

THIAMIN DEFICIENCY IN THE RHESUS MONKEY

CLINICAL, METABOLIC AND HEMATOLOGIC OBSERVATIONS

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AN IMPORTANT advance in nutritional research was the development of an essentially synthetic diet adequate for study of single deficiencies in the monkey.¹ It seemed most timely to restudy the vitamin deficiencies in a primate whose metabolic processes might be expected to approximate those of man most closely. This report is concerned with the study of thiamin deficiency. Seven rhesus monkeys (*Macaca mulatta*) were subjected to one or more episodes of thiamin deficiency. Observations were made on food consumption, weight, clinical behavior, thiamin metabolism and the blood picture. Finally the animals were sacrificed and detailed pathologic examinations were made. In this report the experimental method is reported together with the clinical metabolic and hematologic observations enumerated above. The pathologic findings resulting from recurrent thiamin depletion with particular reference to characteristic degenerative changes occurring in the heart muscle and severe retrogressive changes in the nuclear structures of the central nervous system have been reported^{2, 3} and will be detailed elsewhere.

EXPERIMENTAL METHOD

The diet used in these experiments was a modification of the M-3 diet of Waisman et al⁴ and consisted of powdered sucrose 73, vitamin test casein (General Biochemicals) 18, Hawk and Oser salt mixture 4, and corn oil 2. Sulfited liver extract equivalent to 100 grams of Wilson Laboratories L fraction prepared according to the method of Kline and co-workers⁵ was added to each 4 kilograms of diet. The diet was dried, granulated and following the addition of 1 per cent calcium stearate compressed into tablets weighing approximately 2 grams.* The basal diet was fed ad libitum. The diet in pellet form had the advantage of curtailing waste and facilitating the estimation of the daily food consumption. A vitamin tablet containing daily dosages similar to those of Waisman et al was fed each day. Each vitamin tablet contained the following: nicotinic acid, 5 mg.; riboflavin, 1 mg.; pyridoxine hydrochloride, 1 mg.; calcium pantothenate, 3 mg.; choline dihydrogen citrate, 100 mg.; paraminobenzoic acid, 100 mg.; inositol, 100 mg., and ascorbic acid, 25 mg., plus sufficient powdered sugar to make a tablet weighing 1.5 to 2 grams. The monkeys accepted these vitamin tablets willingly and consumed them eagerly. Control monkeys were also given 1 or 0.5 mg. thiamin chloride daily. In addition the monkeys received by mouth 5 drops of vitamin A and D concentrate twice weekly and additional sulfited liver extract (equivalent to 2.5 Gm. daily) twice or thrice weekly as a source of biotin and folic acid. Some animals also received 5 drops of mixed tocopherols (Napco) once a week.

During the course of these studies the weights and daily food consumption were followed carefully. Blood was taken by venipuncture at approximately weekly intervals for the determination of thiamin

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levels and for hematological studies. At autopsy portions of several tissues were removed from each monkey and prepared for analysis of thiamin and riboflavin content. The methods used for the analysis of thiamin of blood and tissues have previously been described.⁶ The method for the estimation of riboflavin will be reported in another article. The animals were tuberculin tested by injection with old tuberculin in the eyelids. Positive reactors were rejected. The monkeys were then placed on the purified diet with complete supplementation for one to two weeks, and control tests were carried out so that each monkey could serve as his own control. The occasional animal who failed to adapt itself to the diet or failed to gain in weight during the control period was rejected. With the exception of one monkey all the animals used weighed between 1800 and 3600 grams. The one exception was an older animal weighing 7000 grams which had previously been used in some other studies. In all, 7 monkeys were employed in this study and were subjected to one or more periods of depletion.

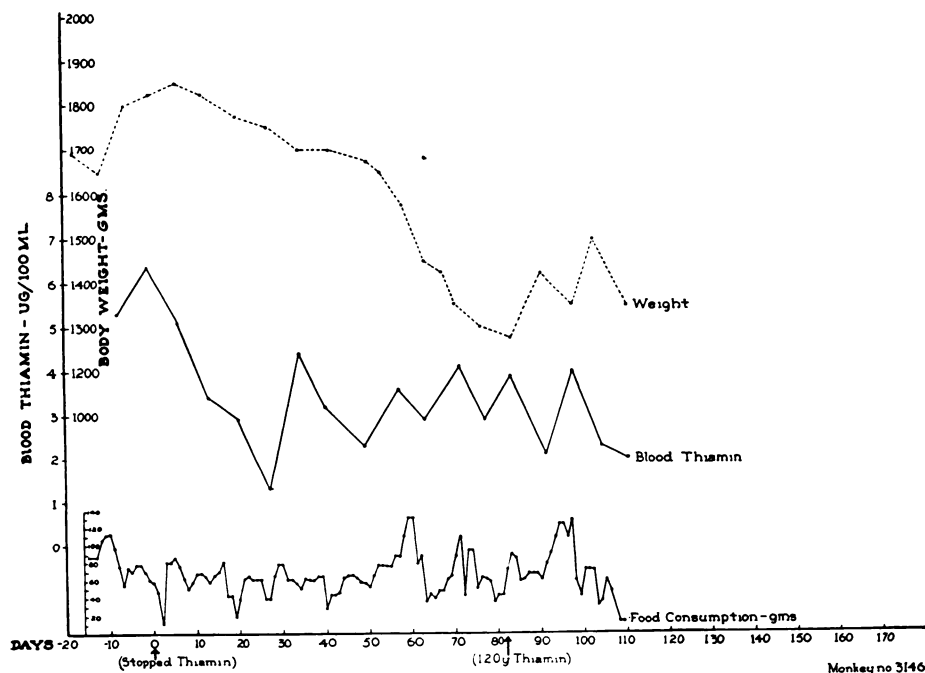


FIG. 1. THIAMIN DEFICIENCY IN MONKEY No. 3146
Graph of food consumption, blood thiamin, and weight

CLINICAL OBSERVATIONS

The clinical behavior of the animals was in most respects similar to that reported by Waisman and McCall.⁴ In general the monkeys ceased gaining after two weeks on the thiamin deficient diet. This was either followed by a plateau of the weight curve for several days or by loss of weight. The weight loss was usually associated with a decreased food consumption and marked lowering of the blood thiamin as is shown in figure 1 and 2, which are representative of the changes observed in monkeys on the thiamin deficient diet. As the deficiency progressed the animals continued to lose weight, became apathetic and inactive, and weakness was evident. Finally, if the depletion period were prolonged, the animals became ataxic. Some developed ptosis and tremors. Retching was observed on several

occasions. The monkey would make every attempt to prevent the escape of vomitus from his mouth by trapping it in his buccal pouches and would ultimately reswallow it. Convulsive movements have been observed in one or more of the animals. If thiamin were administered at this stage a dramatic response was observed in twenty-four to forty-eight hours. The improvement in locomotion, alertness and appetite was striking. On the other hand, if the period of thiamin deprivation were not interrupted at this stage it was but a matter of a few days until the monkey was unable to sit on its perch or even stand upon the floor of the cage without difficulty.

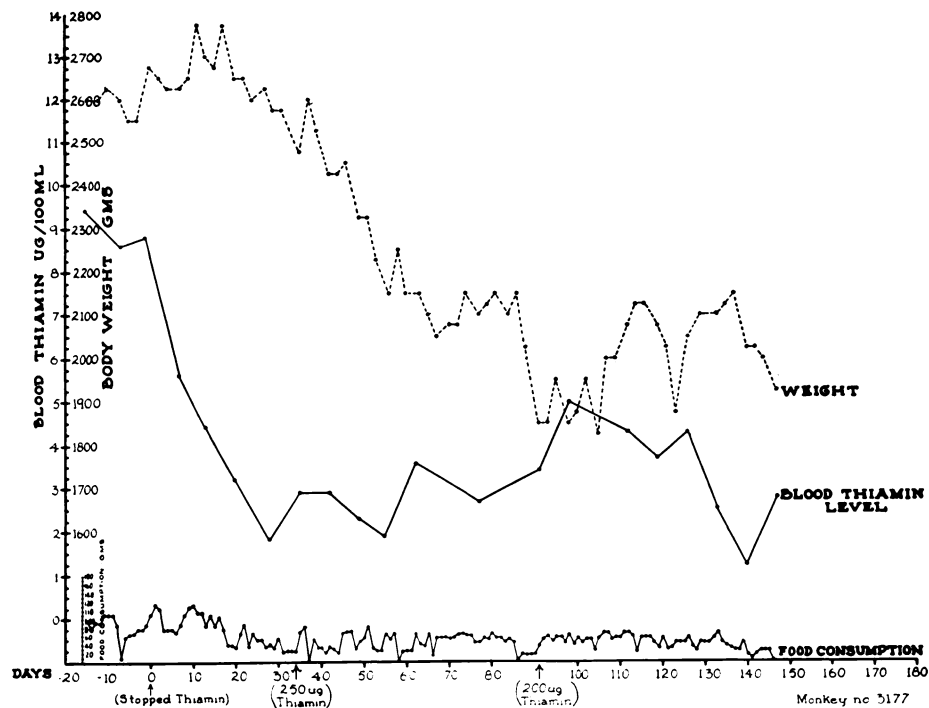


FIG. 2. THIAMIN DEFICIENCY IN MONKEY No. 3177
Graph of food consumption, blood thiamin, and weight

At times there appeared to be a paralysis of the hind legs. The animal could climb or move in the cage only by the use of its forelegs. Occasionally the onset of acute thiamin deficiency was so sudden that no manifestations were evident, aside from weight loss, mild anorexia and decreased activity, until the animal became ataxic, followed by a state of collapse. Edema was observed in only one animal during the period of depletion. However, following administration of thiamin to acutely deficient animals we have in several instances observed the appearance of edema. The control animals continued to gain weight (although not as rapidly as animals we have had on our stock diet), and to remain strong and healthy during the course of the experiment. We have maintained control animals on the complete diet

for a year or longer without any obvious alterations in their strength or vigor. One control animal was sacrificed and autopsied after having been on the diet for a period of six months and no pathologic changes were in evidence during either gross or microscopic examination of the tissues.

THIAMIN METABOLISM

Blood thiamin. During the period while the monkeys were on the complete diet including the thiamin supplement, the blood thiamin levels ranged from 5.5 to 10 micrograms per 100 ml. of whole blood. This range of values is similar to that observed by us in healthy human beings. Following withdrawal of the thiamin, and simultaneously with the first fall in weight and food consumption, the blood thiamin usually dropped to values of 4 micrograms or less. Except for some minor fluctuations these values remained low. When sufficient thiamin was administered, this was reflected in the blood level by a significant rise. The alterations of the

TABLE 1.—*Thiamin Requirement of Monkeys*

Monkey no.	Wt.	Dose of thiamin administered	Elapsed time	Minimum requirement micrograms/kg./day
	kg.	μg.		
245	5	4000	36	22
69	2.75	1000	50	7.5
3192				
1st-2nd depletion	1.9	200	11	9.6
2nd-3rd depletion	1.7	500	12	24.6
3rd-4th depletion	1.7	250	8	18.3
73	2.57	2500	91	27.5
Average.....				15.5

blood thiamin levels are charted in figures 1 and 2. The blood thiamin levels of control animals remained well above 5 mg. per 100 ml. during the course of the experiment.

Minimal thiamin requirement. A rough estimate of the minimum thiamin requirement of the monkey can be obtained by observing the time required to replete an acutely deficient animal following the administration of a small dose of the vitamin. Although there is a possibility that a portion of the vitamin may pass through the gastrointestinal tract unabsorbed (if administered by mouth), or that a portion may be eliminated in the urine if the dose is too large, for this calculation the assumption is made that the full dose is retained. If the total dose is divided by the product of the elapsed time in days and the weight in kilograms, one obtains a value for the minimum daily requirement per kilogram of body weight. Calculations of this type have been carried out on 4 individual monkeys and are summarized in table 1. In the case of Monkey No. 3192 which was carried through four depletion periods, three different observations on this same animal are recorded. The values recorded show considerable variation. However, the average

value obtained is 15.5 micrograms per kilograms per day and is in close agreement with the value reported by Waisman and McCall.⁴ It should be pointed out that this does not represent an intake adequate for normal metabolism.

Tissue thiamin. Certain tissues were removed at the time of autopsy and subjected to thiamin and riboflavin analysis. In nearly all cases (with the exception of one) the deficient animals were sacrificed in the terminal stage of deficiency by the administration of chloroform. For purpose of comparison a control animal was sacrificed at the termination of the study. The latter was in excellent health after having been maintained on this purified diet for a period of six months. In table 2 we have summarized the results of our analyses. These data show that there is a marked lowering of the thiamin content of every tissue examined in the deficient animals.

In the case of riboflavin the heart, kidney and liver concentrations of the deficient animals were found to be slightly higher than those observed in the control

TABLE 2

Mon-key no.	Control (C) or defc. (D)	Skeletal Muscle		Brain (cortex)		Heart (ventr.)		Kidney		Liver	
		thiamin	ribo-flavin	Thiamin	Ribo-flavin	Thiamin	Ribo-flavin	Thiamin	Ribo-flavin	Thiamin	Ribo-flavin
		$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$	$\gamma/gm.$
3163	C	0.9	3.1	1.6	2.5	3.5	6.8	3.5	19.6	2.1	18.9
3175	D	0.4	—	0.3	—	0.3	6.6	0.5	17.8	0.7	24.1
3192	D	0.2	1.8	0.4	2.8	0.3	8.7	0.6	26.3	0.5	21.4
3177	D	0.3	1.7	0.5	2.5	0.3	10.0	0.9	23.1	0.7	26.8
3146	D	0.5	1.8	—	—	0.4	10.2	0.6	22.0	0.6	25.4
69	D	0.2	1.5	—	—	0.9	9.6	1.0	24.8	0.8	21.4
73	D	0.2	1.2	—	—	0.3	7.3	1.3	21.3	0.9	18.5
245	D	0.3	1.2	—	—	0.3	8.2	0.8	25.2	0.9	18.4

animal. However, this is offset by the greater concentration of this vitamin in the skeletal muscle of the control animal.

Although it is difficult to compare tissue vitamin levels of one species of mammal with those of another species since the intake is known definitely to influence the concentration of the vitamins, we can point out that the control levels found in the monkey are similar in some respects to those found in the rat and in man. Figures⁶ obtained in our laboratory on the rat on a 20 γ per day level are of the same order for heart and muscle, but higher values for kidney and lower values for liver are found in the monkey. In general, rats with acute thiamin deficiency show lower figures for the tissues analyzed than those found in the monkey during the acute stage of deficiency. The control thiamin values for monkey's skeletal muscle and heart are not significantly different from those of humans who have died of accidental death. Liver and kidney of human origin have shown lower values than those found in monkeys. The tissue thiamin values recorded in the control monkey probably represent saturation values in as much as the animal had received daily thiamin in excess of its metabolic requirement.

HEMATOLOGIC OBSERVATIONS

It is generally considered that thiamin deficiency per se has little if any influence in hematopoiesis.^{7, 8} Our data indicate a significant influence on erythropoiesis as is illustrated in figure 3. It will be seen that with depletion for thirty days there was a slight but definite reduction in the red blood cell count and hemoglobin. At forty days this was obscured presumably by dehydration. However, at this time the reticulocyte count had fallen to zero. On administration of small subcurative doses of thiamin there were definite reticulocyte responses. Interestingly this was accompanied by a fall in the red blood cell count and hemoglobin which was probably brought out by correction of dehydration. A second

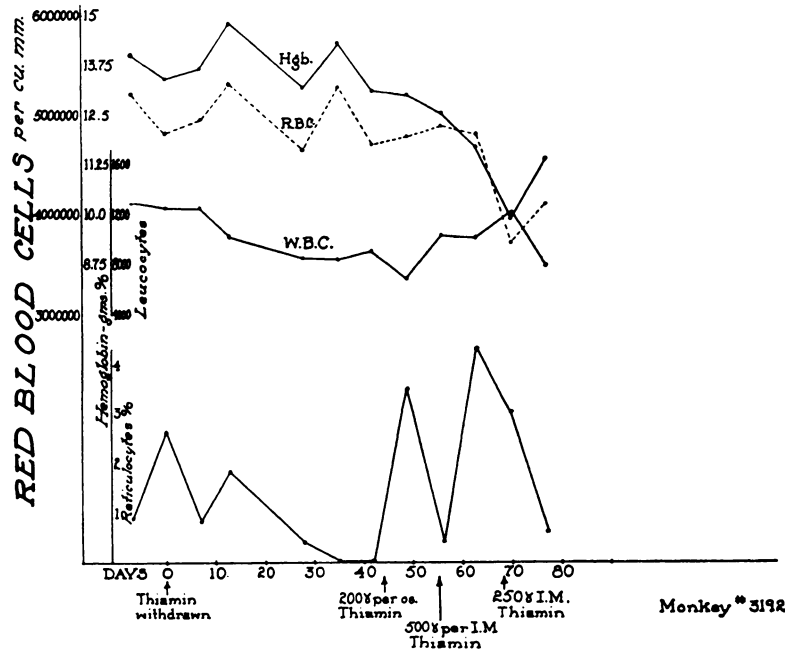


FIG. 3. BLOOD IN THIAMIN DEFICIENCY IN MONKEY No. 3192

animal showed a progressive fall of the red blood cell count and hemoglobin beginning at thirty days and continuing to the termination of the experiment at fifty days. In this animal the red blood cell count fell from 4.7 million to 3.6 million and the hemoglobin fell from 13.4 grams to 8.75 grams. The reticulocyte count during the early phase of the experiment approximated 1 per cent falling to zero between thirty two and fifty days. The complete suppression of circulating reticulocytes is of particular interest and is unique in our experience, not occurring in other deficiencies of the vitamin B factors studied. The small bleedings for metabolic studies probably contributed very little to the anemia. Such anemias did not develop in control animals. The cases cited are typical of the 4 animals in which hematologic examinations were made. The conclusion seems justified that thiamin

deficiency in the rhesus monkey will cause anemia and that the mechanism is evidently due to suppression of reticulocyte formation.*

SUMMARY

Seven rhesus monkeys were subjected to one or more episodes of acute thiamin depletion. It is clear that significant metabolic inadequacies preceded demonstrable structural changes. Diminished food consumption and weight loss were manifest about two weeks after thiamin was removed from the diet. When the deficiency was prolonged the animals became apathetic, inactive and progressively weaker. This was followed by ataxia and at times ptosis and tremors. Even in such advanced states of depletion, administration of thiamin produced dramatic improvement in locomotion, appetite and reactivity. The blood thiamin content of normal monkeys ranged from 5.5 to 10 γ per 100 ml. of whole blood, values which are comparable to those of healthy human beings. Following withdrawal of thiamin the blood concentration fell to values of 4 γ or less. The tissue content of thiamin was correspondingly reduced in depleted animals. The minimum daily requirement for thiamin calculated on the basis of the time required to replete a deficient monkey following a small dose of thiamin was approximately 15 γ per kilogram body weight. Characteristic degenerative changes in the heart muscle and severe retrogressive changes in the nuclear structures of the central nervous system previously reported were noted. Based on careful hematologic studies in 4 animals it is concluded that thiamin is essential for normal erythropoiesis. Acute or chronic depletion results in anemia due to suppression of red blood cell formation as indicated by severe depression or absence of reticulocytes in the blood.

ACKNOWLEDGMENT

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* Since this article was written we have found a report by Fornaroli (*Arch. fisiol.* 41: 276-285, 1941) in which he records "a large reduction" in reticulocytes in vitamin B₁ deficient rats.