

ATTEMPTS TO PRODUCE A NIACIN DEFICIENCY IN THE RHESUS MONKEY¹

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THREE FIGURES

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There are comparatively few reports in the literature dealing with the production of niacin deficiency in laboratory animals fed purified rations. Schaefer et al. ('42) demonstrated a niacin deficiency in dogs which were maintained on a niacin-low purified ration. Briggs et al. ('42) reported that the chick requires niacin for optimal growth and for the prevention of chick "blacktongue" when fed a highly purified ration. Wintrobe et al. ('45) in a recent paper on the niacin requirements of the pig reviewed the literature on niacin deficiency in pigs and concluded that most of the results of the earlier workers were equivocal since the natural rations used were low in proteins and probably some of the B vitamins. They (Wintrobe et al.) made an extensive study of niacin deficiency in pigs, and reported that a dietary source of niacin was not required by pigs which were fed a purified diet containing high amounts of protein. Deficiency symptoms did, however, appear when the protein level was low. Wooley and Sebrell ('45) showed that niacin was a dietary essential for rabbits which were fed a purified ration. Although it has been

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shown (Birch, '39) that the rat does not require niacin when fed a purified ration, Krehl, Teply and Elvehjem ('45) found that a deficiency of this vitamin could be produced in the rat when the purified ration was supplemented with corn grits to the extent of 40%. The production of a niacin deficiency in rhesus monkeys has been reported by Harris ('37) and by Chick and Hume ('20) but the rations employed by both these groups of workers were lacking in adequate protein and B vitamins. In this paper we wish to present the results of attempts to produce a niacin deficiency in monkeys using purified rations alone and purified rations with added corn.

EXPERIMENTAL METHODS

The method of handling the monkeys has been described previously (Waisman et al., '43). The basal ration (M-2) consisting of sucrose 73, Labco casein 18, salts IV 4, cod liver oil 3, and corn oil 2 was fed ad libitum. Adequate amounts of ascorbic acid and all the B vitamins (thiamine, riboflavin, pyridoxine, pantothenic acid, choline, i-inositol, p-aminobenzoic acid and biotin) except niacin were fed daily as a separate supplement. Folic acid was also supplied as a niacin low norite eluate concentrate prepared according to the directions of Krehl et al. ('45) at a level equivalent to 5 gm original solubilized liver (fraction L) per day. In later experiments the synthetic *Lactobacillus casei* factor replaced the norite eluate concentrate and was fed at a level of 100 µg per day. Microbiological assays (Krehl et al., '43) showed that the basal ration together with the supplement contained less than 50 µg of niacin per 100 gm.

Studies with purified rations

Four young monkeys (nos. 186, 187, 188 and 189) were placed on the niacin low purified ration. The monkeys continued to grow at a slow rate for several months without showing any deficiency symptoms. At the end of the fifth month, monkey 188 started to lose weight, and niacin therapy at a level of 10 mg per day was instituted. During the next 4

days on this regimen the monkey continued to lose weight rapidly and 25 mg of niacin amide was administered by intraperitoneal injection. The niacin and niacin amide therapy were completely ineffective and the monkey died the next day. Autopsy revealed no gross lesions.

At the end of the sixth month, monkey 189 showed some weight loss. The hemoglobin values also began to decrease and reached a value of 9 gm per 100 ml of blood. Niacin given at a level of 10 mg per day for 1 week proved ineffective in preventing the weight loss, the decrease in hemoglobin content of the blood and the anorexia. The basal ration was

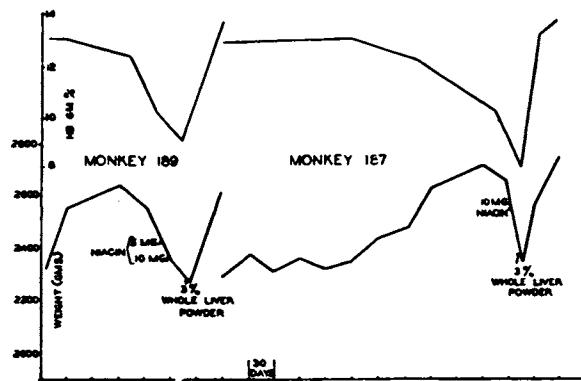


Fig. 1 The response in hemoglobin and weight to niacin and whole liver powder in monkeys which failed on a niacin low purified ration.

supplemented with 3% whole liver powder and fed via stomach tube. Within a week the animal's appetite improved sufficiently to discontinue the tube feeding. Shortly thereafter a sharp increase in weight occurred which was followed by an increase in hemoglobin content of the blood.

Monkey 187 showed a similar history although the animal continued to grow for 14 months before deficiency symptoms appeared. Again niacin was ineffective in correcting the weight loss and drop in hemoglobin, but when the basal ration was supplemented with 3% whole liver powder there was a prompt remission of these symptoms. The data for these animals are summarized in figure 1.

It is apparent from these results that a niacin deficiency can not be readily produced in monkeys fed a purified ration. Since the syndrome precipitated by the niacin low ration was completely reversed by feeding 3% whole liver powder, it is quite probable that a deficiency of the monkey anti-anemia factor existed. These observations are similar in many respects to riboflavin (Cooperman et al., '45b), vitamin B₆ (McCall et al., '46), pantothenic acid (McCall et al., '46), and folic acid (Cooperman et al., '46) deficiencies in the monkey which are complicated by a concomitant deficiency of the monkey anti-anemia factor. There is, however, one distinct difference in the case of niacin, namely, niacin therapy has no beneficial effect upon the deficient animals whereas in the case of riboflavin, vitamin B₆, pantothenic acid, and folic acid there is an initial response to the respective vitamins. The length of time required for the animals to become deficient on the niacin low ration probably depends upon the previous body stores of the monkey anti-anemia factor.

Studies with high corn rations

After having been on the niacin low purified ration for 15 months, monkey 186 still showed no untoward effects. Since Krehl et al. ('45) had demonstrated that rats required niacin when the basal ration was supplemented with 40% corn grits, an attempt was made to produce a niacin deficiency through this means. After 6 weeks on the corn containing ration, monkey 186 began to lose weight rapidly. Twenty-five mg of niacin per day was given for 4 days without any effect on the rate of growth or decrease in hemoglobin. Since Krehl, Teply, Sarma and Elvehjem ('45) had demonstrated that tryptophane could replace niacin as a growth factor for rats when fed the corn grits basal, the monkey was given 300 mg dl-tryptophane per day in addition to the daily supplement of niacin without any beneficial effect. The monkey was then returned to the M-2 basal without the corn grits for 1 week but became progressively worse. The M-2 basal was supplemented with 3 gm of lyophilized liver per day fed via stomach

tube. After 3 days the animal regained its appetite and the tube feeding was discontinued. This treatment was followed by a sharp increase in weight, the animal gained 300 gm in 1 week and the hemoglobin content of the blood increased to 13.5 gm per 100 ml during the same period. The data are summarized in figure 2. The corn grits, in all probability, precipitated a deficiency of the monkey anti-anemia factor since lyophilized liver is an excellent source of this factor (Cooperman, McCall and Elvehjem, '45a).

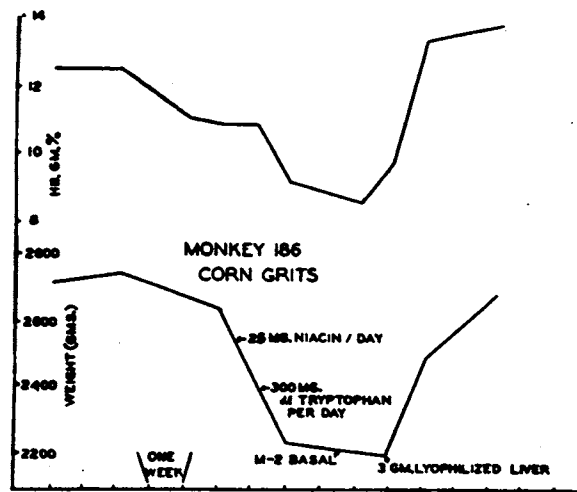


Fig. 2 The effect of feeding niacin, tryptophane, the M-2 basal, and lyophilized liver on the weight and hemoglobin of a monkey which failed on the corn grits basal.

In order to repeat this work with young monkeys, experiments were started in which newly acquired monkeys were placed on the corn grits basal directly. Three young monkeys (nos. 247, 248, and 275) were given the M-2 basal supplemented with 40% corn grits and all the B vitamins except niacin. The hemoglobin content of the blood and differential leucocyte counts were determined regularly on the animals since these are affected in a deficiency of the monkey anti-anemia factor (Cooperman et al., '46; McCall et al., '46). The results with

a typical monkey (no. 248) will be described since all the animals showed a very consistent picture. This animal grew at a very slow rate for 5 weeks at which time growth ceased entirely. The hemoglobin value was 10.6 gm % at this time. The monkey then started to lose weight and at the end of 8 weeks the hemoglobin had decreased to 8.1 gm % and differential leucocyte counts showed a definite reversal in the neutrophile-lymphocyte ratio, the usual syndrome in the monkey anti-anemia factor deficiency (Cooperman et al., '46; McCall et al., '46). At this time 10 mg niacin per day was given for 10 days but this proved inadequate in ameliorating the

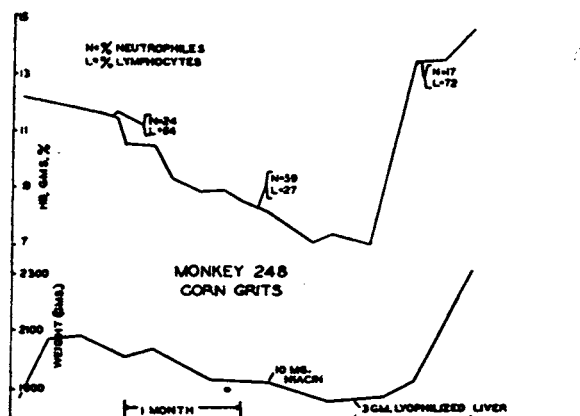


Fig. 3 The effect of niacin and lyophilized liver on the weight, hemoglobin and differential leucocyte counts of a young monkey fed the corn grits basal.

weight loss or the blood dyscrasia. The ration was then supplemented with 3 gm of lyophilized liver per day and within 3 days there was a perceptible change both in the weight and blood picture. At the end of 3 weeks the hemoglobin value reached a level of 14.7 gm % and the neutrophile-lymphocyte reversal was corrected. The data for this animal are summarized in figure 3.

In the case of monkey 247, the corn grits basal was supplemented with both niacin and tryptophane when the weight loss and blood dyscrasia became evident, but this combination also proved ineffective.

Two other young monkeys (nos. 276 and 277) were given the corn grits basal supplemented with 300 mg dl-tryptophane per day and all the B vitamins except niacin. Again growth was poor and the typical blood dyscrasia appeared. Supplementing the ration with 10 mg niacin had no beneficial effect either on the weight or blood condition. One hundred and twenty-five micrograms of crystalline vitamin B₆ per day were given to monkey 276 by intramuscular injection for 1 week but this too proved ineffective.

Three monkeys (nos. 295, 296, and 297) were started on the corn grits basal, which was supplemented with all the B vitamins including niacin. A weight plateau appeared at the end of 6 weeks and soon thereafter the typical blood dyscrasia appeared.

Since the typical syndrome of a deficiency of the monkey anti-anemia factor is readily produced when monkeys are fed a 40% corn grits basal, this affords an easy method for the production of assay monkeys. Our previous assay depended upon the weight and hemoglobin response in monkeys which failed to show complete recovery from a riboflavin deficiency after riboflavin therapy (Cooperman, McCall and Elvehjem, '45). Experiments in this laboratory (unpublished data) have shown that both of these types of assay monkeys (corn grits and riboflavin deficient) respond similarly to materials rich in the monkey anti-anemia factor and in no case has any material shown activity in one type without giving a response in the other.

It is interesting to note the different effect the corn grits ration has on the rat and monkey. Whereas in the rat either niacin or tryptophane will correct the deficiency, in the monkey neither tryptophane, niacin nor both are of value in preventing the onset or curing the deficiency.

It will be difficult to ascertain whether the monkey requires niacin until a concentrate of the monkey anti-anemia factor low in niacin is available.

SUMMARY

Monkeys on a purified ration extremely low in niacin develop a deficiency which does not respond to niacin amide therapy. Whole liver powder, a good source of the monkey anti-anemia factor, causes a prompt remission of the deficiency symptoms.

When monkeys are fed a ration containing 40% corn grits, a deficiency characterized by a loss in weight, suboptimal hemoglobin, and a reversed neutrophile-lymphocyte count develops. Neither niacin, tryptophane, nor a combination of the two ameliorate the syndrome. Lyophilized liver or whole liver powder correct the deficiency.

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