

## CLXXII. VITAMIN P

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SZENT-GYÖRGYI and his co-workers [Armentano *et al.* 1936] reported the presence in extracts of Hungarian red-pepper ("vitapric") and, later, in lemon juice of a substance other than ascorbic acid which could control the number of haemorrhages occurring in the course of certain clinical conditions. The clinical material upon which this original observation was made included three cases of vascular purpura, four cases of thrombocytopenic purpura, seven cases of infectious diseases, one case of myxoedema, and two cases of diabetes mellitus. In these cases the capillary fragility (resistance) was determined by the method of Borbély [1930] and the capillary permeability by an adaptation of the method of Landis, a detailed account of which is given [Armentano *et al.* 1936].

In two brief communications Bentsáth *et al.* [1936; 1937] claimed to have demonstrated by experiments with guinea-pigs the vitamin-like nature of this substance (or substances). As a result of this work they claimed to have determined the existence of a vitamin P which "brought back the fragile and permeable capillaries to their normal state" [Szent-Györgyi, 1937].

It was subsequently stated that the active material was flavanone in nature [St Rusznyák & Szent-Györgyi, 1936] and in a further communication by Bruckner & Szent-Györgyi [1936] it was claimed that the active fraction consisted of a mixture of hesperidin and eriodictyol glucoside. Contradictory statements as to the relative activities of these two fractions have been made [Bruckner & Szent-Györgyi, 1936; Szent-Györgyi, 1937].

The experiments on guinea-pigs were stated to show that the time of survival of animals on a scorbutic diet (Sherman-La Mer-Campbell) was prolonged from 28.5 days to 44 days by supplements of vitamin P given orally as 1 mg. daily of an active fraction prepared from lemon juice. It was further stated that at autopsy the animals which had previously received the vitamin P supplements showed fewer haemorrhages. Zilva [1937] and Moll [1937] have been unable to confirm the work on guinea-pigs, and even Szent-Györgyi [1937; 1938] has been unable to repeat the experiments.

Accordingly, since conclusions in support of the existence of vitamin P cannot be drawn from guinea-pig experiments, evidence for the existence of such a substance rests entirely upon the original clinical observations. The present author's considerable experience of the determination of capillary fragility in a large number of diseases in the human subject has shown that Szent-Györgyi's cases were unsatisfactory for the purpose. In vascular and thrombocytopenic purpura gross alterations in capillary fragility occur as a result of a series of events characteristic of these conditions and independent of the possible existence of vitamin P. The explanation of these variations will be given in another place; for present purposes it is sufficient to make the point that conclusions as to the efficacy of any substance in controlling bleeding or in decreasing the capillary fragility in purpura must be drawn with the greatest

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caution. Hence experiments on patients with purpura cannot be regarded as satisfactory in deciding the question whether there is a vitamin P.

During the last few years various workers have reported their inability to relate increased capillary fragility to states of vitamin C subnutrition. Attention has already been directed to this point by Scarborough & Stewart [1938] in whose paper appropriate references are given. In that preliminary communication the investigation of the capillary fragility in six cases of generalized vitamin deficiency was reported. It was shown that the oral administration of "hesperidin" could control the number of induced petechial haemorrhages in such subjects. In the present paper results of a more extensive investigation are presented. The purpose of the communication is to demonstrate the existence of a substance (or substances), present in fruits and extracts prepared from them, which does in fact control capillary fragility. A brief summary of the paper has already appeared [Scarborough, 1939].

#### EXPERIMENTAL

*Clinical material.* All subjects used in this investigation had abnormally increased capillary fragility. During the experimental period every case was the subject of a more or less severe degree of vitamin deficiency as a result of circumstances which are stated subsequently. It is held that the increased capillary fragility is an expression of this vitamin deficiency.

*Test material.* There is some confusion in the available literature regarding the nomenclature of the materials used in vitamin P studies and it is therefore desirable to describe briefly the nature of the material used in this investigation. Three different extracts containing the flavanones from certain fruit sources have been used:

Fraction C. A crude yellow powder, free from ascorbic acid, deposited spontaneously during the desiccation of Californian Valencia orange juice.

Fraction H. A colourless powder obtained from "C" by extraction with pyridine. Glaxo Laboratories Ltd. have generously provided these two fractions and I am indebted to them for the following account of the preparation of the material:

In a particular experiment 400 ml. of pyridine were used for the extraction of 36 g. of crude material. The dark brown solution was concentrated under diminished pressure to 100 ml. and this was then diluted with 1 l. of boiling water. After standing overnight 30 g. of slightly yellowish crystalline material were deposited. This crop was recrystallized twice more from a mixture of about 1 part pyridine to 5 parts water. The product so obtained consisted of colourless needles of m.p. 255–256°.

Both C and H are only slightly soluble in water. Accordingly, they were administered as a suspension in water in doses of 1.0 g. per day orally.

Fraction E.G. A solution (50 mg. in 1 ml.) of flavanones prepared from orange peel according to the method of Szent-Györgyi. It has been stated [Szent-Györgyi, 1938] that this material consists of a mixture of "eriodictyol glucoside" (a glycoside of 5:7:3':4':tetrahydroxyflavanone) and "hesperidin" (a glucoside of 4'-methoxyeriodictyol). Acknowledgement is due to Roche Products Ltd. for supplies of this preparation which is called by them "Citrin". Glaxo Laboratories Ltd. have also kindly supplied a similar material.

This fraction was administered intramuscularly in doses of 1–2 ml. per day (50–100 mg.).

*Determination of capillary fragility.* The capillary fragility (the ease with which

capillary walls burst in response to the application of pressure) was determined frequently, in the majority of cases daily, by one of two methods. A full account of the techniques involved in this determination is being prepared for publication. At the moment it is sufficient to explain that in the "positive pressure method" the fragility is determined in terms of the number of burst capillaries occurring during standard conditions in response to an increased intracapillary pressure, whereas in the "negative pressure method" the fragility is evaluated as the amount (in mm. Hg) of suction which is required to burst a single capillary loop, the suction being applied over a small area of skin. Several standard areas are used and these are appropriately numbered in the charts which follow. When the former method is used, improvement, that is to say, decreased fragility or an increased resistance, is indicated by a fall in the graph; in the suction method decreased fragility is indicated by a rise in the curve.

The charts given in this paper are typical examples; they are not to be regarded as representing isolated experiments.

### Results

#### *Exp. 1. Fig. 1. Negative pressure method*

Female, aged 53. Nutritional anaemia; haemoglobin 9.3 g. per 100 ml. (58%); subsistence for over a year on a diet which on analysis was found to be deficient in first-class protein, minerals and especially vitamins. At point A the diet was adjusted to contain fresh fruit, including in particular three oranges daily. The effect on the capillary fragility in response to the consumption of fruit is shown.

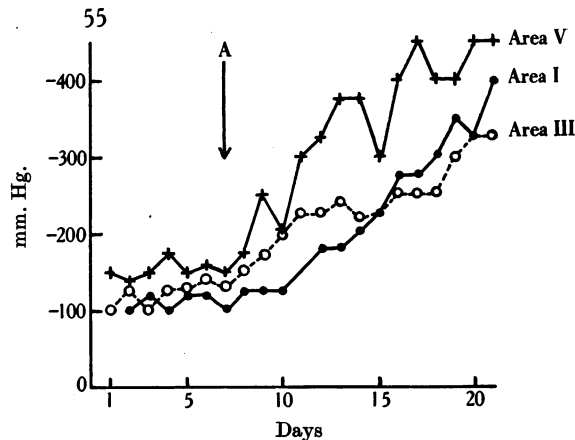


Fig. 1.

It is clearly possible that this improvement, which has been obtained in a number of individuals, may have been brought about by ascorbic acid. The next experiments bear upon this point. A second criticism is evidently that the coincident improvement in the anaemia may have occasioned the decrease in capillary fragility. In point of fact, however, in another investigation it has been found that an increase in blood haemoglobin concentration *per se* has no effect in decreasing capillary fragility.

*Exp. 2. Fig. 2. Positive pressure method*

Male, aged 56. Symptoms of generalized vitamin deficiency were occasioned by his having taken for many years a diet almost entirely lacking in fresh food. During the experimental period the subject was maintained on a similar diet and vitamin preparations were added one at a time. In spite of adequate doses of vitamins A (oral), B<sub>1</sub> (parenteral), C (oral) and D (oral) the capillary fragility

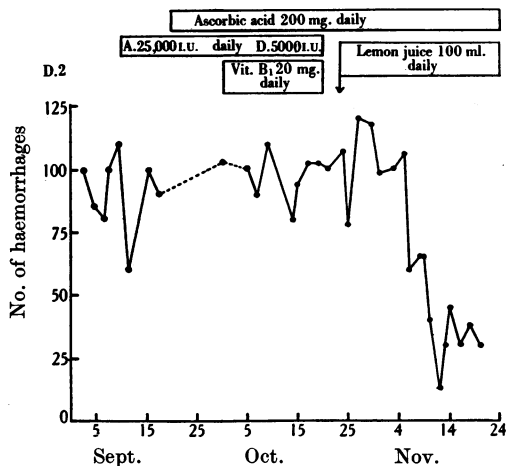


Fig. 2.

remained grossly increased. Lemon juice was now given as a supplement in doses of 100 ml. daily. The effect on capillary fragility is clearly shown. This experiment strongly suggests that lemon juice contains a substance (or substances) other than ascorbic acid which can control capillary fragility.

*Exp. 3. Fig. 3. Negative pressure method*

Male, aged 40. Dyspepsia; haemoglobin 13.6 g. per 100 ml. (85%). On account of his symptoms this patient had for 13 months been taking a diet of poor quality, deficient in fruit and vegetables. The capillary fragility was increased and remained high while he was taking the experimental diet (S. 1) which contains no fruit or vegetables. He was then given a diet (S. 2) differing from S. 1 in that it contained orange pulp and orange juice. It will be observed that while he was taking this diet the capillary fragility gradually decreased. Diet S. 1 was then substituted for diet S. 2 and ascorbic acid was given daily by mouth in amounts roughly equivalent to the vitamin C content of diet S. 2. In spite of the supplement of ascorbic acid the capillary fragility again increased. The flavanone fraction (E.G.) was then injected and a prompt and definite decrease in capillary fragility was obtained.

It was felt that this experiment was not completely satisfactory in that other unknown factors may possibly have contributed towards producing the initial increased capillary fragility. Furthermore, although the absorption of ascorbic acid was controlled by estimation of its elimination in the

urine, it is possible that ascorbic acid might have been effective had it been administered parenterally. Accordingly, an experiment was performed to test this point.

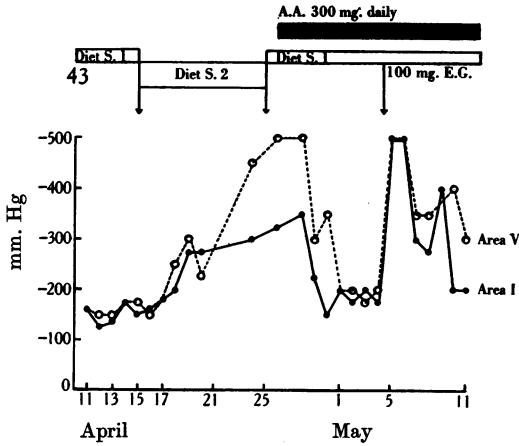


Fig. 3.

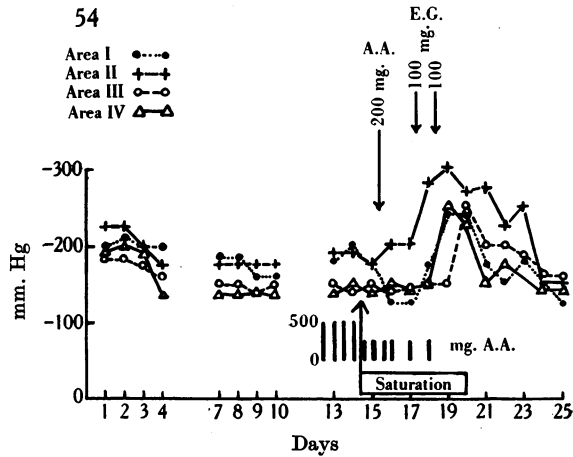


Fig. 4.

*Exp. 4. Fig. 4. Negative pressure method*

Male, aged 30. In this experiment an increased capillary fragility was induced in an otherwise healthy individual by placing him upon a diet containing neither vegetable nor fruit; all other food in it was twice cooked. Supplements of ascorbic acid were then added to the diet to the point of saturation (in the Harris and Ray sense) with no improvement in capillary fragility. 200 mg. of ascorbic acid were then injected intramuscularly without effect. 200 mg. of flavanone fraction (E.G.) were then given intramuscularly with the effect clearly indicated in Fig. 4.

*Exp. 5. Fig. 5. Negative pressure method*

Male, aged 50. Malignant stricture of the oesophagus resulting in marked interference with passage of food into the stomach. In such circumstances the increased capillary fragility is to be explained on the basis of a conditioned deficiency. As a result of treatment the subject became able to swallow liquids and accordingly 300 ml. of orange juice were given. There was a definite effect upon capillary fragility. Thereafter, 200 mg. of flavanone fraction (E.G.) were given in solution in 0.9% saline into the rectum with the result indicated in Fig. 5.

It will be clear from the above experiments that there is material present in orange and lemon juice which is capable of increasing the resistance of abnormally fragile capillary walls. This material is not ascorbic acid. It is present in flavanone fraction (E.G.) which is highly active when injected into human subjects suffering from pure or conditioned vitamin deficiency. It is now important to determine whether it be active by mouth.

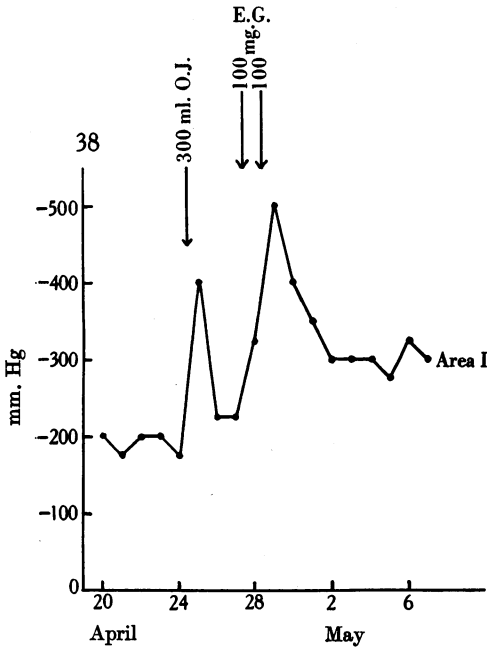


Fig. 5.

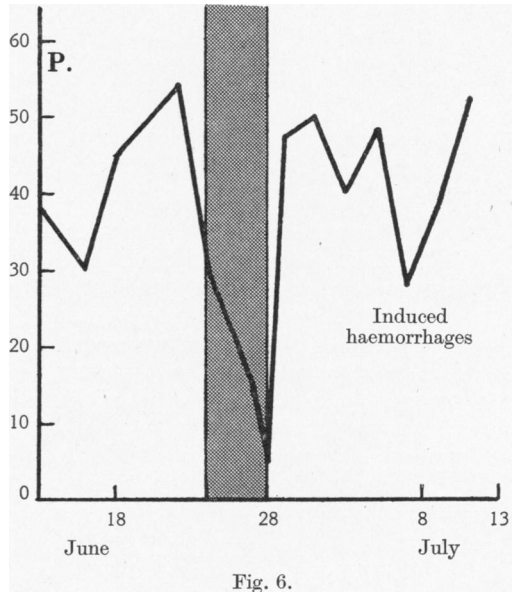


Fig. 6.

*Exp. 6. Fig. 6. Positive pressure method*

Female, aged 37. Vitamin deficiency on account of bad dietary habits. During the shaded period indicated in Fig. 6 flavanone fraction (C) was given by mouth in doses of 1 g. per day. Its effect in decreasing capillary fragility is evident.

*Exp. 7. Fig. 7. Positive pressure method*

Male, aged 58. Severe and generalized hypovitaminosis occasioned by economic and financial stringency. Flavanone fraction (H) was given orally in doses of 1 g. per day. The reduction in capillary fragility in response to the administration of flavanone material is again clear.

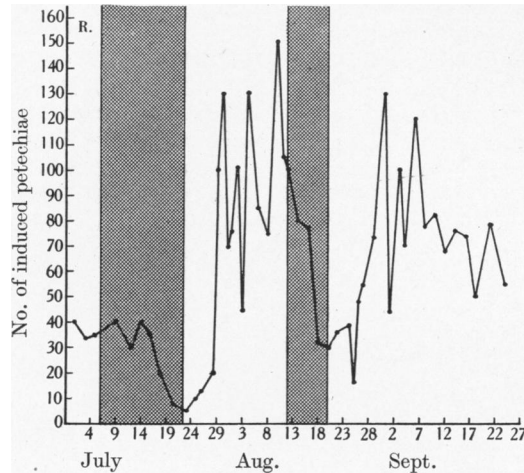


Fig. 7.

The latter two charts are to be regarded as typical of the results obtained in a series of 12 subjects.

*Conclusions*

In human subjects suffering from multiple vitamin deficiency of varying degrees of severity the capillary fragility was found to be increased. This increase has been determined in spontaneous, conditioned and induced deficiencies. There is present in orange and lemon juices, and in certain extracts made from them, a substance (or substances), flavanone in nature, which can increase the resistance of capillary walls to the application of pressure. The precise nature of the material producing this effect has not been determined. It is active when given by mouth, by intramuscular injection or through the rectum. The decrease in capillary fragility (or increased capillary resistance) has been produced in every case even when ascorbic acid, by mouth or by injection, has failed to produce this effect.

## SUMMARY

The evidence upon which the existence of vitamin P is based has been reviewed and it has been shown that a conclusion as to the reality of such a vitamin cannot be maintained on the basis of the published work. Evidence is now adduced from experiments on human subjects which establishes the existence of a factor decreasing capillary fragility.

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