsy the intensity of the haemorrhages in the animals which received vitamin P was claimed to be markedly less than in those of the negative control group.

Further chemical investigation revealed [Bruckner & Szent-Györgyi, 1936] that "citrin" was not a pure substance but consisted of a mixture of crystals of hesperidin and an eriodictyol glucoside with the former predominating. It was found in addition [Bentsáth *et al.* 1937] that hesperidin or an impure sample of demethylated hesperidin, i.e. the mother-liquor of "citrin", possessed vitamin P activity in guinea-pigs whilst the chemically related quercitrin did not.

The general conclusions drawn by Szent-Györgyi and his colleagues from these experiments are that the syndrome of experimental scurvy is due to a combined deficiency of vitamins C and P. Pure vitamin C deficiency can be observed only when vitamin P is administered to guinea-pigs on a scorbutic diet. Pure vitamin P deficiency cannot, on the other hand, be demonstrated in guinea-pigs.

In this communication are described tests on guinea-pigs which were designed as a repetition of the crucial animal experiments mentioned above.

Experiment 1

"Citrin", for which I am indebted to Drs S. Smith and G. E. Foster of the Wellcome Chemical Works, was prepared according to the method described by Armentano *et al.* [1936]. 0.6-0.7 g. of crystalline material, m.p. 240-250°, was

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obtained from 40 l. of lemon juice (South African lemons) as the first crop. It gave on analysis the following figures: Ash, 1.03; MeO, 5.16; C, 54.82; H, 5.98% (figures corrected for ash). This crude preparation was used for the biological tests. On purifying this product by dissolving it in hot glacial acetic acid, adding an equal volume of water and allowing to crystallize, fine almost colourless needles were obtained, M.P. 265° (decomp.). (Found: C, 54.9; H, 5.95; MeO, 5.72%. Hesperidin $C_{50}H_{60}O_{27}$ requires C, 54.95; H, 5.55; MeO, 5.68%.) This suggested that the first crop of crystals ("citrin") consisted most probably of impure hesperidin.

In this and in the following tests guinea-pigs weighing about 300 g. were utilized. Their basal diet, usually employed in this laboratory, consisted of bran 6 parts, barley meal 2 parts, middlings 3 parts, fish meal 1 part, crushed oats 4 parts by volume. In addition 60 ml. of autoclaved milk made up from a dried full-cream milk powder were offered.

Szent-Györgyi and his collaborators do not describe the method of administration of the dose. "Citrin" is practically insoluble in water. It dissolves in hot glacial acetic acid but on dilution with water it separates out. It is also soluble in alkali, in which solution it tends, however, to undergo oxidation. The "citrin" was, therefore, suspended in distilled water before the daily oral administration (dose 1 mg. of "citrin" in 2 ml. of water). Fig. 1 gives a graphic representation of the experiment. In weight, onset of scorbutic signs, and in the picture at the post-mortem examination, this group of guinea-pigs did not deviate from negative control animals and no diminution in the occurrence and in the intensity of the haemorrhages was observed.

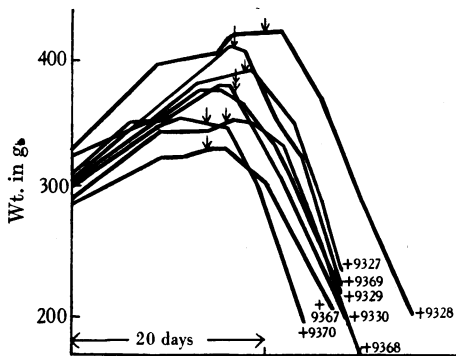


Fig. 1.

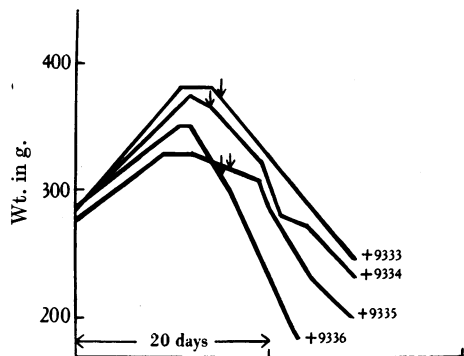


Fig. 2.

Fig. 1. Daily dose "citrin" 1 mg.

c. = killed by chloroform. + = died.
 ↓ = onset of clinical symptoms of scurvy.

Fig. 2. Daily dose $\left\{ \begin{array}{l} \text{hesperidin } \frac{2}{3} \text{ mg.} \\ \text{eriodictyol } \frac{1}{3} \text{ mg.} \end{array} \right.$

Symbols have the same significance as in Fig. 1.

Experiment 2

This experiment was performed after the appearance of Bruckner & Szent-Györgyi's note [1936] in which they asserted that "citrin" was a mixture of hesperidin and an eriodictyol glucoside. A mixture of hesperidin and eriodictyol was therefore tested for its vitamin P activity, the daily dose being constituted of $\frac{2}{3}$ mg. of hesperidin and $\frac{1}{3}$ mg. of eriodictyol. The hesperidin used in this

experiment was a commercial preparation m.p. 225–230° (C, 54.6; H, 5.7; MeO, 5.0 %).¹ For the eriodictyol, m.p. 265–270° (decomp.) I am indebted to Dr T. A. Henry of the Wellcome Chemical Research Laboratories. (Found: C, 62.2; H, 4.4 %.) Power & Tutin [1907] give the m.p. as 267° and the formula $C_{12}H_{12}O_6$, which requires C, 62.5; H, 4.2 %, for the material they prepared. The compounds being almost insoluble in water, the dose was suspended in 2 ml. of distilled water before administration. The behaviour of the test guinea-pigs was similar to that of negative control animals (Fig. 2) and the picture at autopsy was that of typical scurvy with an abundance of fresh haemorrhages especially in the femoral and gastrocnemius muscles.

Experiment 3

In view of the final note of Bentsáth *et al.* [1937], in which it is stated that pure hesperidin can function as vitamin P, a sample of the pure compound was tested for this potency. The commercial sample used in the preceding experiment was purified by solution in alkali and reprecipitation by acid, followed by crystallization from acetic acid. The purified product, needles, m.p. 260° after softening a few degrees lower, gave C, 54.5; H, 5.9; MeO, 5.3 %; ash nil. Hesperidin, $C_{50}H_{60}O_{27}$, requires C, 54.95; H, 5.55; MeO, 5.68 %. 1 mg. of this compound suspended in 2 ml. of distilled water was administered to the guinea-pigs. The

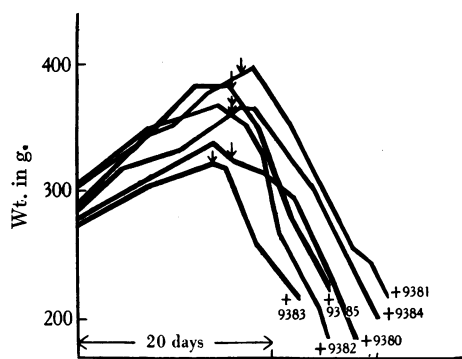


Fig. 3. Daily dose 1 mg. hesperidin.

Symbols have the same significance as in Fig. 1.

experimental animals behaved as if they subsisted on the basal diet only (Fig. 3). At the post-mortem examination the usual picture was obtained with extensive and numerous fresh haemorrhages especially in the femoral and gastrocnemius muscles.

Experiment 4

The pathological condition of the guinea-pigs receiving 1 mg. of "citrin" per day, i.e. animals suffering from a pure vitamin C deficiency, described by Bentsáth *et al.* [1936] brings readily to mind its resemblance to the condition which these animals display when existing for some weeks on a dose of vitamin C insufficient to protect them from scurvy but high enough to delay the fatal termination of the disease. In such cases hardly any fresh haemorrhages can be observed at the post-mortem examination. Instead there are foci of discoloration

¹ Analyses kindly carried out by Mr A. Bennett and Mr H. C. Clarke of the Wellcome Chemical Works.

at the knee joints and at the costochondral junctions—evidence of absorbed haemorrhages. This picture is no doubt quite familiar to many workers experienced in examining guinea-pigs which had received graded doses of vitamin C such as are used in testing an unknown substance for antiscorbutic potency. To demonstrate this point an experiment was performed in which, besides the basal diet, one group of animals received a daily dose of 0.1 mg. and the other 0.2 mg. of ascorbic acid.¹ Of the former group all the animals began declining in weight at the usual time and, with the exception of one, died of scurvy within 40 days as against about 30 days for negative control animals. Of the latter group only two died after about 40 days; the remaining three animals were killed by chloroform after 43 days. Only in one animal (9257, Fig. 4) out of the eleven were marked

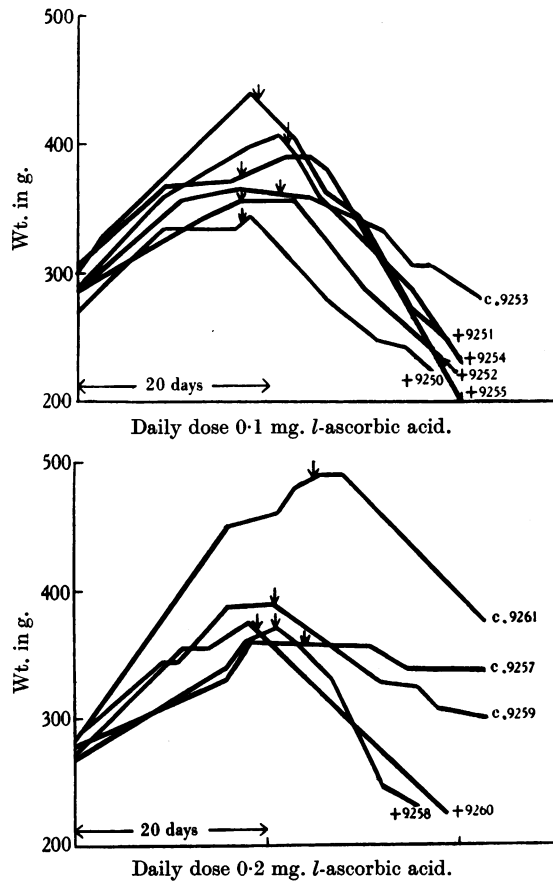


Fig. 4.

Symbols have the same significance as in Fig. 1.

haemorrhages observed. In three (9251, 9254, 9259, Fig. 4) just a few petechiae were found, but the remaining 7 animals showed no new haemorrhages at all, and all animals were found to possess the discolorations mentioned above. On the

¹ Kindly supplied by Messrs Hoffmann-La Roche.

other hand, of all the guinea-pigs which received the "citrin", the mixture of hesperidin and eriodictyol or the hesperidin, 18 animals in all, 15 showed very marked fresh haemorrhages at autopsy. In two only (9380, 9382, Fig. 3) were fresh haemorrhages found in the bladder and at the costochondral junctions and not as is usual in the femoral and gastrocnemius muscles, and in one (9328, Fig. 1) only a few petechiae at the knee joints besides haemorrhages at the costochondral junctions. The post-mortem picture of these animals was, as already mentioned, in fact quite characteristic of that found in negative control animals. On the other hand the post-mortem picture of the guinea-pigs which received 0.1 and 0.2 mg. of ascorbic acid was, as far as one could judge from their description, similar to that found by Bentsáth *et al.* [1936] in the case of the animals which received a daily dose of 1 mg. of "citrin". A very crude preparation of "citrin" might conceivably be contaminated with traces of ascorbic acid so as to bring about in guinea-pigs the pathological condition described by them, but the behaviour of their guinea-pigs on pure hesperidin cannot be thus explained. The discrepancy between the results obtained by Szent-Györgyi and his colleagues and the results of the present experiments will no doubt be elucidated by further work.

SUMMARY

The administration of a daily dose of 1 mg. of "citrin", of a mixture of $\frac{2}{3}$ mg. hesperidin and $\frac{1}{3}$ mg. of eriodictyol, or of 1 mg. of purified hesperidin did not delay the onset of scurvy or the fatal termination of the disease in guinea-pigs on a scorbutic diet. The post-mortem examination of these animals revealed typical scurvy characterized by extensive fresh haemorrhages.

The administration of a daily dose of 0.1 or 0.2 mg. of ascorbic acid, doses lower than the minimum prophylactic dose, to guinea-pigs on a scorbutic diet produced a pathological condition resembling that obtained by Szent-Györgyi and his colleagues by the administration of a daily dose of 1 mg. of "citrin" or of hesperidin to these animals when maintained on a scorbutic diet.

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