THE RELATION OF VITAMIN C DEFICIENCY TO NUTRITIONAL ANEMIA [1]

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ONE FIGURE

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The occurrence of anemia in scurvy has been recognized for a long time. However, the degree of anemia very rarely parallels the other symptoms which are regarded as characteristic for scurvy (Hess and Fish, '14; Hess, '20; Aron, '22; Mettier, Minot and Townsend, '30; Rohmer and Bindschedler, '32; and Parsons and Smallwood, '35).

The frequent and simultaneous occurrence of anemia and scurvy has an important bearing on the cause of nutritional anemia. A study by Aron ('27) of the cases of nutritional anemia treated between 1908 and 1926 in the University Children's Hospital at Breslau revealed that during the period of frightful shortage of food material, 1921 to 1923, there was an extraordinary increase in the number of cases admitted for severe anemia coincident with a hitherto unknown number of cases of scurvy observed during the same period. Fifteen cases of anemia were carefully examined for scurvy according to clinical symptoms and x-ray findings. Such symptoms were found in seven instances (Aron, '22). It is remarkable that Rohmer and Bindschedler ('32), recording the results of blood examinations in fifteen cases of infantile scurvy, found anemia in 'only seven instances.'

Before ascorbic acid was discovered only so-called 'anti-scorbutic foodstuffs,' like orange, lemon, or other fruit juices,

[1] This investigation was conducted under a grant from the Emergency Committee in Aid to Displaced Foreign Medical Scientists.

green vegetables or germinated oats, all of them containing a number of other constituents, could be used in clinical or experimental investigations. Therefore, it was not possible to decide which constituent of the so-called 'antiscorbutics' was the active factor causing anemia by deficiency and acting as a hemopoietic when added to the diet.

After vitamin C became available in chemically pure form as ascorbic acid, some investigations were made concerning its influence upon the blood, especially upon the red blood cells and reticulocytes. However, the results reported thus far are contradictory in several respects and the fundamental problems are not satisfactorily solved (Jonas, '23; Meyer and McCormick, '28; Mettier, Minot and Townsend, '30; Mettier and Chew, '32; Rohmer and Bindschedler, '32; Dunlop and Scarborough, '35; Parsons and Smallwood, '35; Euler and Malmberg, '36, '37, '38; Aszodi, '37; Kenney and Rapoport, '38; Mettier, '38, and Ungley, '38).

Since the fundamental problem of the relationship of ascorbic acid to blood formation and to anemia cannot be solved by clinical studies alone, a systematic experimental investigation is necessary. There are very detailed procedures and rigid rules for the study of the different scorbutic symptoms in experimental scurvy of guinea pigs. These include a study of the body weight, the post mortem findings on the various organs, the bones and even the histological structure of the teeth. However, until now no attempt has been made to include a study of the hemoglobin and the blood cells in the 'biological assay' for vitamin C. It is obvious that for the purpose of 'biological assay' such blood examination if applicable would offer certain advantages over methods based only on post mortem findings or histological examination.

EXPERIMENTAL PROCEDURE

a. First series. In the first series of experiments the method for biological standardization of vitamin C was strictly followed (Coward, '36, '38; King, '38).

The scorbutogenic diet (diet sc.) used throughout all experiments was prepared every 10 to 20 days by mixing the following components:

Heated skimmed milk powder [2]	300
Ground whole oats	590
Melted butterfat, strained	90
Cod liver oil	10
NaCl	10

In addition to diet sc., 5 gm. dry oats were given daily in the first and second series of experiments.

Four male guinea pigs (nos. 3, 4, 11 and 12) somewhat over 200 gm. in body weight were kept on a diet of oats and carrots for a preliminary period. Hemoglobin and red blood cells were checked at least twice. When the animals had reached weights of 265, 275, 280 and 300 gm. respectively they were transfered to the scorbutogenic diet.

The scorbutogenic diet produced the well-known signs of scurvy. A distinct reduction of hemoglobin with a slight reduction of the R.B.C., however, developed in only one of these animals. The other three animals showed no distinct anemia in spite of the fact that they had lost nearly 25% of their body weight. None of those four animals could be saved even by the administration of ascorbic acid subcutaneously or orally in doses of 25 mg. daily. These experiments agree fully with those performed by Dr. M. I. Pierce [3] in this laboratory.

From earlier experiments (Aron, '22, and Jonas, '23) we had the impression that young guinea pigs or guinea pigs of lower body weight do not become anemic as readily as do older ones of higher body weight. A critical analysis of the experiments published by Meyer and McCormick ('28) seemed

[2] The skimmed milk powder was heated in a very thin layer in flat pans for at least 4 hours. During the heating process the powder was stirred and turned over several times. The heating was continued until the milk powder showed a pronounced brown color. The purpose of this was to destroy completely vitamin C and such other heat labile factors as might be present, as e.g. the 'grass juice factor' (Koehler, Elvehjem and Hart, '38).

[3] Unpublished results.

to confirm this belief. For this reason in the next series of experiments the guinea pigs were not put on the deficiency diet before they had grown to about 500 gm. and were at least 4 months of age.

b. Second series. This experiment included six guinea pigs, all of them male (nos. 1, 2, 5, 7, 6, 10). In a preliminary period carrots and oats with milk were given. Then the animals were transfered to the scorbutogenic diet which for a limited period was supplemented either by addition of 5 gm. fresh germinated oats [4] daily, or by 12.5 mg. ascorbic acid [5] dissolved in 2 to 3 cc. of water and fed orally.

On the one hundred and first day the administration of vitamin C was discontinued and dry oats was substituted for germinated. Following this change a distinct anemia was produced in all six animals as will be seen from table 1. All these animals were in the ultimate stage of vitamin C deficiency. When we attempted to cure the anemia even amounts up to 100 mg. ascorbic acid, given either by mouth or by subcutaneous injection, did not produce a remission of the anemia. Four animals, nos. 1, 2, 5 and 7 succumbed within 3 to 6 days. However, pigs nos. 6 and 10 which on the fourth day were placed on a diet of germinated oats and concentrated milk could be saved.

In spite of the fact that the quantity of ascorbic acid contained in the germinated oats was less than 5 mg. there was a marked improvement immediately after this change of diet was introduced. After the animals had been on this supplement of 5 gm. germinated oats for a period of 10 days, the germinated oats were replaced by ascorbic acid. At first 25 mg., later 2 mg. daily were fed orally to each animal.

Before the guinea pigs had again reached their original weight the hemoglobin content of the blood was within normal limits.

[4] Five grams germinated oats, according to our chemical determination, have an average content of about 2 mg. ascorbic acid.

[5] We wish to express our appreciation to Merck and Co., Rahway, N. J., for furnishing the ascorbic acid (Cebione) used in this investigation.

c. A third series of experiments was initiated with the intention of starting the supplement with the anemic animals before they reached the ultimate (fatal) stage. Six male guinea pigs all of whom had been watched closely for more than 100 days were fed our scorbutogenic diet (diet sc.) with an addition of either ascorbic acid (Cebione) or a juice pressed from germinated oats for 53 to 68 days respectively.

TABLE 1

NUMBER OF ANIMAL	SUPPLEMENT TO DIET SC.	AVERAGE BEFORE WITHDRAWAL OF ANTISCORBUTIC	LOWEST VALUE AFTER WITHDRAWAL OF ANTISCORBUTIC
1	68th to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms. 14.9	8.2
		Ery. mill. 4.5	2.2
2	68th to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms. 14.8	12.4
		Ery. mill. 4.6	3.7
6	82nd to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb gms. 14.2	11.1
10	82nd to 100th day: 5 gm. dry oats +12.5 mg. ascorbic acid	Hgb. gms. 14.5	10.6
5	68th to 100th day: 5 gm. germinated oats	Hgb. gms. 15.4	11.6
		Ery. mill. 4.9	4.3
7	68th to 100th day: 5 gm. germinated oats	Hgb. gms. 15.9	11.8
		Ery. mill. 4.8	4.3
Average	Hemoglobin, grams	14.9	11.0 = 26% reduction
	Erythrocytes, mill.	4.7	3.7 = 21% reduction

The life duration of a red blood cell hardly amounts to more than 50 days. Consequently we are fully justified in stating that the red blood cells of these guinea pigs and therefore the hemoglobin within these cells was built up entirely while these animals were on diet sc. with the respective supplement.

As the anemia produced by the withdrawal of vitamin C is always of a distinctly hypochromic type, the decrease in

hemoglobin is comparatively greater than that in the number of red blood cells (see also table 1). For this reason it seemed highly important to concentrate our studies upon the hemoglobin changes. In order to make the hemoglobin determinations as accurate as possible they were performed in duplicate with two different types of hemoglobinometers, the one checking against the other (Newcomer and Hellige or Sahli Leitz). Blood was drawn at intervals from 2 to 5 days.

In guinea pigs nos. 6 and 10 the withdrawal of the ascorbic acid supplement produced anemia for a second time; the loss of hemoglobin amounting to 24% and 30% respectively.[6] No attempt was made to cure these animals which were made anemic for a second time.

With the other four animals of this group, however, we gave the ascorbic acid anew in sufficient time to cure the animals. Once or twice daily 50 mg. of ascorbic acid were given either subcutaneously[7] or orally. With these large doses all four animals were saved.

Following this administration of ascorbic acid, a surprisingly fast regeneration of hemoglobin was observed. When the weight curves began to rise, the hemoglobin levels had regained their normal values. The most important figures are compiled in figure 1.

Throughout all experiments it was noticed that the more the anemia develops the more difficult it becomes to obtain sufficient amounts of blood for hemoglobin determinations by pricking the ears of the guinea pigs. However, within a few days after ascorbic acid is given anew, there is again ample bleeding from the same ears from which a short time before, with exactly the same technic, blood could be obtained only with great difficulty. This may be due to a reduction of the total

[6] These animals in addition to the scorbutogenic diet were given an extra supplement of iron from the time the first decline in hemoglobin was noticed. Iron pyrophosphate in doses of 0.2 to 0.5 gm. daily were given in the drinking water. This mode of iron administration was adopted since the animals while declining in weight as well as hemoglobin still drank some water, while the food was hardly touched.

[7] 'Cenolate' kindly furnished by Abbott Laboratories, North Chicago, Ill.

blood volume. It may also be due to desiccation. Such desiccation would explain why some investigators have reported an increase of red blood cells and of hemoglobin in clinical as well as in experimental scurvy (Hess and Fish, '14; Hess, '20 and Aszodi, '37). In our experiments there was also a slight rise noted in some instances after the lowest values for hemoglobin were obtained.

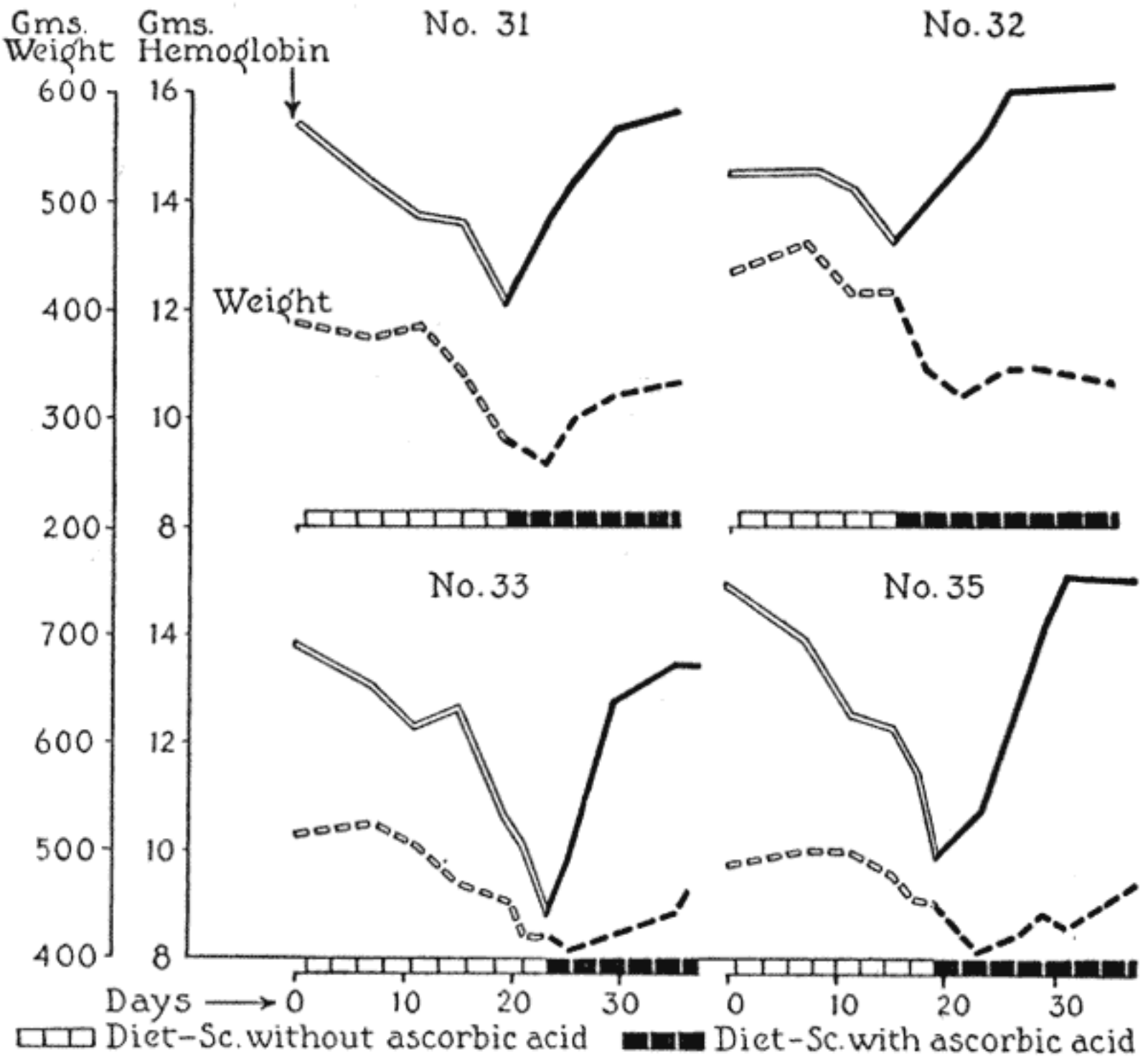

Fig. 1 For each graph: Upper curve: Hemoglobin. Lower curve: Weight.

SUMMARY

1. When guinea pigs are fed a scorbutogenic diet with an addition of ascorbic acid for 50 days or more, a period longer than the life cycle of the erythrocyte, normal blood formation takes place.

2. When the ascorbic acid supplement is withdrawn, guinea pigs 4 months of age having a body weight of 450 gm. or more

show a distinct reduction in the hemoglobin content of the blood within 20 days. A supplement of iron does not prevent this decline in hemoglobin. Younger animals, of 200 to 300 gm. body weight, may also become anemic. However, they very rarely do so because they usually succumb before a distinct anemia develops.

3. Guinea pigs made anemic by the withdrawal of ascorbic acid from their diet can be cured by administration of ascorbic acid in large amounts either orally or subcutaneously. This cure, however, is successful only in animals which have lost not more than about 25% of their body weight or one-third of their hemoglobin. The rise in hemoglobin induced by the ascorbic acid medication takes place much faster than the rise in body weight. This indicates that the anemia is cured long before the repair of the other body tissues is accomplished.

4. While these experiments give unquestionable evidence that ascorbic acid is a factor of deciding influence on hemoglobin formation in the guinea pig, they do not prove that ascorbic acid is the only active factor present in fresh green vegetables or in germinated oats as used in this experiment.

The problem of comparing the activity of ascorbic acid present in fresh green foods with the ascorbic acid in chemically pure form will be taken up in another paper.

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