NUTRITIONAL CYTOPENIA IN MONKEYS RECEIVING THE GOLDBERGER DIET*

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(Received for publication, June 25, 1940)

In 1938 (1, 2) we reported detailed experiments on monkeys which developed nutritional cytopenia while receiving a diet of purified foodstuffs. Supplementing this diet with ascorbic acid, thiamin chloride, riboflavin, and nicotinic acid failed to prevent the leucopenia, anemia, gingivitis, diarrhea, and death. Consequently, we suggested the existence of an unidentified component of the vitamin B complex, and we tentatively proposed the term vitamin M for this nutritive factor required by the monkey. Topping and Fraser (3) and Tomlinson (4) have reported careful studies of mouth lesions in monkeys receiving diets deficient in the various vitamins of the B complex, as well as in other vitamin deficiencies. Although their interest was primarily in the oral pathology, they reported finding some anemia associated with a moderately severe leucopenia in the monkeys receiving the vitamin B2 complex deficient diet. Janota and Dack (5) reported experiments on rhesus monkeys given our diet 600, supplemented in various ways. Leucopenia and diarrhea were encountered in animals receiving the deficient diet, and "Bacterium dysenteriae (Flexner)" was isolated from the stools of most of the animals in the terminal stages of the deficiency.

In our previous paper (2) we reported hematological data on one monkey

* Research Paper No. 523, Journal Series, University of Arkansas. Read before the sixth annual meeting of the American Institute of Nutrition, Toronto, April 26, 1939. Aided by grants from the Ella Sachs Plotz Foundation, the Committee on Scientific Research of the American Medical Association, and the Committee on Meat Board Grants of the National Research Council. The authors are indebted to Dr. B. A. Rhinehart and Miss Ila Wright for the x-ray examination of certain monkeys. The authors wish to acknowledge the assistance of the following companies in supplying liberal quantities of the materials indicated: liver extract and thiamin chloride from Eli Lilly and Company; riboflavin from Merck and Company and from the Department of Medical Research of Winthrop Chemical Company; and cod liver oil from the E. L. Patch Company.
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(No. 28) which was given a slight modification of the Goldberger diet. In this present report we are presenting data on 17 monkeys which received this same modification of the Goldberger diet, supplemented in various ways.

EXPERIMENTAL

The young immature monkeys (*Macaca mulatta*) were purchased from animal dealers, as in our previous experiments. Our methods of selection of animals, caging, care, and feeding have been described previously (2, 6). Radiographic examinations of the chests of many of the animals were made when they were received. The techniques for blood determinations were the same as those described formerly (7).

The diet given these animals was a modification of the Goldberger black tongue-producing diet No. 268 (8). The quantities fed each monkey per day are as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, white, ground</td>
<td>40</td>
</tr>
<tr>
<td>Cowpeas, black-eyed, ground</td>
<td>5</td>
</tr>
<tr>
<td>Casein, washed with dilute alcohol</td>
<td>10</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>3</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>3</td>
</tr>
<tr>
<td>Salt mixture, Hubbell, Mendel, and Wakeman</td>
<td>3</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2</td>
</tr>
</tbody>
</table>

The ingredients, except for the cottonseed oil and cod liver oil, were cooked in an enameled boiler for 2 hours. After cooling, the cottonseed oil and cod liver oil were added.

As described in the discussions of individual animals, various supplements were given to this basal diet. 10 mg. of ascorbic acid daily was fed in tablet form, except as otherwise indicated. Thiamin chloride, when given, was fed daily as tablets containing 1 mg. In most cases riboflavin was administered parenterally in a solution which was supplied to us in sterile ampules by the manufacturer. In certain cases, riboflavin was given orally in tablets containing 2 mg. Nicotinic acid was given orally in two different ways: certain animals were given 10 mg. amounts daily which were added to the basal diet before feeding in the form of aliquots of a standard solution; other animals received tablets containing 50 mg. each, three times a week. Certain animals were given parenteral injections of nicotinamide daily in the form of a sterile solution. The amounts of these addenda and the modes of administration are explained in the discussions of individual animals. Two samples of nicotinic acid were purchased from Eastman Kodak Company and one sample, which had previously been shown to be effective in curing black tongue in dogs, was kindly supplied by Dr. W. J. Dann. A part of the nicotinamide used was synthesized in this laboratory, and part of it was purchased from the S. M. A. Corporation. A sample of this latter preparation was kindly used by Dr. Dann and found to be effective in curing black tongue in dogs.

1 Casein, edible, muriatic, manufactured by the Casein Company of America; washed with dilute alcohol in this laboratory according to the method of Sherman and Spohn (9). Monkeys 5-9, 6-3, 6-5, 6-8, 7-2, and 7-4 received the Casein Company's Labco brand of vitamin-free casein during the latter part of the experiments.
RESULTS

The hematological and other data on 11 of the animals are presented here as charts and these data are discussed in the following paragraphs. The data for the 6 animals which are not given in detail in this report are briefly discussed.

Monkeys 4-8 (Not Illustrated) and 4-9 (Fig. 1).—These animals received the Goldberger diet supplemented with 10 mg. of nicotinic acid daily, mixed with the diet. From Fig. 1 it will be seen that the blood of monkey 4-9 exhibited a progressive decrease in all cellular elements after the 55th day, and death occurred on the 85th day. During the last 2 weeks of experiment 7 blood counts were made, and 6 of these counts showed the total number of white blood cells to be less than 2,500 per c.mm. The erythrocytes totaled 2,000,000 or less per c.mm. in 4 of these counts. Monkey 4-8 received the same diet and supplements and showed a similar blood picture. The survival period was 89 days. Data for this monkey are not presented in detail here.

It is evident from these two experiments that the Goldberger diet supplemented with 10 mg. daily of nicotinic acid is not adequate for the maintenance of life or a normal blood picture in the young monkey.

Monkeys 4-7 (Fig. 2).—This monkey received the Goldberger diet without supplement. As will be seen from the chart, there was a progressive decrease in all cellular elements, so that by the 94th day the erythrocyte count had fallen to one million per c.mm., the hemoglobin to about 3 gm. per 100 cc., and from the 78th to the 94th day 8 total white...
counts had been below 3,000, and one was as low as 1,000 per c.mm. The stools were watery and some edema of the face was evident.

On the 95th day supplements of 5 gm. daily of liver extract (Lilly) were mixed with the diet. This was followed by a marked increase in white cells and a reticulocytosis, the reticulocytes reaching a maximum of 28 per cent on the 101st day (6 days after liver feeding was started). The erythrocytes and hemoglobin slowly returned to normal and the facial edema subsided. The stools were formed on the 4th day following liver feeding.

On the 112th day of experiment the gums of the monkey appeared spongy, and upon handling the animal gave evidence of muscular pain. We interpreted these findings as possible scurvy, and consequently added 50 mg. of ascorbic acid daily to the diet. This supplement was followed by the disappearance of the gum changes and the evidence of muscular tenderness. It should be pointed out, however, that the improvement in blood picture followed liver feeding, in the absence of an antiscorbutic agent.

The supplement of liver extract was subsequently reduced to 2 gm. daily and the supplement of ascorbic acid to 10 mg. daily. The animal is still alive and healthy with a normal blood picture, on the 630th day of experiment.

Monkey 5-5 (Not Illustrated).—This animal received the Goldberger diet supplemented with 10 mg. of ascorbic acid, 1 mg. of thiamin chloride, and 2 gm. of liver extract daily. It is still alive and in excellent condition after 580 days on such a regimen. During this period 82 complete blood counts were made, and all showed the blood picture to be normal without question. The total white blood cell counts were between 11,000 and 26,000 per c.mm., the total erythrocyte counts were between 4.8 and 6.6 million per c.mm., and the hemoglobin levels were between 11.1 and 15.1 gm. per 100 cc. of blood. All of these values fall within the normal ranges as previously established.
for young monkeys of our colony (7). During this period the animal has gained in weight from 2,530 gm. to 6,768 gm., and dentition has proceeded normally with loss of deciduous teeth and eruption of permanent teeth. During the first 583 days of this experiment the monkey received the daily quantity of basal diet indicated in an earlier paragraph, and during that time gained in weight from 2,530 gm. to 4,467 gm. There seems little doubt, therefore, that the amount of basal diet fed in these experiments is adequate to meet the energy and protein needs of monkeys weighing 2 to 3 kg. Since the 483rd day the monkey has received double portions of the basal diet, and, as would be expected, the gain in weight has been more rapid. There has been no evidence of diarrhea, ulceration of the gums, or edema of the face. The absence of diarrhea deserves comment, since the animal has been in the same room with animals receiving

![Graph](image)

**Fig. 3. Data on monkey 6-2.**

the deficient diet without liver, most of which developed diarrhea, and among which several exhibited signs of dysentery and yielded pathogenic organisms on stool culture.

*Monkey 6-2 (Fig. 3).—This experiment was designed to indicate whether or not we were dealing with a deficiency of an inorganic element. Other experiments have demonstrated (monkeys 4-7 and 5-5) that the Goldberger diet with daily supplements of 10 mg. of ascorbic acid and 2 gm. of liver extract is capable of maintaining health and growth and a normal blood picture over long periods of time. Monkey 6-2 received the Goldberger diet plus daily supplements of 10 mg. ascorbic acid, 1 mg. of thiamin chloride, and the ash of 4 gm. of liver extract. This ash of liver extract was prepared by heating weighed amounts of the powder in silica crucibles in an electric muffle furnace. The resulting white or grayish-white ash was treated with sufficient dilute hydrochloric acid to dissolve all of the readily soluble part of the ash, and the resulting digest (both soluble and insoluble parts) was mixed with the monkey’s diet before feeding. The animal showed the typical progressive cytopenia, and died on the 106th day.
The Hubbell, Mendel, and Wakeman salt mixture, in the amounts fed (3 gm. daily), is presumably adequate to supply all of the inorganic elements needed by the monkey. This experiment is further evidence that the deficiency leading to nutritional cytopenia and death in our animals is not the result of a deficiency of an inorganic element.

*Monkey 6-0 (Fig. 4).*—This animal received the Goldberger diet supplemented with 1 mg. of thiamin chloride and 50 mg. of ascorbic acid daily. It exhibited a progressive anemia, mild leucopenia, necrotic gums, and diarrhea. Just before death there was a pronounced increase in the leucocyte cell count to 30,000 per c.mm., most of the cells being granulocytes. The animal developed a bloody diarrhea on the 102nd day, and *Shigella paradysenteriae* was isolated from the stools.

Since this animal received relatively large amounts of ascorbic acid, but nevertheless developed nutritional cytopenia, it would seem to indicate that vitamin C deficiency is not the cause of changes seen in our animals.

*Monkey 5-6 (Fig. 5), Monkey 5-8 (Not Illustrated), and Monkey 6-8 (Fig. 6).*—These animals received essentially the same diet and exhibited similar pictures, so will be considered together. Each received the Goldberger diet supplemented with 10 mg. of ascorbic acid and 1 mg. of thiamin chloride daily. Monkey 5-6 received in addition 20 mg. nicotinamide parenterally daily, monkey 5-8 received 4 mg., and monkey 6-8 received 40 mg. nicotinamide in the same manner. Monkeys 5-6 and 6-8 each developed mild anemia, with the lowest erythrocyte counts around 3 million per c.mm. and lowest hemoglobin values about 8 gm. per 100 cc. Before death monkey 6-8 had a white cell count of 3,000 per c.mm., while monkey 5-6 showed a low count of 700 white cells per c.mm. Both of these exhibited necrotic areas on the gums, and No. 5-6 developed a diarrhea. Attempts to isolate pathogenic organisms from the stools failed, however.
Monkey 5-8 died on the 18th day of experiment with necrotic gums, bloody diarrhea, and upon autopsy, changes in the colon suggestive of dysentery were found. It did not develop an anemia but the white cell count before death was 2,000 per c.mm. Monkey 6-8 developed an edema of the lips before death, but did not exhibit diarrhea at any time during the experiment.

It is evident from these three experiments that relatively large doses of nicotinamide given parenterally did not protect the animals from the nutritional cytopenia.

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2 Anhaemin is a preparation of the anti-pernicious anemia factor, which the manufacturers state was prepared by the method of Dakin, Ungley, and West (11). This was kindly supplied by the British Drug Houses.
anemia, but a decided leucopenia before death, the final white count being 2,000 cells per c.mm. The gums were necrotic and a diarrhea was present for several days before death.

Monkey 7-3 (Fig. 7).—This animal received daily supplements of 10 mg. of ascorbic acid and 1 mg. of thiamin chloride by mouth, and 1 mg. of riboflavin parenterally. It exhibited the usual picture of leucopenia, anemia, gingivitis, diarrhea, and death. A stool culture taken on the day of death showed the presence of *Shigella paradysenteriae*.

It is obvious that riboflavin in conjunction with ascorbic acid and thiamin did not prevent any of the signs of the deficiency.

The 6 animals discussed in the following paragraphs (5-7, 6-4, 5-9, 6-5, 7-2, 7-4) each re-
Monkey 5-7 (Fig. 8).--In addition to the ascorbic acid and thiamin chloride, this animal received 20 mg. of nicotinic acid daily and 1 mg. of riboflavin weekly. It developed mild anemia and leucopenia, diarrhea, and necrosis of the gum margins, and died on the 72nd day. This experiment was complicated by pulmonary tuberculosis.

Monkey 6-4 (Fig. 9).--From the beginning of experiment this monkey received ascorbic acid, thiamin chloride, 10 mg. of nicotinic acid daily, and 1 cc. of Anhaemin weekly, the latter given parenterally. There was a marked anemia and leucopenia, as shown in the chart, the erythrocytes falling to 2 million per c.mm., and the white blood cells to a low of 2,600 per c.mm. On the 54th day of experiment parenteral administration of riboflavin was begun, and thereafter the animal received 3 doses of 1 mg. each in that manner each week. The riboflavin administration was followed by an increase in hemoglobin and erythrocytes, but death occurred on the 81st day, nevertheless. The animal did not exhibit diarrhea at any time during the experiment, but did have an edema of the lips and face from the 59th day of experiment on.

Monkey 6-5 (Fig. 10).--This animal was given at the outset the Goldberger diet supplemented with 10 mg. of ascorbic acid and 1 mg. of thiamin chloride daily. There was a moderate progressive decrease in erythrocytes, hemoglobin, and leucocytes. On the 54th day the blood picture was as follows: erythrocytes, 3.19 million per c.mm.; hemoglobin, 8.42 gm. per 100 cc.; and white blood cells, 7,000 per c.mm. At this point in the experiment parenteral administrations of 40 mg. of nicotinamide daily and 1 mg. of riboflavin twice weekly were started. There was an increase in reticulocytes to a peak of 8.5 per cent of circulating erythrocytes on the 63rd day (9th day following administration of nicotinamide and riboflavin). As the reticulocyte response subsided the erythrocytes and hemoglobin slowly increased to levels in the lower normal range, where they remained until death of the animal.

Following the administration of nicotinamide and riboflavin there were several transitory increases in total white cell counts, which may be, but probably are not, significant.
Fig. 10. Data on monkey 65. "

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The curve for total white cells then flattened out, showing numerous counts between 5,000 and 7,000 per c.mm. During the final week of experiment the total white cell count fell to 2,000 per c.mm., and the animal showed some recession of gums and diarrhea. The animal died on the 218th day.

*Monkey 5-9 (Not Illustrated).*—This experiment was very similar to the one on monkey 6-5. From the start of experiment the monkey received 10 mg. of ascorbic acid, 1 mg. of thiamin chloride, and 40 mg. of nicotinamide daily, the latter given parenterally. A mild anemia and leucopenia developed. On the 58th day parenteral injections of riboflavin were begun, and thereafter the monkey received 2 mg. each week by this route. This was followed by a reticulocyte increase to 4.2 per cent, and an increase of erythrocytes to about 4 million per c.mm. The level of hemoglobin remained between 7 and 8 gm. for the greater part of the experiment, however, thus giving evidence of a mildly hypochromic anemia. 59 complete blood counts were made on this animal. They showed a consistent mild leucopenia after the first few weeks of experiment, most of the white counts being between 3,000 and 8,000 per c.mm. The animal developed a diarrhea on the 406th day and died on the 419th.

*Monkey 7-2 (Fig. 11).*—This animal received daily supplements of 10 mg. of ascorbic acid and 1 mg. of thiamin chloride by mouth, and 40 mg. of nicotinamide and 1 mg. of riboflavin parenterally. This animal maintained normal erythrocyte and hemoglobin levels. After the 43rd day, however, the white cell count remained at about 7,000 per c.mm. until 2 days before death, when a count showed only 1,700 white cells per c.mm. The terminal condition was characterized by diarrhea and loss of appetite. *Shigella paradysenteriae* was isolated from the stools on the 87th day and from an ulcer in the colon at autopsy.

*Monkey 7-4 (Not Illustrated).*—This animal received 10 mg. of ascorbic acid and 1 mg. of thiamin chloride daily, and three times a week received 2 mg. of riboflavin and
50 mg. of nicotinic acid in the form of tablets. This monkey did not exhibit an anemia, and only a very mild leucopenia. A bloody diarrhea developed on the 64th day and the animal died on the 66th. Although a stool culture failed to yield pathogens, it is possible that bacillary dysentery was a contributing factor in the death of the animal.

**DISCUSSION**

It is evident from these experiments that monkeys which were given the Goldberger diet developed a condition characterized by leucopenia, anemia, gingivitis, diarrhea, and ultimate death. This syndrome appears identical with that observed in monkeys which were given our diet of more or less purified foodstuff (1, 2, 6), and which we have designated by the term "nutritional cytopenia." Although it cannot be said to be proved, it appears probable that both diets are deficient in the same essential substance, which we have termed vitamin M. The average survival period for animals receiving the Goldberger diet was somewhat longer than for monkeys receiving diet 600, indicating that the Goldberger diet may contain a small amount of that essential substance.

It appears clear from the data presented here that a combination of ascorbic acid, thiamin chloride, and nicotinic acid (or amide) is incapable of preventing the syndrome. Also a combination of ascorbic acid, thiamin chloride, and riboflavin is likewise incapable of preventing the nutritional cytopenia. However, at least in certain animals receiving the Goldberger diet, a combination of nicotinic amide and riboflavin (with ascorbic acid and thiamin chloride) seemed to maintain erythrocyte and hemoglobin levels at or just under the lower normal levels (monkeys 5-9, 6-5, 7-2). In fact, the addition of riboflavin and nicotinamide to the diet of animals with mild anemia (5-9, 6-5) resulted in distinct evidence of stimulated erythropoiesis. However, the combination of nicotinamide and riboflavin was incapable of maintaining normal leucocyte levels, and the animals all ultimately succumbed to the deficiency.

An animal with profound anemia and leucopenia (monkey 4-7) recovered on the addition of a crude liver extract to the diet, and this animal is still alive and in excellent health after 2 years. Another animal (monkey 5-5) maintained a normal blood picture, and doubled its weight in 530 days on the Goldberger diet supplemented with liver extract, ascorbic acid, and thiamin chloride. It is thus evident that the Goldberger diet supplemented with ascorbic acid, thiamin chloride, and liver extract in the amounts given is adequate for the maintenance of a normal blood picture, and for health, growth, and development of the young rhesus monkey.

On the other hand, a highly purified preparation of the anti-pernicious anemia fraction of liver (Anhaemin), which was stated by the manufac-
turer to be prepared by the method of Dakin, Ungley, and West (11), did not protect against nutritional cytopenia or significantly prolong life (monkeys 6-3, 6-4). This would appear to indicate that we are not dealing with a deficiency of the anti-pernicious anemia factor. It is interesting to note that Wills and Evans (12), likewise found Anhaemin ineffective in their experimental anemia in the monkey and in tropical macrocytic anemia in man.

We do not know to what extent the presence of bacillary dysentery and other possible pathogenic organisms has been a contributing factor in the changes seen in our animals. Although stool cultures were made on several animals which exhibited severe diarrhea, such examinations were not made as frequently as in the studies of Janota and Dack (5). The experiments of those investigators "suggest that Bact. dysenteriae (Flexner) may live a saprophytic existence being present in the intestine in numbers which escape detection, even where a better type of selective medium . . . is used for isolation." Consequently, many of our experimental animals may have been infected with bacillary dysentery even though we isolated pathogenic organisms from only 3 animals. In this connection it is interesting to note that Topping and Fraser (3) reported that all of their 16 monkeys receiving the vitamin B_2 complex deficient diet "developed a severe watery diarrhea, yet in only 2 of these was a possible etiological cause demonstrated." Elsewhere (page 419) they state that Shigella paradysenteriae was recovered from the stools of certain animals. The experiments of Janota and Dack strongly suggest that vitamin M deficiency may be the predisposing factor in the development of the dysentery infection in such experiments in monkeys. It has been our observation that the leucopenia usually precedes any clinical manifestations of dysentery, when such signs are seen. We have never succeeded in isolating pathogens from animals which did not exhibit diarrhea. Three of the 16 animals in this series (monkeys 4-8, 6-4, 6-8) failed to develop a diarrhea at any time during the experiments, although each exhibited a marked leucopenia before death. Further studies are needed to clarify the relationship between the deficiency, the infection, and the blood picture.

All of the animals in this series which did not receive liver extract exhibited some pathological changes of the gums. This varied from mild recession of the gingival line in some animals to extensive necrosis of the gums in others. None of the animals in this particular series showed extensive involvement of the cheek such as that described by Topping and Fraser (3) and termed noma. We have, however, seen such a case of noma in a monkey receiving our diet 600 in a series of experiments as yet
unreported. As with the dysenteric infection, it is difficult with the evidence available at present to evaluate the relationship between the deficiency, the oral lesions, and the blood picture. This much seems evident, however: a deficiency of what we term vitamin M results in a lowered resistance to infection of the mucosa of the alimentary tract. Thus, one may observe lesions of the mouth or large intestine, or of both, which appear to be brought about by organisms which would seem to have been present in those locations prior to the deficient feeding, but which were not causing pathological changes.

Other laboratories have reported anemia or anemia and leucopenia in monkeys receiving diets deficient in one or more of the unidentified B vitamins (13-16). Somewhat similar experiments have been reported on dogs (17-20), on rats (21-25), on pigs (26-29), and on pigeons (30, 31). It is impossible to say at the present time which of these experimental blood dyscrasias may be the result of the deficiency that we are dealing with in our experiments on the monkey.

**SUMMARY**

Experiments are reported upon young *rhesus* monkeys which were given a diet essentially the same as the Goldberger black tongue-producing diet, supplemented in various ways. Those receiving the unsupplemented diet developed the syndrome characterized by leucopenia, anemia, gingivitis, diarrhea, and death, which has been previously described in monkeys receiving our diet of refined foodstuffs.

An animal receiving the Goldberger diet supplemented with ascorbic acid and liver extract exhibited normal growth and development and has maintained a normal blood picture for approximately 2 years.

Likewise, the feeding of a crude liver extract to an animal with profound anemia and leucopenia was followed by a dramatic reticulocyte response and ultimate recovery. However, the ash of liver extract failed to maintain a normal blood picture or to prolong life.

Supplementing the diet with ascorbic acid, thiamin chloride, nicotinic acid (or amide), and riboflavin failed to prevent the leucopenia, gingivitis, diarrhea, and death. The combination of nicotinic acid and riboflavin, however, appeared to have a definite erythropoietic effect. *Shigella paradysenteriae* was isolated from the stools of several of the animals which received the deficient diet. Further studies are needed to clarify the relationship between the deficiency, the infection, and the blood picture.

Three of the animals exhibited edema of the face.
It is evident that the Goldberger diet, even when supplemented with nicotinic acid, riboflavin, thiamin, and ascorbic acid, is inadequate for maintenance of health in the young monkey. The nature of the deficiency manifestations would indicate that the diet is deficient in the substance or substances which we have previously termed vitamin M.

BIBLIOGRAPHY