Experimental Nutritional Megaloblastic Anemia and Scurvy in the Monkey

III. Protoporphyrin, Coproporphyrin, Urobilinogen and Iron in Blood and Excreta

By Elsa C. Proehl, M.A. and Charles D. May, M.D.

The early descriptions of scurvy comment upon the conspicuous pallor and the frequency of anemia. For many years consideration has been given to whether ascorbic acid is directly and specifically involved in hematopoiesis or is only indirectly a cause of anemia through hemorrhage or disturbance in metabolism of other nutrients. More precise knowledge of a disturbance in hematopoiesis may be obtained by examination of the bone marrow along with the peripheral blood. Reticulocyte response following therapy is an unreliable criterion for determining the specific relation of substances to anemia in scurvy because of the spontaneous bleeding and reticulocytosis characteristic of the disease. A limited number of descriptions of the bone marrow in human or experimental scurvy have been reported. Neither a consistent nor a definitive picture of the marrow or anemia in scurvy has developed from these studies. Scurvy in humans usually develops under circumstances that lead to the ingestion of a diet inadequate in several categories. One gains the impression that when anemia occurs in scurvy the type of marrow or the response to therapy depends on other deficiencies accompanying the deficiency of ascorbic acid.

The observations contained in this report were derived from a study of experimentally induced megaloblastic anemia accompanying ascorbic acid deficiency in the monkey. In reports of the nutritional and hematologic aspects of the experiments it was concluded that the megaloblastic pattern in marrow was due to a disturbance in the metabolism of pteroyl-glutamic acid (PGA), indicating an interrelationship with ascorbic acid. Besides the customary morphologic observations on the blood and marrow, which are included in separate reports, certain measurements relative to the metabolism of pyrrole pigments were made, namely erythrocyte and fecal protoporphyrin, erythrocyte, urinary, and fecal coproporphyrin, urinary and fecal urobilinogen, and plasma iron.

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We are indebted to Dr. Cecil J. Watson and Dr. Samuel Schwartz of the Department of Medicine for guidance in use of methods and helpful suggestions.
Measurements of the blood pigments were included to make the characterization of the experimentally induced anemia more complete and also to detect any effect of ascorbic acid or PGA either in the metabolism of pyrrole pigments or in hematopoiesis.

**PLAN OF EXPERIMENTS**

The monkeys used in these experiments (immature animals, about 15 months of age, averaging 2 Kg. in weight) were fed, as a basal diet, whole dried cows' milk reconstituted with water to normal composition and treated to destroy its ascorbic acid content. This was supplemented with iron, copper, vitamins A, D, and members of the B complex except PGA and B2. The animals thrive on this diet when adequate ascorbic acid is provided. Details of the diet and manifestations of the animals have already been published.7, 8 The numerical designations of the animals in the present report refer to the animals so designated in the earlier papers. When this basal dietary regimen is employed without supplementary ascorbic acid the monkeys become noticeably depleted in ascorbic acid in 30 days as revealed by retardation of growth, reduction in urinary ascorbic acid excretion to a minimum, and marked lowering of the concentration of ascorbic acid in tissues (liver and adrenals). Consequently the animals were classified as “ascorbic acid deficient” after 30 days without ascorbic acid. When clinical signs of scurvy appeared (periorbital hemorrhages, etc.), and while the marrow remained normoblastic they were designated as “scurburtic (normoblastic).” The marrow usually became megaloblastic after about two weeks of scurvy; then the animals were classed as “scurburtic (megaloblastic).” Our other studies have demonstrated that the appearance of a megaloblastic marrow is due to a deficiency of or disturbance in the metabolism of PGA. Monkeys fed simultaneously with the same basal diet and supplements plus ascorbic acid were termed “controls.”

The data to be presented were obtained from 21 controls, 13 ascorbic acid deficient, 14 scurbutic (normoblastic) and 7 scurbutic (megaloblastic) monkeys. The therapeutic or prophylactic effects of ascorbic acid, PGA, folic acid (citrovorum factor), vitamin B2, and iron compounds were also determined in the latter two groups in an effort to distinguish the contribution of each of these substances to the abnormalities which may be encountered during the progressive development of scurvy. It was possible to obtain rather complete serial determinations in two of the monkeys, before and after therapy, and these will be given separate consideration.

**METHODS**

**Porphyriins**

Free erythrocyte protoporphyrin was determined by the method of Grinstein and Wintrobe9 as modified by Schwartz10; the procedure was changed to include a measurement of erythrocyte coproporphyrin on the same sample. Coproporphyrin is extracted from the ethyl acetate just prior to the final extraction of erythrocyte protoporphyrin. This is done with 0.5 to 1.0 cc. quantities of 0.33N HCl, two to three times or until no fluorescence is seen in the acid wash. The acid extract is then diluted with distilled water to 0.13N HCl and shaken with small quantities of chloroform to remove any protoporphyrin taken out with the coproporphyrin. The chloroform is added to the ethyl acetate solution and the procedure for protoporphyrin is completed as described by Grinstein and Wintrobe. The 0.13N HCl solution of coproporphyrin is made to 0.33N by the addition of 1.65N HCl and fluorescence of the solution is measured in a fluorimeter* using the filter combination suggested by Schwartz, et al.12 In addition, all distilled water washings were replaced by a 3 per cent sodium acetate solution to avoid the loss due to the slight solubility of porphyrins in water.

The method employed for urinary coproporphyrin is the “five ml.” method of Schwartz, et al.13 Fecal protoporphyrins and coproporphyrins were measured with slight modifications.

* A special fluorimeter* with photomultiplier tube was made available to us through the kindness of Dr. S. Schwartz.
One gram of wet feces is triturated with a small amount of acetic acid and then extracted with ethyl ether. This is repeated until no red fluorescence is seen in the ethyl ether wash. The combined extracts are washed with a 3 per cent sodium acetate solution. The pH of the washings must lie between 4 and 7. If they are too acid they will remove porphyrins. In such a case the wash water is returned to the separatory funnel and a small quantity of saturated sodium acetate is added to raise the pH to the desired range. The ethyl ether is then extracted with 1.65 N HCl until no fluorescence is seen in the extracts. An aliquot is diluted to 0.13 N HCl, shaken with chloroform to remove protoporphyrin and deuteroporphyrins, neutralized with buffered acetic acid, and then extracted with ethyl acetate. From this solution, the coproporphyrin is removed with 0.33 N HCl and the fluorescence measured. The chloroform solution is diluted twenty to thirty times its volume with ethyl acetate and extraction of the protoporphyrin is made with 8.25 N HCl. Absorption of the solution is measured in the Beckman Spectrophotometer (Model DU) at a wave length of 411 m. and the concentration calculated from a standard curve. Fluorimetric measurement is preferred but at the time of these studies, conditions of fluorimetric stability had not been standardized.

Urobilinogen

The quantitative estimation of urinary and fecal urobilinogen was carried out according to the methods of Schwartz, Sborov and Watson.

Plasma Iron

A semi-micro method for the determination of plasma iron was employed, using o-phenanthroline as the color developing agent. The method described by Barkan and Walker was modified to deal with a smaller amount of plasma. Five-tenths cc. of plasma is pipetted into a small test tube, to which 0.25 cc. 0.4 N HCl is added. After thorough mixture is effected, the solution is incubated at 37 C. for at least one hour. Subsequently, 0.25 cc. 20 per cent trichloroacetic acid is added and after thorough mixing the suspension is allowed to stand for one hour. The precipitate is then spun down in a centrifuge for a half-hour at about 1500 r.p.m. The protein-free filtrate is decanted into a clean dry test tube and a maximum aliquot transferred to a glass stoppered volumetric tube calibrated at 2 cc. The solution is made up to 1.0 cc. by the addition of double distilled water. To this 0.25 cc. saturated sodium acetate, 0.25 cc. acetate buffer (pH 4.5) containing 1 per cent hydrazine sulfate, and 0.25 cc. 0.2 per cent o-phenanthroline in double distilled water are added. The solution is allowed to stand overnight (at least 12 hours) for color development. Before the colorimetric reading is made the solution is diluted to the 2.0 cc. mark with double distilled water. A blank is prepared with each run by placing a 0.5 cc. aliquot of a 1:1 mixture of 0.4 N HCl and 20 per cent trichloroacetic acid into a 2 cc. volumetric tube and continuing as with the unknown. Duplicate samples checked within ±10 per cent.

A standard curve is prepared by treating solutions of known concentration of ferric ion in the same manner as the unknown. The range of concentrations in the final 2 cc. volume was 12.5 to 100 gamma per cent ferric ion with the optimal range from 25 to 75 gamma per cent.

The absorption of the solution is read against the blank at a wave length of 509 m. in a specially constructed micro cell and housing fitted to a Model DU Beckman spectrophotometer. The cell is constructed out of a block of highly polished plastic (Lucite). Three grooves, measuring 1 mm. in width, 12 mm. in depth, and 50 mm. in length, are evenly spaced across the block. Highly polished plastic windows are held in place at either end of the cell block by steel plates machined with the plastic block when the grooves were cut. The cell is securely fitted into an adapter. This unit rests in the housing which replaces the cell housing of the Beckman spectrophotometer.

RESULTS

The data from the experimental animals obtained during the "control" period, the periods designated according to the above definitions as "ascorbic
### Table 1.—Data Obtained from Monkeys during the Experimental Periods Indicated

<table>
<thead>
<tr>
<th></th>
<th>Erythrocyte Protoporphyrin (μg. per 100 cc, packed cells)</th>
<th>Erythrocyte Coproporphyrin (μg.)</th>
<th>Plasma Iron (mg.%.)</th>
<th>Hemoglobin (Gm. %.)</th>
<th>Reticulocyte (%)</th>
<th>Fecal Urobilinogen mg./24 hrs.</th>
<th>Urinary Urobilinogen mg./24 hrs.</th>
<th>Urinary Coproporphyrin mg./24 hrs.</th>
<th>Fecal Coproporphyrin mg./24 hrs.</th>
<th>Fecal Protoporphyrin mg./24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
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</tr>
<tr>
<td>Average</td>
<td>31 (30)*</td>
<td>2.9 (5)</td>
<td>180 (9)</td>
<td>12.7 (32)</td>
<td>0.8 (28)</td>
<td>0.24 (18)</td>
<td>0.05 (13)</td>
<td>0.019 (18)</td>
<td>0.044 (12)</td>
<td>0.048 (10)</td>
</tr>
<tr>
<td>Range</td>
<td>7-62</td>
<td>2.4-3.5</td>
<td>110-287</td>
<td>10-14.1</td>
<td>0.2-1.2</td>
<td>.04-.74</td>
<td>.02-.17</td>
<td>.003-.048</td>
<td>.009-.096</td>
<td>.009-.133</td>
</tr>
<tr>
<td><strong>Ascorbic acid deficient</strong></td>
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<tr>
<td>Average</td>
<td>55 (22)</td>
<td>1.5 (4)</td>
<td>96 (15)</td>
<td>13.2 (18)</td>
<td>1.1 (20)</td>
<td>0.32 (17)</td>
<td>0.08 (10)</td>
<td>0.02 (12)</td>
<td>0.034 (8)</td>
<td>0.033 (8)</td>
</tr>
<tr>
<td>Range</td>
<td>30-85</td>
<td>1.2-1.94</td>
<td>45-220</td>
<td>10.4-14.4</td>
<td>0.4-2.8</td>
<td>.03-.80</td>
<td>.02-.16</td>
<td>.008-.020</td>
<td>.008-.044</td>
<td>.007-.062</td>
</tr>
<tr>
<td><strong>Scorbolute (normoblastic)</strong></td>
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<tr>
<td>Average</td>
<td>170 (32)</td>
<td>5.1 (16)</td>
<td>85 (22)</td>
<td>9.5 (31)</td>
<td>7.3 (26)</td>
<td>1.7 (22)</td>
<td>1.14 (11)</td>
<td>0.015 (4)</td>
<td>0.036 (3)</td>
<td>0.08 (3)</td>
</tr>
<tr>
<td>Range</td>
<td>75-396</td>
<td>1.3-9.8</td>
<td>40-176</td>
<td>5.1-10.7</td>
<td>2.6-11.3</td>
<td>.2-.2.5</td>
<td>.06-.25</td>
<td>.003-.024</td>
<td>.027-.052</td>
<td>.026-.115</td>
</tr>
<tr>
<td><strong>Scorbolute (megaloblastic)</strong></td>
<td></td>
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<tr>
<td>Average</td>
<td>154 (11)</td>
<td>5.0 (6)</td>
<td>90 (8)</td>
<td>7.3 (10)</td>
<td>8.6 (11)</td>
<td>1.2 (1)</td>
<td></td>
<td>0.04 (1)</td>
<td>.056 (1)</td>
<td>.100 (1)</td>
</tr>
<tr>
<td>Range</td>
<td>70-372</td>
<td>2.4-35</td>
<td>20-183</td>
<td>4.4-8.6</td>
<td>1.3-32</td>
<td></td>
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</tbody>
</table>

* Figures in parentheses are the number of determinations included in the average value and the range.
acid deficiency," "scorbutic (normoblastic)" and "scorbutic (megaloblastic)"
are summarized in table 1.

It may be seen that as scurvy progresses there are no marked variations from
the controls in the excretion of coproporphyrin or protoporphyrin in any period.
The average erythrocyte protoporphyrin level which increased slightly during
the "ascorbic acid deficiency" period shows a five-fold increase over the controls
during the "scorbutic (normoblastic)" period. The erythrocyte protoporphyrin
levels in the "scorbutic (megaloblastic)" period indicate that the advent of the
megaloblastic pattern in the marrow has no additional effect.

The wide range of the values of erythrocyte protoporphyrin which are aver-
aged in table 1 can be understood if the individual determinations are plotted
in relation to the duration of ascorbic acid deficiency and scurvy as in figure 1.
It is clear that there is an abrupt trend towards higher values in the scorbutic
periods. Also, one may note the corresponding abrupt fall of hemoglobin and
increase in fecal urobilinogen which occurs simultaneously with the clinical
signs of hemorrhage due to scurvy. This leads to the consideration that the
advent of hemorrhage into the tissues, intestine and bladder in the scorbutic
periods produces a profound effect on the values obtained for the blood pig-
ments as ascorbic acid deficiency progresses. This same consideration must be
kept in mind in regard to all the determinations.

The average erythrocyte coproporphyrin level also shows a rise above the
"controls" in the scorbutic periods. Schwartz' and Watson' have found that
erthrocyte coproporphyrin in general varies directly with the reticulocyte
count. The coproporphyrin usually was elevated above control values during
reticulocytosis. That strict parallelism with the reticulocyte count was not
found may be due to measurements not being frequent enough to detect the
peak values of erythrocyte coproporphyrin in relation to the peak of the reticulo-
cytosis.

Urobilinogen excretion in the urine and feces is not greatly increased above
the control level in the "ascorbic acid deficiency" period. During the scorbutic
periods average fecal excretion of urobilinogen shows a five-fold increase. This
rise may be attributed to increased destruction of blood which extravasated
into the tissues. Urinary excretion of urobilinogen in the scorbutic periods is
elevated only slightly above the controls. This may be interpreted as indicating
that liver function was adequate to cope with the increased load of blood pig-
ment presented for excretion.

Now consideration may be given to serial determinations made before and
after treatment in 2 monkeys. Figure 2 illustrates the course of events in Monkey
No. 51 maintained in a scorbutic condition for a considerable length of time.
Megaloblastic anemia developed on the eighteenth day of scurvy. Treatment
with folinic acid, which caused a remission of the megaloblastic anemia but did
not affect the scurvy, was instituted on the thirtieth day of scurvy and continued
until the forty-seventh day when the animal was killed. Erythrocyte protopor-
phyrin rose to 235 gamma per cent in the scorbutic periods and remained at
this level until the time of treatment. Two days after institution of therapy a
marked increase in reticulocytes occurred. The erythrocyte protoporphyrin then
rose to 455 gamma per cent and remained close to this value until the end of
the study. The reverse is true of plasma iron levels: as the protoporphyrin levels rose the iron values fell. Hemoglobin concentration began to decline after the onset of scurvy, and had fallen to 5.5 Gm. per cent when therapy was begun.

In the following seventeen days the level rose 1 Gm. per cent. The mean corpuscular hemoglobin concentration showed a downward trend after therapy and the marked reticulocytosis. The MCHC was 28 before therapy and 23 after seventeen days of therapy. This lowering of the mean corpuscular hemoglobin concentration is consistent with a lowered availability of iron for hemoglobin.
synthesis. Furthermore, the erythrocytes in this period appeared hypochromic. It can also be seen in figure 2 that fecal urobilinogen excretion became very high during scurvy, probably indicative of destruction of blood which had extravasated into the tissues. Erythrocyte coproporphyrin rose during the reticulocytosis.

Monkey No. 33 (fig. 3) developed megaloblastic anemia ten days after the onset of scurvy. At this time therapy with ascorbic acid was begun. The duration of the scurvy, was, therefore, shorter than in the case of Monkey No. 51. Its erythrocyte protoporphyrin level just prior to treatment was only slightly

![Experimental Megaloblastic Anemia](image)

Fig. 2.—Monkey No. 51 fed the basal diet without ascorbic acid, treated when megaloblastic with 30 μg. folinic acid daily. (See reference 8 for complete protocol.) Note erythrocyte protoporphyrin increases as plasma iron decreases; concomitantly, as hemoglobin declines fecal urobilinogen increases. Deductions concerning relationships between time of occurrence of peak values of reticulocytes, E.P. and E.C.P. cannot be made from this chart as the measurements are not sufficiently frequent to assure the peak values were detected.

Elevated (80 gamma per cent packed red cells) and plasma iron was within normal limits. Five days after administration of ascorbic acid was begun a marked reticulocytosis occurred; erythrocyte protoporphyrin then rose though the plasma iron remained normal. This is to be expected because reticulocytes contain considerably more protoporphyrin than mature erythrocytes. Hemoglobin formation took place readily; in five days the level rose 4 Gm. per cent and within the next ten days it had reached 12.9 Gm. per cent. In this animal in which the plasma iron remained normal the mean corpuscular hemoglobin concentration also remained normal (30 before therapy and 32 after fifteen days therapy).
Three monkeys (Nos. 30, 37, 39) were maintained on a scorbutogenic diet and given PGA prophylactically. Scurvy developed in the usual time. As seen in table 2 after severe scurvy had persisted for twenty-five days while the marrow remained normoblastic the erythrocyte protoporphyrin was elevated, the plasma iron decreased, and the fecal urobilinogen excretion increased, just as in the "scorbutic (megaloblastic)" animals. The erythrocytes in the peripheral blood were hypochromic. As these animals were not deficient in PGA, the abnormal

values in the scorbutic megaloblastic monkeys (table 1) must be attributed to the effects of ascorbic acid deficiency.

A principal effect of the ascorbic acid deficiency may well be due to the hemorrhage into the tissues in scurvy. Data concerning the fecal excretion of urobilinogen in five scorbutic monkeys is presented in table 3. Four day collections of feces were analyzed at intervals as the signs of scurvy appeared and progressed. The outpouring of urobilinogen in the feces coincided with the appearance of periorbital hemorrhage. The periorbital hemorrhage was seen clinically to occur in bursts and tended to subside between bursts. The undulations in the excretion of urobilinogen in the feces correspond to the appearance

![Experimental Megaloblastic Anemia](image-url)
of hemorrhages. Unchanged bilirubin was demonstrable in only one instance in fecal collections in the scorbutic period.

Just before the appearance of scurvy 3 monkeys were found to have low levels of plasma iron. At this time 30 mg. of Fe (as saccharated iron oxide) were given intravenously to one and 50 mg. to the other two. When scurvy appeared

<table>
<thead>
<tr>
<th>TABLE 2.—Average Values in Monkeys 30, 37 and 39 Twenty-five Days After Onset of Scurvy. PGA Was Given Prophylactically and the Marrows Remained Normoblastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocye Protoporphyrin mg./100 cc. packed/cells</td>
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<td>-------------------------------------------------</td>
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<tr>
<td>260</td>
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<thead>
<tr>
<th>TABLE 3.—Fecal Urobilinogen Excretion in Scurvy* (mg. per 24 hours)</th>
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<tbody>
<tr>
<td>Days Without Ascorbic Acid</td>
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<tr>
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<tr>
<td>45</td>
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<tr>
<td>50</td>
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<td>55</td>
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<td>110</td>
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<tr>
<td>115</td>
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<tr>
<td>120</td>
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</tbody>
</table>

* Values are average per day obtained from a four-day collection. Controls excreted an average of 0.24 mg. per day. (See table 1)
† Clinical appearance of first periorbital hemorrhages.

about a week later the plasma levels of iron were again determined in these monkeys and were found to be as low as before intravenous iron was given.

**Discussion**

Wohlbach and Bessey have described the tissue changes occurring in ascorbic acid deficiency. They regard these as being due to a failure of formation and maintenance of intercellular material with consequent weakness of supporting tissues. These changes offer an explanation of the occurrence of tissue hemorrhages in scurvy. In scorbutic monkeys we also observed an external loss of blood.
from the gums, intestine and bladder. The decline in hemoglobin values paralleled the severity of the scurvy. With the external loss of whole blood there would be a loss of hemoglobin iron. The manifestations noted in the scorbutic animals pointing to iron deficiency are: a lowered plasma iron, elevated erythrocyte protoporphyrin, hypochromic cells and a decrease in mean corpuscular hemoglobin concentration.

In the megaloblastic anemia of pernicious anemia the erythrocyte protoporphyrin is not elevated. The elevation of the erythrocyte protoporphyrin we found in the majority of the scorbutic-megaloblastic monkeys may be correlated with the low plasma iron and the elevated reticulocyte values associated with the hemorrhage of scurvy. By reference to Monkey No. 33 (fig. 2) it may be seen that when the plasma iron had not become markedly lowered by the time megaloblastosis developed the erythrocyte protoporphyrin was more nearly normal.

It is noteworthy that even though supplementary oral iron was included in the diets of these experimental animals prior to and during the development of scurvy, the plasma level of iron could not be maintained. It has been shown that ascorbic acid enhances the absorption of iron in normal individuals. The decline in plasma iron levels in the “ascorbic-acid-deficient” period before clinical signs of scurvy and hemorrhage or increased fecal urobilinogen were observed may be a reflection of defective absorption of iron. It is also possible that other factors that cause a decrease in plasma iron such as those operating in chronic infection are responsible. Studies are in progress in our laboratory concerning the functional capacity of the adrenal glands in scorbutic monkeys which may have a bearing on the metabolism of iron in such animals.

**Conclusions**

A megaloblastic type of anemia developed during scurvy in monkeys on a diet devoid of ascorbic acid and containing only a small amount of folic acid.

Supplementary folic acid prevented megaloblastosis during scurvy and then a normoblastic anemia developed.

As scurvy progressed hemoglobin and plasma iron decreased, while erythrocyte protoporphyrin and coproporphyrin increased. Fecal urobilinogen became elevated in association with clinical signs of tissue hemorrhage. No significant change was noted in urinary urobilinogen and coproporphyrin, or fecal coproporphyrin and protoporphyrin. The most likely explanation for these findings is the development of iron deficiency because of loss of blood externally from the gums, intestine and urinary tract. Defective absorption of iron from ascorbic acid-deficient diets is another factor to be considered.

The requirements for two important hematopoietic substances, folic acid and iron, are apparently increased during scurvy. Either megaloblastic or normoblastic anemia may be encountered in scurvy depending upon which deficiency predominates. This will be determined by various conditioning factors such as composition of the diet, duration of the scurvy, the extent of blood loss, etc.

No direct specific function of ascorbic acid in hematopoiesis, comparable to that of folic acid or iron, has been detected by these experiments.
ADDENDUM

After this manuscript was submitted for publication additional information was obtained by determining the concentration of iron in the livers of control and scorbutic monkeys.

Samples of liver from 5 control monkeys, 4 which had been scorbutic for thirty to thirty-five days, and 4 which had been scorbutic for thirty-five to fifty-five days were pooled and homogenized. Aliquots of the homogenates were analyzed for iron by the method of Schaefer. Five to seven analyses were done on the aliquot from each pool with the following results:

<table>
<thead>
<tr>
<th></th>
<th>Average mg. Fe/100 gm. wet liver</th>
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<tbody>
<tr>
<td>Controls</td>
<td>23.3</td>
</tr>
<tr>
<td>Scorbatic 30-35 days</td>
<td>16.9</td>
</tr>
<tr>
<td>Scorbatic 35-55 days</td>
<td>16.8</td>
</tr>
</tbody>
</table>

* Statistical analysis revealed the difference between the control and scorbutic averages to be significant at the 1 per cent level in both cases.

As noted previously both control and scorbutic monkeys received 25 mg. Fe++ as a daily supplement to the diet throughout the experiments.

These data are in agreement with the other indications pointing toward the development of iron deficiency in these scorbutic monkeys.

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