THE ANEMIA OF SCURVY

EFFECT OF VITAMIN C DIET ON BLOOD FORMATION IN EXPERIMENTAL SCURVY OF GUINEA PIGS*

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PLATE 36

(Received for publication, March 14, 1932)

During recent years numerous articles have appeared in the literature concerning the various food factors, both organic and inorganic in nature, which influence hemoglobin and red blood cell production in animal and man. The effect of certain food factors upon blood regeneration in some types of anemia has been described by various investigators. It is now known that liver is effective in the treatment of the experimentally induced anemia of dogs (1), and in the treatment of pernicious anemia (2). It is also known that iron (3, 4), and perhaps other metals (5), may play an important rôle in the treatment of certain sorts of secondary anemia. There is, however, relatively little to be found in the literature concerning the effect that the vitamins may have on blood formation. There is evidence (6) that anemia of variable degree occurs in a large percentage of adults suffering from chronic lack of vitamin C, and that a reticulocyte response can be induced in patients with the anemia of scurvy following the administration of orange juice. In addition, it has been reported (6) that the altered function of the bone marrow may be in a large measure directly dependent upon the prolonged lack of vitamin C from the diet. Since it has been shown by Meyer and McCormick (7) and others that

* Read before the Section on Medicine of the American Association for the Advancement of Science, Pasadena, California, June 17, 1931.

A preliminary report of this study is given in Proc. Soc. Exp. Biol. and Med., 1931, 29, 11.
anemia is of regular incidence in the experimental scurvy of guinea pigs, we became interested in determining the effect of vitamin C on blood formation in the experimental animal.

The following experiments were performed: (1) to ascertain the effect of a diet deficient in vitamin C on blood formation in guinea pigs; (2) to ascertain the effect of a diet rich in vitamin C on blood formation when administered to animals with manifest scurvy.

Methods and Materials

Adult guinea pigs weighing 300 to 550 gm. were used throughout the experiment. Animals of this weight are better suited than smaller animals to this type of investigation, as they are less likely to succumb to infection during the development of scurvy. The males were segregated from the females to insure against pregnancy, and all animals were observed over a period of from 1 to 3 weeks before being put to experimental use, to be sure, in so far as possible, of an infection-free stock.

The diet used in the experiment to produce scurvy was considered to be adequate in all food factors with the exception of vitamin C-containing substances. It consisted of rolled oats, boiled milk, cod liver oil, and small portions of yeast daily. At first a little bran was added to the milk to prevent bowel obstruction from the formation of hard fecal impactions, but it was later learned that the ingestion of small bits of filter paper was more effective. Throughout the experiment a similar diet was fed to three animals used as controls, but in addition they received daily 3 cc. of vitamin C-containing orange juice or canned tomato juice. The latter substances were fed to the animals through a medicine dropper.

One series comprising eleven animals were studied while being fed the diet deficient in vitamin C. Every other day the weight of the animal was recorded and total red cell counts and hemoglobin determinations were made. The hemoglobin concentration was determined from a Sahli hemoglobinometer. In addition, counts were made on the reticulocytes in the circulating blood. The reticulocyte counts were made approximately at the same hour each day on smear preparations stained with brilliant cresyl blue and counterstained with Wright's stain.

With the exception of two animals, one of which died of spontaneous tumor and the other of pneumonia, all animals developed manifest scurvy, at which time they were either sacrificed or permitted to die of the disease. Autopsy was performed on all. Sections of the various viscera and of muscle were removed for sectioning and study under the microscope to determine the presence or absence of infection or other complicating disease and the degree of hemorrhage. Specimens of bone marrow from the femora were fixed in Zenker's solution, and after being set in paraffin blocks, sections were cut and stained with eosin and methylene blue (methylthionine chloride, u.s.p.). The examination of the bone marrow was made
with attention to the following points: the degree of cellularity of the tissue; the relative amount of erythropoiesis and the stage of maturation of the cells of the erythrocyte series as denoted by the number of mitotic figures appearing in a field of the oil immersion lens.

In order to determine the effect of a diet rich in vitamin C on blood formation as determined by the course taken by the reticulocytes (immature erythrocytes), a second series comprising six animals was given a diet similar to that outlined above. After the appearance of signs of scurvy in these animals, they were given 3 cc. of orange juice daily. Three of these animals were permitted to regain a normal state of health, and the other three animals were sacrificed after the appearance of a distinct rise in the reticulocytes in the circulating blood. Specimens of femoral bone marrow were removed from these animals for study.

RESULTS

The results in the guinea pigs placed upon the scurvy-producing diet were almost similar in all animals. The animals appeared well for about 12 to 15 days after being placed upon the experimental diet, when they began to show signs of inanition and a marked loss in weight. This loss of weight progressed in all instances, although the animals continued to consume the oats and milk. In most animals, subcutaneous hemorrhages appeared, especially on the ventral surface of the body, but in none of the animals was there evidence of extensive hemorrhage, and in some animals there was no gross evidence of hemorrhage. Other evidences of scurvy were to be found in the thickening about the ends of the carpal and tarsal bones and along the costochondral junctions.

Results of the effect of the scurvy-producing diet on weight and blood formation are recorded in Table I. After about 12 to 15 days, there was a slow drop in the number of red blood cells and in the hemoglobin content until within a few days before death, when the drop became more rapid. There was a decrease in the number of red blood cells of from 1 to 2.5 million per c.mm. below the average normal obtained for each individual animal before being deprived of vitamin C. The change in the hemoglobin content of the blood did not take place simultaneously with the red blood cell reduction. The time interval between the feeding of the diet and the hemoglobin decrease was usually 5 to 10 days longer than that necessary to produce a change in the total number of red blood cells. There was a drop in the hemoglobin concentration ranging from about 2.5 to approxi-
mately 7.1 gm. per 100 cc. below the normal. Thus, in all animals showing signs of scurvy, there developed a distinct anemia.

After both the red blood cell and hemoglobin concentrations had become definitely lowered, there was a considerable alteration in the character of the red blood cells. Polychromatophilic and stippled erythrocytes appeared in the peripheral blood, and there was slight poikilocytosis and anisocytosis. These changes are in accord with those of numerous other reports (8, 9).

### TABLE I

**Effect of Diet Deficient in Vitamin C on Weight and Blood Formation in the Guinea Pig**

<table>
<thead>
<tr>
<th>Guinea pig No.</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>Duration</th>
<th>Initial R.B.C. determination</th>
<th>Final R.B.C. determination</th>
<th>Initial Hb determination</th>
<th>Final Hb determination</th>
<th>Final reticulocyte count</th>
<th>Comment</th>
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<tr>
<td>112</td>
<td>460</td>
<td>330</td>
<td>21</td>
<td>5.2</td>
<td>2.5</td>
<td>86</td>
<td>12</td>
<td>43</td>
<td>6.2</td>
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<tr>
<td>111</td>
<td>505</td>
<td>325</td>
<td>22</td>
<td>4.9</td>
<td>3.0</td>
<td>82</td>
<td>11.5</td>
<td>40</td>
<td>5.7</td>
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<tr>
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<td>519</td>
<td>316</td>
<td>25</td>
<td>5.9</td>
<td>3.2</td>
<td>82</td>
<td>11.5</td>
<td>43</td>
<td>6.2</td>
</tr>
<tr>
<td>127</td>
<td>300</td>
<td>170</td>
<td>23</td>
<td>5.1</td>
<td>4.1</td>
<td>84</td>
<td>11.6</td>
<td>58</td>
<td>8.1</td>
</tr>
<tr>
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<td>500</td>
<td>340</td>
<td>23</td>
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<td>2.6</td>
<td>90</td>
<td>12.5</td>
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<td>5.4</td>
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<tr>
<td>116</td>
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<td>305</td>
<td>18</td>
<td>4.8</td>
<td>3.8</td>
<td>85</td>
<td>12</td>
<td>47</td>
<td>6.6</td>
</tr>
<tr>
<td>119</td>
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<td>220</td>
<td>35</td>
<td>5.1</td>
<td>3.6</td>
<td>85</td>
<td>12</td>
<td>54</td>
<td>7.5</td>
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<td>288</td>
<td>17</td>
<td>4.7</td>
<td>3.8</td>
<td>76</td>
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<td>57</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>Died</td>
</tr>
<tr>
<td>129</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died of pneumonia</td>
</tr>
</tbody>
</table>

The particularly interesting result of the observations is the fact that beginning 3 to 4 days prior to the death of the animal from scurvy, there appeared moderately increasing numbers of reticulocytes in the peripheral circulation and decreasing numbers of mature red blood cells. Data recorded in Table I show that there was a terminal rise in the reticulocytes from a low or normal level of about 0.2 per cent to as high as 9 per cent. Thus it seemed apparent that with failure of the bone marrow to maintain a normal number of erythrocytes in the
peripheral circulation, there was a release of cells of a relatively younger stage of maturation. This replacement, however, was only relative with regard to total numbers of cells and was never adequate in lessening the degree of anemia in the animal with scurvy. This would seem to suggest that a state of retarded erythrocyte maturation had been induced following the withdrawal of vitamin C-containing substances from the guinea pigs’ diet.

Effect of Diet Containing Vitamin C on Blood Formation

An example of the effect of vitamin C-containing food on blood formation when administered to an animal with scurvy, is shown in Text-figs. 1 and 2. The results obtained in the four other animals with scurvy were similar, following the ingestion of orange juice. Definite responses of the bone marrow indicated by the course of the reticulocytes were obtained promptly after the administration of 3 cc. of orange juice daily, and this usually began about the 3rd day after the treatment was started. The reticulocytes increased in number very rapidly and reached the peak of production within 5 to 7 days, and then subsided to within normal limits. Following the reticulocyte response, there was an increase in the red blood cell and hemoglobin concentrations and a disappearance of the signs of scurvy.

The duration, as well as the degree of the reticulocyte response, varied in each animal, so that the relation between the severity of the anemia and the peak of reticulocyte production could not be adequately studied. This is mentioned because it has been shown by Minot (10) and his associates that the reticulocyte response in patients with pernicious anemia is inversely proportional to the degree of anemia. An entirely comparable situation was not met with in our animals. In one animal with an anemia of 3,000,000 red blood cells per c.mm., the reticulocyte production was less pronounced than in another animal with a slightly greater number of red blood cells per c.mm. However, the rapid liberation of large numbers of reticulocytes in the peripheral circulation soon after the administration of orange juice, appeared significant, and would seem to indicate that a substance necessary for red blood cell production had been supplied. The effectiveness of the substance administered was further indicated by the subsequent return of the adult red blood cells and hemoglobin to normal concentrations.
TEXT-FIG. 1. An example typical of the response of the reticulocytes to vitamin C-containing orange juice in a guinea pig with scurvy.

TEXT-FIG. 2. Response of the reticulocytes to orange juice. Autopsy indicates time of removal of bone marrow illustrated in Fig. 3.
In order to gain further information concerning the effectiveness of vitamin C on blood formation, a contrast study was made of the cytological changes in the bone marrow of animals with scurvy before and after treatment. The medullary cavities in the long bones of animals that died with scurvy were filled with a uniform grayish red tissue that was somewhat soft in consistency. Specimens appropriately prepared showed, when examined under the microscope, a marked increased cellularity over the normal. An example of a specimen of bone marrow removed from a normal animal is shown in Fig. 1 and from an animal with scurvy in Fig. 2. There was an almost complete disappearance of fatty tissue from the scurbyc marrow which was replaced largely by cells of the erythropoietic series. Nucleated red blood cells appeared in large numbers and were mainly of the normoblastic variety. In spite of the increased numbers of cells present, there was but little evidence of active cellular maturation. Adult erythrocytes occurred in small numbers, and only an occasional mitotic figure was noted. The development of the cells seemed to be at a standstill.

A somewhat different cellular state was encountered in the bone marrow of three animals that were sacrificed during the reticulocyte response following the administration of vitamin C. The response obtained in one animal is shown in Text-fig. 2, and the character of the bone marrow removed from this animal is shown in Fig. 3. The most striking distinction between the bone marrow of the animals treated with orange juice and those not so treated was the difference in maturation of red blood cells. Adult erythrocytes appeared in much larger numbers in the bone marrow of the animals that had received orange juice therapy than in those dying with scurvy. In the animals showing a beginning remission, an increased number of cells containing mitotic figures were noted. Thus, there was distinct evidence of active red blood cell maturation in those treated with vitamin C; this substance is evidently required in order that a progressive development into mature cells can take place.

Sections of muscle, liver, spleen, lungs, heart, and kidneys showed no pathological change other than an occasional small hemorrhage.
DISCUSSION

It has been held by investigators that the altered rate of red blood cell formation in scurvy is dependent upon various factors. Undernutrition and chronic blood loss undoubtedly contribute to abnormal erythropoiesis, but in the animals studied in this investigation, it could not be demonstrated that sufficiently large hemorrhages had occurred in the various tissues to be responsible for the severe degree of anemia that appeared in most instances. In their report, Meyer and McCormick (7) suggested that increased blood destruction might be held responsible for the anemia of scurvy, but convincing evidence of abnormal destruction was lacking in their experiments. The observations made in the experiments recorded here show a decrease in the number of adult erythrocytes in the peripheral circulation and in the bone marrow and simultaneously the appearance of increasing numbers of immature cells in these situations as the scurvy progressed. This suggests that retarded erythropoiesis may be fundamental. This state of affairs would be comparable to a delay or retardation in maturation of the red blood cell. Such a hypothesis is substantiated by the reticulocyte response induced following the ingestion of a diet containing vitamin C by the animal with scurvy; the return of the bone marrow to a normal state of activity; and the appearance of normal numbers and kinds of red blood cells in the peripheral circulation.

CONCLUSIONS

1. Weight loss, progressive anemia, and a moderate increase in reticulated red blood cells occurred in seventeen guinea pigs on a diet deficient in vitamin C.

2. The histological changes of the bone marrow removed from guinea pigs with scurvy showed large numbers of erythrogenic cells, but scant evidence of active maturation to the adult erythrocyte.

3. A reticulocyte response was induced in guinea pigs with scurvy when fed orange juice daily.

4. The histological changes of the bone marrow removed from guinea pigs during the reticulocyte response showed large numbers of mitotic figures and relatively more adult red blood cells than in the
bone marrow from guinea pigs with scurvy that had not been treated with orange juice.

5. It is concluded from this study that the anemia of experimentally induced scurvy in the guinea pig is largely dependent upon vitamin C deficiency resulting in retarded maturation of the red blood cell.

BIBLIOGRAPHY


EXPLANATION OF PLATE 36

**Fig. 1.** Specimen of bone marrow removed from healthy guinea pig.

**Fig. 2.** Bone marrow from guinea pig with scurvy. Note marked cellularity and absence of mitotic figures.

**Fig. 3.** Bone marrow from guinea pig with scurvy removed during reticulocyte response to orange juice. Arrows denote mitotic figures.