

## A CRITERION OF HEMORRHAGIC DIATHESIS IN EXPERIMENTAL SCURVY\*

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The purpose of this report is to describe the results of a simple experiment designed to investigate the hemorrhagic diathesis in scurvy.

The hemorrhagic diathesis is perhaps the most striking clinical feature of scurvy. The anatomic study of the disease, however, has centered chiefly about the changes in the skeletal system, and, lately, the teeth. This is due, in part, at least, to the absence of definite or characteristic lesions in the vessels.

The pathologic features of the terminal vessels have been described as thickening or proliferation of the cells of the intima and, by some, adventitia as well. Several pathologists consider them to be inflammatory (1, 2). According to this view of the hemorrhagic diathesis the vitamin deficiency is responsible for a nutritive state in which organisms normally non-pathogenic incite inflammatory changes. Meyer and McCormick have characterized the vessel lesions as "autolysis of small arteries (3)." Höjer (4) considers the effect of the deficiency to be an atrophy of certain types of cells and his hypothesis suggests that the vessel lesion is likewise primarily an atrophy.

Hess (7) has shown that the Rumpel-Leede phenomenon is positive in scurvy and that the tendency of the skin capillaries to rupture during stasis is responsive to treatment.

Aschoff and Koch (5) demonstrated that the skeletal lesion was a disturbance in the intercellular substances and reasoned that a like disturbance probably was present in the smaller vessels and that rupture was the result of deficient or inferior cement substances. This opinion was given support by Wolbach and Howe (6) who showed that in scorbutic animals the healing of soft tissues occurred without the formation of new capillaries though no impairment of the proliferation of endothelial cells was present.

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Our failure to find a satisfactory explanation of the hemorrhages in the histology of the small vessels lead to a search for another method of approach. The purpose of the following experiment was to determine whether the resistance of the vessels to rupture is decreased in experimental scurvy, and, if so, what relation this change bears to the course of the disease.

### Method

Scurvy was produced in young healthy guinea pigs weighing from 250 to 350 gm. by feeding the following diet which is deficient only in vitamin C.

	<i>per cent</i>
Baked skim milk powder (baked in shallow pan for 2 hours at 110°C.).....	30
Butter fat (butter melted and filtered at 45-50°C.).....	10
Rolled oats and bran (equal parts by volume).....	58
Sodium chloride.....	1
Cod liver oil.....	1

During the course of the disease (which, under these conditions, is approximately 3 weeks) the fragility of the skin vessels was tested by determining their resistance to negative pressure. The skin of the belly was used because other skin surfaces overlie bony structures which greatly interfere with the application of the suction cup when the animal is emaciated, as in late scurvy. The hair was removed with a barber's clipper and the skin greased with petrolatum.

The apparatus used consisted of a glass tube of 1 cm. inner diameter connected with a mercury manometer and a small hand pump. We found it advisable to use a tube with edge turned out at right angles for a breadth of 0.75 cm. to avoid constriction and afford good contact. A partial vacuum was created and maintained for 1 minute and the minimal negative pressure producing definite petechiae was taken as an expression of the vessel resistance. The 1 minute period was arbitrarily chosen. A similar apparatus was used by Hecht (9) in 1907 in distinguishing between certain erythematas and has lately been modified by Zilva-Mello (10).

A group of four animals completely deprived of vitamin C was tested every 3rd day throughout the course of the disease. On the 19th day of the diet they were given 2 cc. of orange juice by mouth from a pipette and greens *ad lib.* for the following 24 hours. During this latter period the pigs were tested every 6 hours. At the end of the period they were given 8 cc. of orange juice, and, after 6 hours, tested again.

A second group of four animals was similarly dieted but was tested every 2nd day for 2 weeks and was then left undisturbed for 10 days. On the 23rd day of the diet they were tested once to determine the degree of reaction present and then given antiscorbutics. Two animals received 12 cc. of tomato juice by mouth

(forced feeding) during the first 3 hours and tomato *ad lib.* thereafter. A third animal was given banana *ad lib.*, and a fourth received an intraperitoneal injection of an extract of banana in Locke's solution which had been purified by centrifugalization and filtration. Skin samples of areas in which petechiae had been produced were removed from two of the animals before the administration of antiscorbutics and from the same animals after the normal reaction of the vessels had been restored by the antiscorbutics. Three of the animals were tested every hour for 6 hours during the period of forced feeding, and final tests were made on all four after 24 hours. All of the animals were weighed each day they were tested.

Four other animals were tested which were part of a series in another experi-

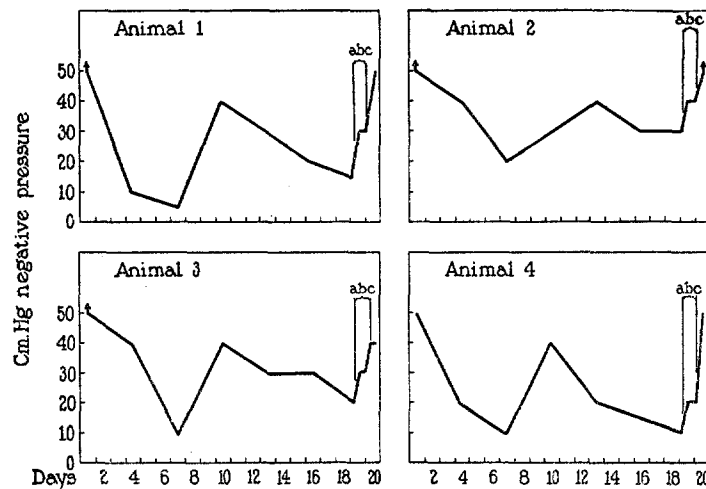


FIG. 1. Time of appearance of induced petechial hemorrhages in the skin of guinea pigs on vitamin C free diet. First group. (a) given 2 cc. of orange juice by mouth by pipette. (b) greens *ad lib.* (c) 8 cc. orange juice by mouth by pipette. (↑) a negative reading at the pressure indicated.

ment and which had received the same basal diet for 6 weeks but with the addition of graded amounts of banana.

#### *Description of the Reaction*

The reading of the typical reaction to the test offered little difficulty. The result of the test was commonly indicated by the appearance of the skin dome as seen through the glass cup immediately after applying the suction. An area which reacted negatively usually became deeply

injected and even cyanotic before the minute occupied by the test elapsed. An area which gave a positive reading usually remained pale, the bright, cherry-red hemorrhages appearing a few seconds after the application of the suction and often increasing slowly in size during the minute interval. However, occasional areas could not be satisfactorily interpreted while under the cup. Hence, readings were always made after the cup was removed from the skin. In very faint reactions correct interpretations could be arrived at most easily by lifting the skin and looking at it by transmitted light.

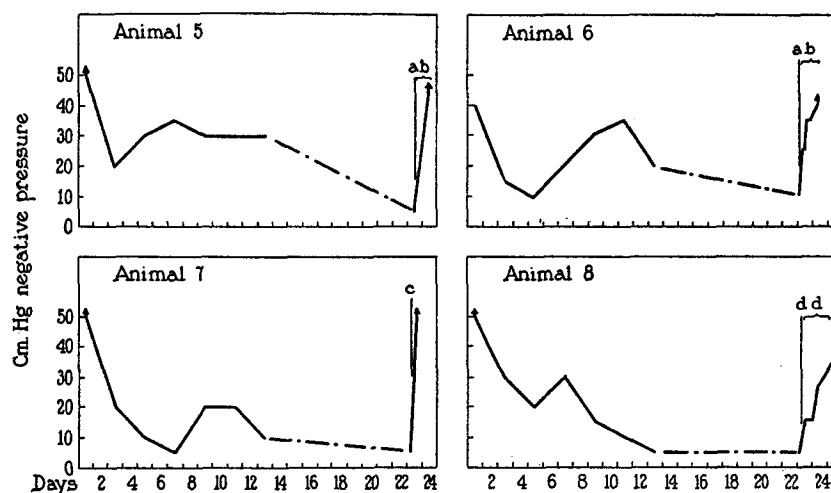


FIG. 2. Time of appearance of induced petechial hemorrhages in the skin of guinea pigs on vitamin C free diet. Second group. (a) given 12 cc. tomato juice by mouth by pipette. (b) tomato *ad lib.* at will. (c) 4 cc. banana extract intra-peritoneally. (d) banana by mouth *ad lib.* (↑) negative reading at pressure indicated. Broken line indicates interval during which no tests were made.

The degree of the reaction varied from a single fine petechia to numerous hemorrhages so large and close together as to appear to be confluent. This extensive reaction looked very much like a strawberry. The severity of the reaction in a series of readings made at one testing was roughly in proportion to the amount of negative pressure applied but since the evaluation of the degree of hemorrhage could only be approximate and it was influenced by other factors (see

further on), the reading of most significance was held to be that of the minimal negative pressure sufficient to produce any hemorrhage whatsoever. When only one pin-point hemorrhage appeared the reading was called plus : minus and a second test made. If one or more hemorrhages appeared in the second reading the result was called positive, if none, negative. These extremely faint reactions were uncommon. The large majority of tests were frankly positive or negative.

#### *Limitations of the Test*

The most unsatisfactory factor in the experiment was the inability to test as often as desired because of the limited area of skin available, since an injured area cannot be tested again for several days. This affected both the frequency of the tests and the number of determinations made at any time.

The chief difficulty in reading positive tests arose in late scurvy when spontaneous skin hemorrhages occur. Fortunately, upon careful observation, the bright red of the new hemorrhages was easily distinguishable from the duller color of the spontaneous petechiae.

Contradictory readings at a single testing occasionally occur in the same animal in different areas submitted to the same negative pressure. This state of affairs is highly erratic in its appearance. An inconsistency appearing in an animal on one day might be found in quite different skin areas or disappear altogether the following day. The tendency bore no relation to the course of the disease.

Excitement of the animal usually affected the degree of reaction but seemed to have slight effect on the pressure at which a positive reading was obtained. This phenomenon is probably due to an increased blood pressure either acting directly to increase the tension on the vessel walls or simply causing engorgement which results in larger hemorrhages when rupture does occur.

Subcutaneous hematomas occasionally occurred in one of our animals and were associated with absence of skin petechiae in the area over the hematomas. This tendency increased with the progress of the disease.

#### RESULTS

The detailed results are presented in the graphs representing the behavior of the individual animals. (Figs. 1 and 2.)

An increased liability to hemorrhage became apparent early in every case. At the end of 4 days the amount of negative pressure necessary to produce petechiae averaged 25 cm. less in one group of animals and 22.5 cm. in the other. In the tests made after 2 days of deficiency there was an increased tendency to hemorrhage in each case. The average reduction of pressure was approximately 26 cm. mercury. This downward trend occupied an average period of 6 days. Increased resistance then set in for an average period of 4 days and was followed in turn by a period of continued decline in resistance. The greatest resistance during the period of transient recovery was never equal to the normal.

In those animals kept 23 days on the scorbutic diet, hemorrhages were produced on the last test day at an average pressure of 6 cm. mercury.

Antiscorbutics were administered to all of the animals and in each case their administration was followed by prompt and definite increase in the resistance of the vessels. The greatest and most rapid improvement was found in an animal in which an aqueous extract of banana was injected intraperitoneally. This animal developed normal resistance within 3 hours of the administration of the antiscorbutic substance. In feeding antiscorbutics, more rapid recovery occurred when the protective substances were forced and in such cases the effects were noted after 6 hours. The degree of recovery, in the limited number of tests made, was proportional to the amount of antiscorbutic given. Animals of the first series showed slight but prompt recovery after 2 cc. of orange juice but ceased to improve further until given an added dose of 8 cc. of orange juice. The resistance then promptly increased again.

In four animals which had been receiving basal diet plus daily graded doses of banana over a period of 6 weeks the readings were made at irregular intervals. The average resistance in the animals receiving 3 gm. daily of banana was 10 cm. mercury, the average in the animal receiving 6 gm. was 23 cm. and the average for two animals receiving 9 gm. was 32 cm.

The skin samples removed were sectioned and stained with Giemsa solution. No differences in the structure of the terminal vessels were noted between those samples removed before treatment and those removed after recovery.

## DISCUSSION

The promptness with which changes in the vessels occur in the early stages of experimental scurvy is in accord with other features of the disease. We have repeatedly been able (10) to recognise lesions in the roots of incisor teeth after 5 days of deprivation of vitamin C. The rapidity of recovery on the administration of antiscorbutic substances is in equal agreement with the histologic changes. Wolbach and Howe (6), for example, reported definite evidence of recovery in the incisor roots within 24 hours after administering orange juice. The greater celerity of the vessel changes may be due to the nature of the test which is probably more searching than microscopic examination.

Previous observation on scorbutic animals had lead us to assume that the disease progressed regularly. The appearance of a transient period of recovery in the damage to the vessels indicates that this may not be the case. This new observation has a parallel in a well recognised phenomenon of scurvy in guinea pigs. It has long been noted that, on complete deprivation of vitamin C, the weight curve uniformly sank rapidly and then, after a few days, rose slowly to a peak greater than the original weight. Emaciation then developed rapidly until death. The transient recovery peak in the graph of vessel resistance occurs simultaneously with this peak of increased weight. We suspect that these phenomena, as yet without explanation, are due to similar causes. It is even probable that other features of the disease accord with this cycle.

The reaction might be a form of compensation due possibly to delayed mobilization of stored vitamin within the body. An observation of a possibly related phenomenon was made in Germany during the war years (11). It was observed that well nourished subjects suddenly transferred to districts in which the ration was extremely poor exhibited higher morbidity and mortality rates than the permanent population of the districts. Soldiers transferred to civil communities in which the diet was almost exclusively vegetable suffered greatly from disease and the death rate from infectious diseases was unduly high. The civil population which had gradually become reduced to the poor fare maintained a relatively great resistance to infection.

The rapidity of the change in the vessel resistance strengthens the

theory that the underlying mechanism in scurvy is a chemical alteration of intercellular substances since it is difficult to suppose that cellular changes can occur so rapidly and since no discernible alteration in vessel structure occurred during recovery. The results of the experiment further suggest that the vitamin probably acts directly. The conception of Wolbach and Howe that the reaction during healing is analogous to the setting of a gel is in accord with our results. The theory that the vessel lesion is inflammatory in nature appears untenable.

The effect of the peritoneal injection of banana extract indicates the action to be independent of the gastrointestinal tract. This was shown by Hess and Unger when they cured scurvy with intravenous administration of antiscorbutic (12).

If the vessels of human cases are as responsive to treatment as those of the guinea pig, the vessel resistance test should have clinical value in the diagnosis of latent scurvy in which the hemorrhagic diathesis is the only sign of the disease.

#### CONCLUSIONS

1. The degree of scorbutic change in the vessels of animals with experimental scurvy can be roughly measured by establishing the amount of negative pressure required to produce petechial hemorrhages in the skin.
2. The test shows that the hemorrhagic diathesis in experimental scurvy develops earlier than any other known sign of the disease and that it persists in some degree throughout.
3. The response of the blood vessels to the administration of antiscorbutic substances is extremely rapid as shown by the test, but it varies with the amount of antiscorbutic given and its method of administration.
4. The changes in the resistance of the vessels follows a curve which rises towards recovery during the end of the 1st week on a scorbutic diet, reaches a peak in the 2nd week and then falls steadily during the remainder of the course of the disease. This indicates that the course of the disease is not constant and progressive.
5. The test may have clinical value in the diagnosis of scurvy.



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