NUTRITIONAL CYTOPENIA (VITAMIN M DEFICIENCY) IN THE MONKEY*

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PLATE 50

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In 1935 it was reported from this laboratory that monkeys given a diet deficient in some factor of the vitamin B complex developed an anemia, leukopenia, and in many cases gingivitis and diarrhea, and that all animals receiving such a diet died from the deficiency (1). The syndrome was prevented by supplementing the deficient diet with yeast or a liver extract (2). We have recently reported the failure of nicotinic acid to prevent this nutritional cytopenia (3). It is the purpose of this paper to describe the manifestations of this deficiency, and to present evidence showing that the syndrome is not a result of a deficiency of any of the chemically known vitamins. We have proposed the designation vitamin M for the substance in yeast and liver which prevents nutritional cytopenia in the monkey (3).

Anemias have been produced experimentally in a number of species by dietary deficiency. Wills and Bilimoria (4), Wills and Stewart (5), and Johnstone and Reed (6) have produced anemias in monkeys by dietary means. Rhoads and Miller (7, 8) have reported the irregular appearance of an anemia in dogs fed a diet productive of chronic black tongue and "apparently lacking a substance closely associated with vitamin B_2G." Spies and Dowling (9) have also noted an anemia

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in dogs on a black tongue-producing diet. Miller and Rhoads (8, 10) have further reported that a certain number of the dogs placed on their black tongue-producing diet developed "an ulcerative stomatitis associated with leucopenia and granulopenia." György *et al.* (11) have produced a somewhat similar syndrome in rats on a diet deficient in some component of the vitamin B complex. More recently he has reported (12) that nicotinic acid prevented this nutritional disturbance. Hogan *et al.* (13) have reported the development of a nutritional anemia in the pigeon and have stated that this condition did not yield to treatment with riboflavin, vitamin B, or an antidermatitis (rat) concentrate, but did respond to the administration of certain curative agents. Fouts *et al.* (14, 15) reported that a rice polish extract rich in vitamin B_6 supplemented a purified diet and prevented the appearance of a severe microcytic hypochromic anemia and death in puppies. Gall (16) and Miller and Rhoads (17) reported that guinea pigs seem to require some one or more substances in the diet for normal hematopoiesis. Kyer and Bethell (18) reported that a nutritional anemia in the rat might be prevented by the administration of a vitamin B_4 concentrate.

EXPERIMENTAL

Young immature monkeys (*Macaca mulatta*) weighing approximately 2 kilos were purchased from animal dealers. Upon arrival they were given a mixed diet consisting of grains, bread, fresh vegetables and fruits, and dried dog food. During a preliminary period of observation of at least 3 weeks, any animals that showed diarrhea, cough, anorexia, or other symptoms which did not abate shortly were discarded. Those animals which appeared to be normal were then separated, weighed, and bled. After this preliminary period of observation at least two blood counts were taken on each monkey before it was placed on an experimental diet. Some of the animals were in our laboratory more than a year before they were started on experiment; during this period each gained approximately 1 kilo in weight.

After an animal was found to be normal it was started on the experimental diet and thereafter was weighed and examined, and blood was drawn for study at weekly intervals, or oftener as the nature of the experiment or condition of the animal required. Some animals were bled daily near death or after the administration of a supplement to the deficient diet. The monkeys were kept in large metal metabolism cages with wire mesh bottoms. No effort was made to prevent coprophagy. The monkeys were housed in a light, airy room, but no attempt was made to expose them to direct sunlight. The temperature of the room was maintained as nearly constant as was possible; the room was heated continuously in winter, and the temperature rarely fell below 20°C. Clean tap water was kept in the cages at all times. The animals were fed once a day, usually about noon, but never before blood was drawn on that particular day. Weighings were made by placing the monkey in a tared cage on a direct reading balance. The techniques for blood determinations were the same as those described elsewhere (19).

Normal animals on a mixed diet were usually kept in the same room simultaneously with the experimental animals. In none of these have we ever observed a symptom complex resembling in any way that produced in our experimental animals. That the hygienic conditions in our quarters are satisfactory for the monkey is evident from the fact that we have kept certain animals in our laboratory for more than 2 years, and they have remained in good health and developed normally.

The experimental diet (No. 600) has been altered only slightly from that previously reported (1). In recent years we have used somewhat larger monkeys and hence felt it advisable to increase the caloric intake. Vitamin C is now supplied in the form of tablets of ascorbic acid (Merck), and in our more recent experiments a more complete salt mixture has been used (20). The quantities fed each monkey per day were as follows:

	gm.
Casein, washed with dilute alcohol[1]	10
Rice, polished, uncoated	50
Ground whole wheat	15
Salt mixture, Hubbell, Mendel, and Wakeman (20)	3
Sodium chloride	2
Cod liver oil	3
Ascorbic acid (Merck)	0.01

The casein, rice, salt mixture, and salt were mixed in an enameled boiler and tap water was added. This mixture was cooked on a steam bath with frequent stirring for 1½ hours. The whole wheat was then added and the cooking continued for ½ hour longer. The diet was then cooled in front of a fan, and the cod liver oil was mixed with the cooled material. The diet was cooked fresh every second day and was stored in a refrigerator until used. The ascorbic acid was fed separately in the form of tablets which the monkeys ate readily. Thiamin chloride was fed to one monkey in the same way. The monkey which was given extralin received 4 pulvules of this preparation before its basal diet, and consumed them readily. All other orally administered supplements were mixed with the diet just before feeding.

The diet, as it was given to the monkeys, had the consistency of a thick paste or mush, and was fed in earthenware dishes. Each animal was offered the amount of diet indicated above and in nearly every case the entire portion was eaten. In a few animals it was necessary to develop a taste for the diet by mixing a small amount of fresh banana with the portion for the first 3 or 4 days, after which they readily ate the diet without the banana. When a supplement was added and the monkey did not eat all of its diet, the amount of basal diet was decreased somewhat to make certain that all of the supplement was consumed.

[1] Casein, edible, muriatic, manufactured by the Casein Manufacturing Company of America, New York; washed with 60 per cent alcohol in this laboratory by the method of Sherman and Spohn (21).

Monkey 28 received the following diet, which is a modification of the Goldberger black tongue-producing diet No. 268 (22).

	gm.
Corn meal, white	40
Cowpeas, black eyed, ground	5
Casein, washed with dilute alcohol	10
Cottonseed oil	3
Cod liver oil	3
Salt mixture, Osborne and Mendel (23)	3
Sodium chloride	2
Ascorbic acid	0.01

The ingredients, except for the cottonseed oil, cod liver oil, and ascorbic acid, were cooked in an enameled boiler for 2 hours. After cooling, the cottonseed oil and cod liver oil were added. The ascorbic acid was fed separately. The monkey was cared for in the same way as were animals on the other diet.

RESULTS AND DISCUSSION

Since space does not permit the presentation of complete hematological data on all of the animals, we are giving in Table I the data on individual animals at the most critical points in the experiments. Hematological data on normal monkeys under the conditions existing in this laboratory have been presented elsewhere (19), but for ready reference the average normal figures and the normal ranges for blood elements are repeated at the beginning of Table I. The text-figures give somewhat more complete results on 5 of the monkeys. In the following paragraphs the data are discussed.

Deficient Diet.—Monkeys 13, 20, 22, 25, 26, and 30 received the deficient diet alone (diet 600). In every case they showed the symptom complex previously described: leukopenia, neutropenia, anemia, and loss of weight. A decrease in packed cell volume (hematocrit) was also seen. There also appeared to be a decrease in the number of platelets, although the data for this blood element are more meager. The dramatic increase in number of platelets to 1,555,000 per c.mm. following the administration of yeast to monkey 27 (Text-fig. 4) would also suggest that a thrombocytopenia is a part of the picture of this nutritional deficiency. No consistent variation in the clotting time has been observed. Ulceration of the gums and diarrhea were less consistently seen than was the cytopenia. The gum ulceration, as would be expected, appeared to be more closely correlated with

TABLE I

Hematological Data on 22 Monkeys at the Most Significant Points in the Experiments. The Average Normal and Normal Range for Blood Elements in Monkeys of This Colony Are Given for Comparison

Monkey No.	Time on diet	W.B.C. per c.mm.	Lymphocytes per c.mm.	Neutrophils per c.mm.	Platelets per c.mm.	R.B.C. per c.mm.	Reticulocytes	Hemoglobin	Hematocrit	Weight	Diet and remarks
	days	*thousands*	*thousands*	*thousands*	*thousands*	*millions*	*per cent*	*per cent*	*per cent*	*gm.*	
19 normal monkeys		15.1 9.7–20.5	8.8 5.0–13.0	5.7 2.0–9.0	475 318–632	5.2 4.6–5.8	0.6 0.56–0.64	12.2 10.9–13.5	40.0 36.4–43.6		Average normal Normal range
13 ♀	3	9.3	5.9	2.9		4.65		11.9		2037	Deficient diet 600*
	64	1.4	1.0	0.4		1.93		5.2		1330	Died
20 ♀	0	10.0	7.0	2.1	234	5.38	0.9	11.8	39.0	3860	Deficient diet 600*
	54	1.1	1.1	0.1	175	3.24	0.6	8.2	26.2	3400	
	55	2.8	2.7	0.6	76	3.48	0.3	8.2	27.5		Died
22 ♂	−2	7.9	3.0	4.2		6.56		12.5		2700	Deficient diet 600*
	61	3.7	3.5	0.1		0.95		2.2	7.2	2405	Died 62nd day
25 ♀	1	16.0	7.8	7.7		4.82		9.9		2215	Deficient diet 600*
	25	0.7	0.5	0.2		2.84		6.6		1715	Died 26th day
26 ♂	1	20.7	8.7	11.2		5.16		12.1		2455	Diet 600*
	27	5.4	3.5	1.6		4.39		12.6			
	42	8.4	4.2	4.0		2.74		6.4	22.8	2060	Died 44th day; photographed
30 ♂	0	17.4	13.4	3.4	725	5.00	1.2	11.1	43.5	2490	Diet 600†
	59	4.7	2.8	1.8	635	3.30	0.3	7.7	26.0	2110	Gums ulcerated; appetite good
	94	0.5				1.40		3.8	11.0	1445	Died
28 ♂	−3	22.8	7.5	14.4	412	5.02	0.8	12.3	41.5	2790	Modified Goldberger diet
	72	6.7	2.0	4.2	463	4.27	0.2	10.8	30.7	2502	Gums ulcerated
	114	4.1	2.4	1.6	635	2.08	0.9	6.5	18.8	2532	Diarrhea
	162	1.2			278	1.77	0.4	5.0	13.0	1719	Died 163rd day
23 ♀	−2	32.0	4.5	25.9		4.63		13.6		2510	Diet 600*
	45	5.4	2.5	2.5		4.21		9.7	32.0	2145	Gums ulcerated. 6 gm. yeast added to diet‡
	53	7.6	1.2	5.4		2.66		9.2	27.2		Died (heart blood)

* Received the original diet (1) which contained Osborne and Mendel salt mixture (23), and 4 gm. of orange daily as a source of vitamin C.

† Received 0.01 gm. of ascorbic acid daily in place of the orange.

‡ Dried brewers' yeast, vita-food, red label, supplied by the Vitamin Food Company, New York.

TABLE I—*Continued*

Monkey No.	Time on diet	W.B.C. per c.mm.	Lymphocytes per c.mm.	Neutrophils per c.mm.	Platelets per c.mm.	R.B.C. per c.mm.	Reticulocytes	Hemoglobin	Hematocrit	Weight	Diet and remarks
	days	*thousands*	*thousands*	*thousands*	*thousands*	*millions*	*per cent*	*per cent*	*per cent*	*gm.*	
24 ♀	0	16.7	9.4	6.7		5.66		11.0		2265	Diet 600* + 10 gm. yeast‡
	200	15.0	9.5	4.4	280	5.69	0.4	12.5	44.0	2849	Yeast reduced to 5 gm. daily
	429	17.8	7.46	9.59	428	6.18	0.7	12.8	45.3		Yeast reduced to 2.5 gm. daily
	664	4.1	1.35	2.54	56	1.61	4.3	5.7	18.5	2890	10 gm. yeast added daily, 5 days prior to death; died 665th day
27 ♂	1	10.6	6.3	4.2		4.6	0.6	10.4	37.0	1760	Diet 600* + 10 gm. skim milk powder daily
	34	4.8	3.5	0.9	280	3.72	0.6	7.5	25.2	1956	Milk withdrawn; 10 gm. yeast‡ added daily
	45	6.1	4.4	1.4	1555	3.9	4.0	10.0	30.3	1860	Stools normal
	498	20.4	16.5	3.7		5.78	0.6	12.3	41.0	3770	
	596	13.0	8.9	4.0	705	5.46		13.0	36.5	3427	Normal this date
29 ♂	1	13.8	5.5	8.0	1115	5.03	0.3	11.7	37.0	3016	Diet 600* + 4 pulvules extralin§
	572	11.8	3.1	8.6	380	4.08	0.7	10.7	32.3	3495	Paralysis; off diet
32 ♀	−2	9.4	7.0	1.8	374	5.27	0.1	12.4	41.0	3275	Diet 600† + 2 gm. Lilly liver extract daily
	630	12.1	7.5	3.6		5.40		11.8		3935	Diet 600‖ + 2 gm. Lilly liver extract. Menstruated
	728	21.1	12.9	6.9		5.17		10.5		4955	Liver extract withheld
	799	4.1	1.4	2.4		4.12		8.5		3300	Gums ulcerated; died 802nd day
34 ♂	0	17.1	10.4	5.3	630	5.66	0.4	14.6	40.6	3080	Diet 600† + 1 cc. Lederle liver extract weekly
	56	8.0	3.5	3.9	510	5.24	0.4	13.2	35.0	3195	
	104	1.6	0.9	0.3		2.69				1579	Died 105th day
31 ♂	0	11.9	6.3	4.9	824	5.36	0.9	13.9	42.0	2667	Diet 600† + 5 mg. Cu daily
	58	9.4	6.9	2.0	221	4.80	0.0	12.6	36.5	2695	Gums necrotic
	99	0.9	0.8	0.1	337	5.6	0.2	10.6	29.3	1817	Gums very necrotic; died 100th day
33 ♀	0	14.1	8.8	4.1	578	4.6	1.0	10.6	38.5	2340	Diet 600†
	34	9.0	4.5	4.4	415	3.16	0.0	10.6	27.5	2295	5 mg. Cu added to diet daily
	48	4.0	3.9	0.1	215	2.98	0.1	7.6	22.5	2285	
	56	3.4	2.4	0.8		0.73			4.5	2365	Died 57th day

§ Extralin is a liver-stomach preparation manufactured by Eli Lilly and Company.

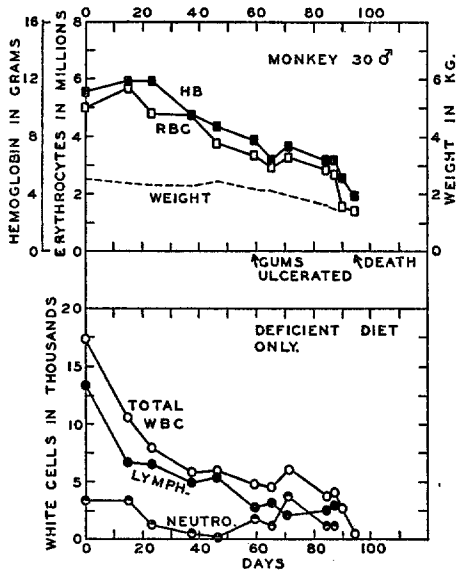
‖ Received the diet of composition indicated in the text, which contained the salt mixture of Hubbell, Mendel, and Wakeman (20).

TABLE I—*Concluded*

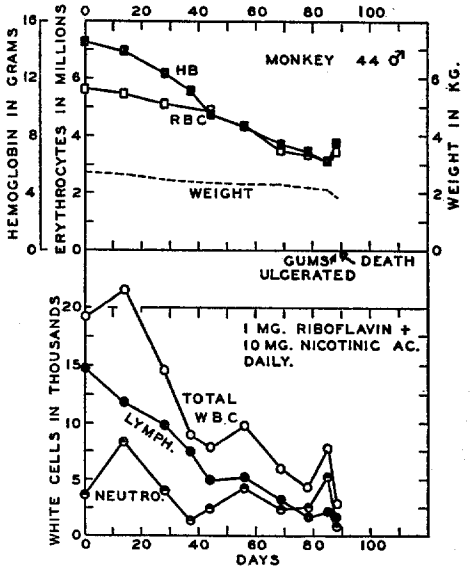
Monkey No.	Time on diet	W.B.C. per c.mm.	Lymphocytes per c.mm.	Neutrophils per c.mm.	Platelets per c.mm.	R.B.C. per c.mm.	Reticulocytes	Hemoglobin	Hematocrit	Weight	Diet and remarks
	days	*thousands*	*thousands*	*thousands*	*thousands*	*millions*	*per cent*	*per cent*	*per cent*	*gm.*	
43 ♂	0	15.0	7.68	5.72		5.72		10.5		2200	Diet 600‖ + 10 mg. nicotinic acid daily
	71	2.2				4.38		8.21		1580	Died; gums necrotic
40 ♀	−1	17.9	10.05	6.28		4.35		11.00		3400	Diet 600‖ + 10 mg. nicotinic acid
	55	4.9	2.47	2.13		3.14		7.52		2825	Alopecia face and chest
	89	3.0	0.94	2.00		2.75	0.5	6.13		2595	Extensive alopecia face; given Dakin-West liver concentrate**
	105	3.5	1.51	1.95		2.89	2.4	6.75		2475	
	120	2.1	1.42	0.66		2.39	0.2	5.98		2235	Died 122nd day
37 ♂	−1	8.7	6.18	2.26		5.39		10.1		3465	Diet 600‖ + 10 mg. nicotinic acid
	74	0.9	0.68	0.17		3.85		10.36		2280	Died
36 ♀	−1	8.0	4.88	2.9		4.93		10.71		3465	Diet 600‖ + 1 mg. riboflavin by mouth
	46	3.4	1.9	1.5		4.6		12.28		2695	No diarrhea; gums necrotic; died 51st day
45 ♀	−8	9.5	5.05	4.10		5.38		10.71		2055	Diet 600‖ + 50 mg. nicotinic acid + 1 mg. riboflavin parenterally
	63	2.4	1.42	0.79		3.67		8.10		1865	0.5 gm. nucleic acid daily added
	76	30.2	10.26	19.32		3.88		9.36		1630	Died 77th day
44 ♂	0	19.3	14.82	3.66		5.63		14.55		2685	Diet 600‖ + 10 mg. nicotinic acid + 1 mg. riboflavin parenterally
	89	3.7	1.70	1.81		3.44		7.18		1845	Died; diarrhea; gums ulcerated
42 ♀		31.7	17.76	11.40		5.53		13.33		2280	Diet 600‖ + 50 mg. nicotinic acid + 1 mg. riboflavin + 1 mg. thiamin
	50	5.4	1.40	3.78		4.58		8.96		1690	Diarrhea; died 57th day; gums necrotic

** Received a preparation from 50 gm. of Lilly's liver extract, prepared by the method described by Dakin, Ungley, and West (26).

the leukopenia and granulopenia than with the anemia (note monkeys 31 and 36). Unless the diet was supplemented with some active material, the animals died in about 100 days.



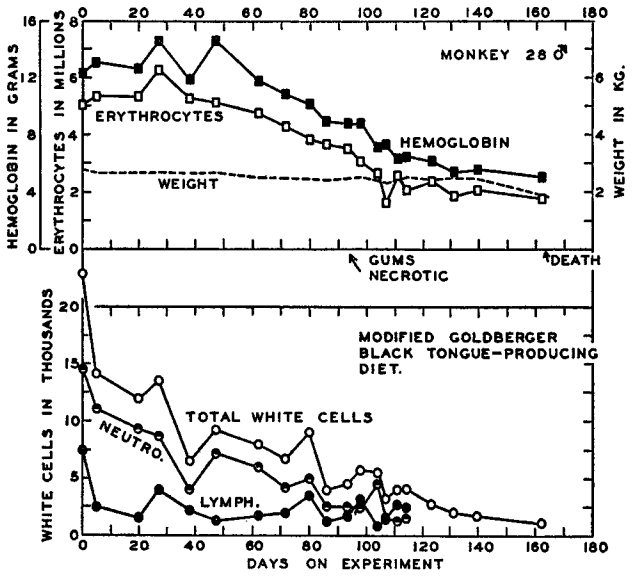
TEXT-FIG. 1. Hematological and weight data on a monkey which received the deficient diet without supplement.



TEXT-FIG. 2. Hematological and weight data on a monkey which received the deficient diet supplemented with 1 mg. riboflavin parenterally and 10 mg. nicotinic acid by mouth each day.

The degree of anemia seemed to vary somewhat with the animal. Erythrocyte counts below 1,000,000 per c.mm. have been found; however, the counts were more frequently around 3,000,000 at or before death. The decrease in hemoglobin appeared to parallel the decrease in erythrocytes. Hence, the color index did not vary greatly from normal for the monkey. The packed cell volume also paralleled the erythrocyte numbers, so the volume index did not depart significantly from normal. A few of the animals died before the anemia had become marked.

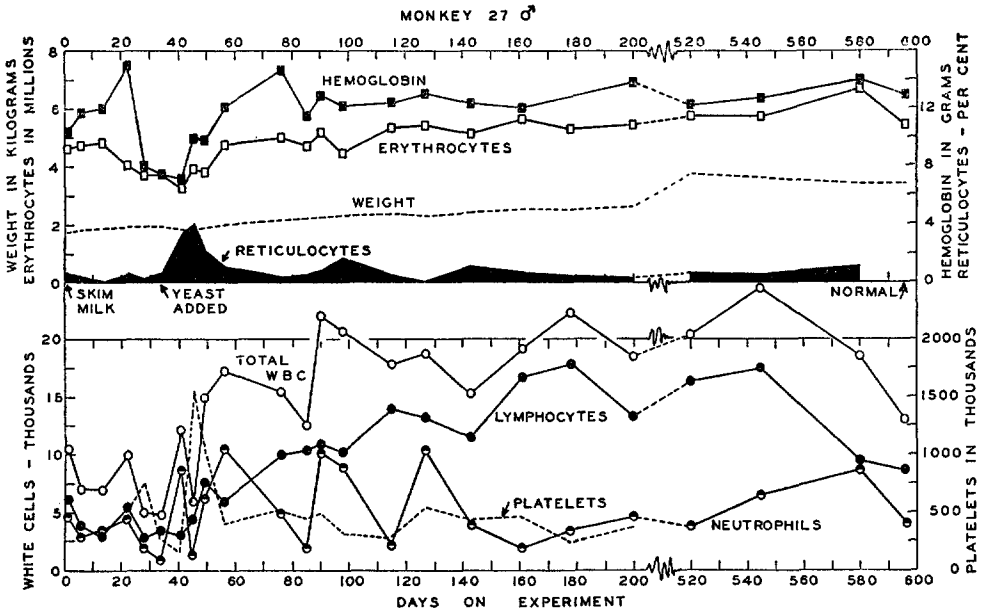
The average total white blood cell count for the monkey (15,000 per c.mm.) is considerably above that of man. Therefore, a total white cell count of 5000 or less would indicate a definite leukopenic state. Several of the monkeys on the



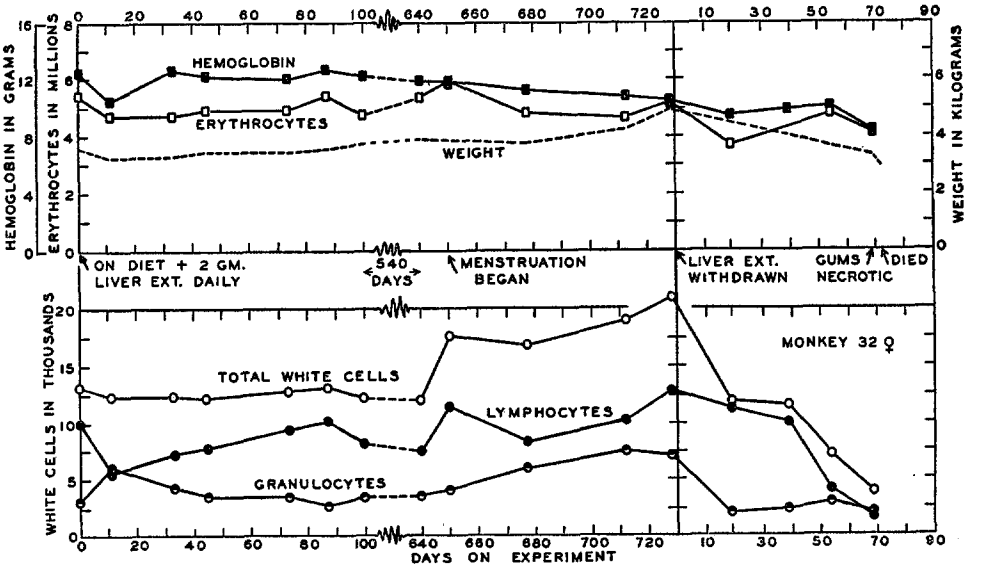
TEXT-FIG. 3. Blood changes in a monkey which received a modification of the Goldberger black tongue-producing diet.

deficient diet exhibited before death white cell counts of less than 1000 per c.mm. The most frequent finding, however, was a total white count of approximately 3000 cells per c.mm. Usually, but not always, there was a relative granulocytopenia. It is suggestive that those animals which survived the deficiency for the longest periods also tended to maintain the normal *relative* distribution of granulocytes and lymphocytes. That the leukopenia was not the result of the gingival infection is suggested by the fact that we have frequently seen animals die with very low white cell counts without showing gum lesions. The converse has not been true, however. Usually the leukopenia appeared to precede shortly the anemia.

Terminal rises in the numbers of all cellular elements have sometimes been



TEXT-FIG. 4. Blood picture of monkey 27. This animal was given the deficient diet supplemented with 10 gm. of skim milk powder daily. A mild anemia and distinct leukopenia had developed by the 34th day. At this point the milk powder was discontinued and replaced by 10 gm. of dried yeast daily. There was an immediate increase in reticulocytes and platelets and the entire blood picture slowly returned to normal.



TEXT-FIG. 5. Hematological and weight data on monkey 32, which received the deficient diet supplemented with 2 gm. of Lilly's liver extract daily. The animal gained in weight and developed normally, and at the end of 2 years the blood picture was still normal. At this time the liver extract was withdrawn, and the animal died in 72 days showing the typical symptoms of weight loss, leukopenia, and ulceration of the gums.

seen in the last two or three days of life. These rises have never increased the counts to normal values, however. They may possibly be explained by dehydration of the moribund animal.

Black Tongue-Producing Diet.—Monkey 28 was fed the modified Goldberger diet. It also developed a severe leukopenia and anemia, with marked ulceration of the gums and a diarrhea. In contrast with the results of Miller and Rhoads in dogs (7, 10) and in swine (24) there appeared to be no tendency to spontaneous remission. Likewise, we have never observed a spontaneous remission in any monkey on diet 600. Also, in contrast with the findings of Rhoads and Miller in dogs (7), we have not observed a significant consistent change in either the color index or the volume index in this deficiency in the monkey. Further study will be necessary to determine whether these differences are due to variations in the response of the several species to the same deficiency, or whether the conditions are manifestations of deficiencies of distinct nutritional factors.

Although it is logical to assume that the blood changes in monkeys on our diet 600 and on this modified black tongue-producing diet are the result of the same deficiency, this point remains to be proved. Experiments are under way to determine the effect of supplementing the black tongue-producing diet with nicotinic acid. The blood findings on the 2 monkeys reported by Johnstone and Reed (6) are strikingly similar to those which we observed.

Yeast.—Monkey 24 was given the deficient diet supplemented with 10 gm. of dried brewers' yeast (vita-food, red label). The animal gained in weight and size, and dentition proceeded normally. After 200 days the blood picture was still normal; at that time the amount of yeast was reduced to 5 gm. daily. On the 429th day the blood picture was still normal, and at that time the daily amount of yeast was reduced to 2.5 gm. Following that reduction the monkey developed a severe cytopenia and died on the 665th day, showing the following terminal values: total white cells, 4100 per c.mm.; platelets, 56,000 per c.mm.; erythrocytes, 1,610,000 per c.mm.; hemoglobin, 5.7 gm. per 100 cc.; and packed cell volume, 18.5 per cent. 5 days before death the daily dosage of yeast was increased to 10 gm. This was followed by an increase in reticulocytes to 4.3 per cent, but the increased amount of yeast did not prevent death. Although 2.5 gm. of yeast daily did not prevent the deficiency manifestations, that amount did appear to prolong life. It appears, therefore, that the minimum protective dose of this preparation of dried brewers' yeast was between 2.5 and 5 gm. daily.

Monkey 27 was started on experiment with the deficient diet supplemented with 10 gm. of skim milk powder daily (see Table I and Text-fig. 4). By the 34th day a mild cytopenia had developed. At this point the milk powder was replaced by 10 gm. of dried brewers' yeast daily. As can be seen by an inspection of Text-fig. 4, there was a sharp rise in reticulocytes to 4 per cent and a simultaneous

increase in platelets to 1,555,000 per c.mm. There was a slower return of white cells, erythrocytes, hemoglobin, and packed cell volume to normal levels. On the 596th day the blood picture was still normal.

It is evident from these two experiments that brewers' yeast contains a factor which is essential in the maintenance of proper hematopoietic function in the monkey. In sufficient quantities yeast is able to prevent nutritional cytopenia in the monkey, and must therefore be regarded as a reliable source of vitamin M.

Liver Extract.—The effectiveness of several liver preparations in the prevention of nutritional cytopenia was studied. Monkey 32 was given the deficient diet supplemented with 2 gm. of Lilly's liver extract (343 powder), which is essentially Cohn's fraction G (25). The animal gained in weight and height, and the blood picture was still normal at the end of 2 years. At the start of the experiment the monkey was juvenile, but during the experiment it passed puberty and began to menstruate. Dentition proceeded normally with the loss of deciduous teeth and the eruption of permanent teeth. At the end of the second year the liver extract was withdrawn. The animal died in 72 days following the withdrawal of the liver extract, after showing loss of weight, a marked leukopenia, and ulceration of the gums. Monkey 29 was given the diet supplemented with 4 pulvules of extralin, a liver-stomach preparation. This animal gained in weight and height, dentition proceeded normally, and the blood picture was still normal at the end of 572 days. The monkey developed a paralysis of unknown etiology during the latter part of the experiment; this paralysis will be discussed later.

Monkey 34 was given the deficient diet supplemented with 1 cc. per week of Lederle liver extract parenterally. This dosage was based upon the amount of the extract necessary to keep a pernicious anemia patient in remission. The monkey developed the severe leukopenia and anemia typical of vitamin M deficiency, and died on the 105th day. On the day before death the total white cells numbered 1600 per c.mm., and the total erythrocyte count was 2,690,000 per c.mm. It is apparent that this amount of Lederle liver solution did not protect against nutritional cytopenia. One of the animals (monkey 40) was given a concentrate from Lilly's liver extract prepared according to the method of Dakin, Ungley, and West (26) after a marked cytopenia had developed. Although this was followed by an increase in reticulocytes to 2.4 per cent, the condition of the animal became progressively worse and it died on the 122nd day.

It is evident from the experiments on monkeys 29 and 32 that certain liver extracts given orally were effective in preventing nutritional cytopenia over long periods of time, and as supplements to the deficient diet were capable of promoting normal growth and development.

The experiments with more concentrated preparations of the anti-pernicious anemia factor were less conclusive.

Riboflavin.—It seemed possible that nutritional cytopenia might be the result of a deficiency of riboflavin; consequently, the effectiveness of synthetic riboflavin was investigated. Diet 600 contains some riboflavin in the whole wheat, but rats on a similar diet containing 35 per cent whole wheat eventually developed the characteristic signs of flavin deficiency (27).

The requirement of the monkey for riboflavin is a matter of conjecture only. Estimates of the human requirement have been from 400 Bourquin-Sherman units for children to 600 units for adults (28). Accepting the tentative conversion factor of 1 unit as equivalent to between 2 and 3 micrograms of riboflavin (29, 30), the human requirement would be between 1 and 2 mg. of riboflavin daily. The requirement of a young 2 kilo monkey should be considerably less. We therefore decided to give 1 mg. of synthetic riboflavin daily to certain monkeys as supplements to the deficient diet. 4 monkeys (36, 42, 44, and 45) were given this dosage either alone or in combination with nicotinic acid, or with nicotinic acid and thiamin chloride. The riboflavin was supplied to us in sterile ampules, each containing 1 mg. riboflavin in 2 cc. of solution. Monkey 36 was given the riboflavin mixed with the basal diet. The somewhat bitter taste of the riboflavin seemed to render the diet unpalatable, however, so the method of administration was changed and the riboflavin was given subcutaneously during the latter part of the experiment. The other 3 animals received it subcutaneously throughout the experiment. Monkey 44 was also given 10 mg. of nicotinic acid daily mixed with the basal diet (Text-fig. 2). Monkey 45 received 50 mg. of nicotinic acid daily, while monkey 42 received 50 mg. of nicotinic acid and 1 mg. of thiamin chloride daily, in addition to the riboflavin.

It is evident from the data given in Table I on these 4 animals that riboflavin, either alone or in combination with nicotinic acid or with nicotinic acid and thiamin chloride, did not prolong life or prevent a marked leukopenia. It is possible that the riboflavin tended to sustain the hemoglobin and erythrocyte numbers, since monkey 36 did not develop an anemia. This would not be surprising in view of the reported value of riboflavin in hemoglobin production (31). The other 3 monkeys showed a distinct anemia, however, and the sustained erythrocyte numbers and hemoglobin in monkey 36 can probably be explained by two facts: this animal was somewhat larger (3.5 kilos) than most of our animals, and the survival period was shorter. We have observed that the anemia appears to develop more slowly than the leukopenia, and that larger monkeys die before they show more than moderate anemia.

Monkey 45 was given 0.5 gm. of yeast nucleic acid daily after marked cytopenia had developed. This was followed by a dramatic increase in leukocytes to a total of 30,000 per c.mm. The animal died on the 77th day, nevertheless. At autopsy numerous small abscesses were found throughout the wall of the intestine and in certain other abdominal viscera. In the presence of this complication it is im-

possible to evaluate the rôle of the nucleic acid. Further investigation of this substance is planned.

It is apparent that riboflavin, either alone or in combination with nicotinic acid and thiamin chloride, was ineffective in the prevention of nutritional cytopenia.

Nicotinic Acid.—In another place (3) we have reported the failure of nicotinic acid to prevent nutritional cytopenia in the monkey, and in that paper detailed hematological data were given on 2 monkeys. Table I gives the most significant results from the 6 monkeys which received nicotinic acid (monkeys 37, 40, 42, 43, 44, and 45) and Text-fig. 2 gives complete data on one of them. These data are also discussed in the paragraph on riboflavin. Although it is conceivable that nicotinic acid amide or some other pyridine derivatives might be effective, it is clear that nicotinic acid is not identical with the substance that prevents nutritional cytopenia in the monkey (vitamin M).

Copper.—Originally we used the salt mixture of Osborne and Mendel (23) as a source of inorganic elements. This mixture furnishes abundant iron, but contains copper only as it is incidentally found as a contaminant of the chemicals used. Therefore, we gave one animal (monkey 31) the deficient diet prepared with this salt mixture but supplemented with 5 mg. of copper daily as copper sulfate. It developed a severe leukopenia (Table I) and marked necrosis of the gums (Figs. 2 and 3), but did not show the severe anemia which is commonly observed. Monkey 33 was then given the deficient diet without copper supplement and allowed to develop a moderate anemia. At this point 5 mg. of copper daily was added to the diet. There was no increase in erythrocytes and no reticulocyte response, and the animal showed an erythrocyte count of 730,000 just before death.

Since October, 1937, all the animals used have received 3 gm. daily of the improved salt mixture of Hubbell, Mendel, and Wakeman (20), and hence have received more than 1 mg. of copper daily from this source alone. Moderately severe anemia has appeared in approximately the same percentage of comparable animals as was observed before the change in salt mixture, and one monkey has exhibited a severe anemia since the change. No appreciable alteration of the total white or differential counts has been observed since the change of salt mixture and the consequent increase in copper content of the diet.

Although the apparent stimulating action on erythropoiesis of moderately large doses of copper sulfate in the one animal requires further investigation, the absence of any such stimulating action by intakes within the suggested range of human requirement (32) and the absence of any leukopoietic effect from any of the doses would indicate that copper deficiency is certainly not the cause of the cytopenia.

General Discussion.—The anemia in puppies reported by Fouts

and associates (14, 15) resulting from vitamin B_6 deficiency was described as a microcytic anemia with no appreciable change in the leukocyte counts. In contrast with that, the condition we are here describing in monkeys appears to be a normocytic anemia accompanied by a profound lowering of the number of leukocytes. The two conditions therefore present distinctly different pathological pictures. Furthermore, calculated on the basis of assays reported by Birch *et al.* (33), our diet 600 contains approximately 28 "rat day doses" of vitamin B_6 per portion, and the modified Goldberger diet that we used contains 80 or more rat day doses of this vitamin. On the other hand, the amount of liver extract necessary to prevent nutritional cytopenia in the monkey contains only 10 rat day doses of vitamin B_6, computed from data from the same laboratory. In the light of the foregoing statements it appears quite improbable that a deficiency of vitamin B_6 could be a causative factor in the production of nutritional cytopenia in the monkey.

Since many of the monkeys ate well up to within a day or two of death, we feel justified in concluding that the cytopenia was not the result of simple inanition.

The experiments on monkeys 24, 27, and 32 demonstrate that the quantity of basal diet was adequate; all of these survived more than 500 days on the usual ration supplemented with a small quantity of an active material (yeast or liver extract), and their blood pictures remained normal. Monkey 32 is of especial interest in this connection. It received the usual amount of basal diet plus 2 gm. of Lilly's liver extract daily. On the 388th day it had reached a maximum weight of 4295 gm. Evidently the usual daily ration was not adequate to support growth in such a large animal, for during the next 288 days the animal declined in weight, although the blood picture remained normal. Consequently, the amount of basal diet was doubled on the 677th day, and by the 728th day the animal had attained a weight of 4955 gm. (Text-fig. 5). On this date the liver extract was withdrawn but the double portion of basal diet was continued. The monkey died 72 days after the withdrawal of the liver extract, having shown the typical leukopenia and gingival lesions characteristic of vitamin M deficiency. These experiments would seem to preclude the possibility that the cytopenia was the result of an inadequate caloric intake.

Monkey 27, which received 10 gm. of yeast daily, and monkey 29, which received 4 pulvules of extralin daily, developed a paralysis after they had been on the experiment for more than a year. Monkey 29 was returned to a mixed stock diet after the 572nd day, and within a short time the paralysis had disappeared;

this would suggest that a dietary factor may have been involved in the etiology of the paralysis. Monkey 32 survived for 2 years on the basal diet supplemented with 2 gm. of liver extract daily and did not show any evidence of paralysis. We have no explanation to offer for the appearance of paralysis in these 2 monkeys, but wish to record the observations in the interest of completeness.

Space will not permit a detailed comparison of these experiments on the monkey with the other experimental anemias of dietary origin described in the literature, but certain brief comments may be appropriate. One cannot avoid being impressed by the similarity of this symptom complex to the anemia and leukopenia which Rhoads and Miller have shown accompany black tongue in dogs (7, 8, 10). Also, the condition reported by Wills and associates (4, 5) in monkeys resembles in many ways the condition that we refer to as nutritional cytopenia. However, their monkeys survived longer, the blood changes appeared much later, and their animals appeared to develop a more severe anemia but milder leukopenia than our animals. Furthermore, the anemia in their animals was reported to be macrocytic, while the volume index and color index in our animals did not deviate greatly from normal. The panmyelophthisis in rats reported by György and associates (11) resembles nutritional cytopenia in the monkey in so many ways that one might be inclined to believe they had a common etiology, were it not that György has reported that nicotinic acid prevented the blood changes in their rats (12).

Although the condition we designate as nutritional cytopenia in many ways resembles certain clinical conditions, as agranulocytosis, aplastic anemia, and possibly others, it is not identical in all respects with any one of them. We feel that it is wise to defer the discussion of these points of similarity and dissimilarity until more evidence is available.

SUMMARY

Young *rhesus* monkeys (*Macaca mulatta*) were given a diet containing casein, polished rice, whole wheat, salt mixture, sodium chloride, cod liver oil, and ascorbic acid. They developed a syndrome characterized by anemia, leukopenia, and loss of weight. Ulceration of the gums and diarrhea were common, and death occurred between

the 26th and 100th day. 4 monkeys were given the deficient diet supplemented with 1 mg. of riboflavin daily, and these developed the characteristic signs and died in periods of time similar to the survival of monkeys receiving the deficient diet alone. Nicotinic acid, either alone or in combination with riboflavin and thiamin chloride, failed to alter appreciably the course of the deficiency manifestations. Thus, it is evident that this nutritional cytopenia is not the result of a deficiency of vitamin B, riboflavin, or nicotinic acid.

The deficient diet supplemented with either 10 gm. of dried brewers' yeast or 2 gm. of liver extract (Cohn fraction G) daily supported good growth, permitted normal body development, and maintained a normal blood picture over long periods. It is obvious that yeast and liver extract contain a substance essential to the nutrition of the monkey which is not identical with any of those factors of the vitamin B complex that have been chemically identified. We have proposed the term vitamin M for this factor which prevents nutritional cytopenia in the monkey.

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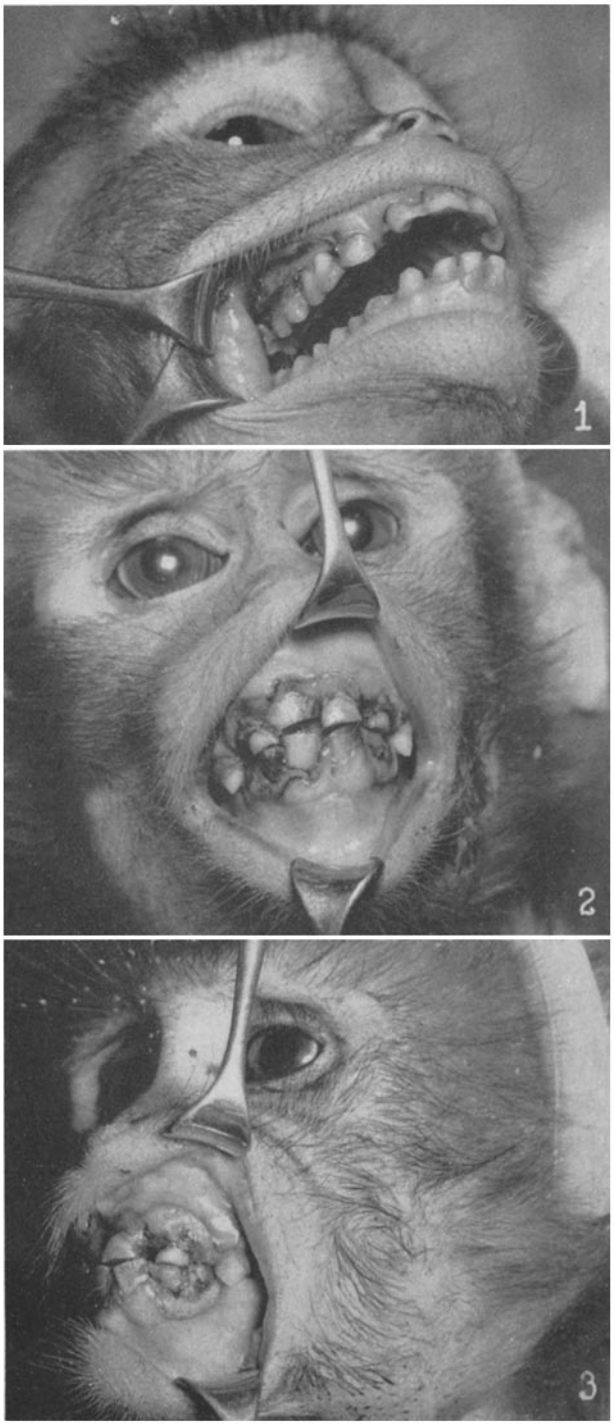
EXPLANATION OF PLATE 50

FIG. 1. Photograph of mouth of monkey 26, showing necrosis of gum with exposure of maxilla. This animal received the deficient diet only. Photographed on the day of death.

FIGS. 2 and 3. Photographs of monkey 31, which received the deficient diet supplemented with 5 mg. of copper (as copper sulfate) daily. Marked ulceration and necrosis of the gums are evident. Photographed on the day of death.



(Langston *et al.*: Nutritional cytopenia in the monkey)