
A STUDY OF THE VITAMIN K REQUIREMENT OF THE MONKEY (MACACUS RADIATA).

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The vitamin K requirements of different species of animals seem to differ markedly. For example, the vitamin K requirements per gramme of the diet are 0.014 microgram for the mink (Travis et al., 1961), 0.1 microgram for the rat (Mameesh and Johnson 1960), and 1 microgram for the chick (Griminger and Donis, 1960). Sells, Walker and Owen (1941) have reported a requirement of 1 microgram 2-methyl-4-amino-1-naphthol (vitamin K₄) per day for the new-born infant.

In recent years, the monkey is being extensively used for biochemical and nutritional investigations. The authors are not aware of any published data regarding the vitamin K requirement of the monkey. Therefore, vitamin K studies were undertaken on the monkey. The first step in such studies obviously is to produce vitamin K deficiency in the monkey.

In the present investigations, attempts have been made to induce vitamin K deficiency in monkeys, with a view to derive information regarding their vitamin K requirement.

MATERIALS AND METHODS.

Animals.—Seven male and 3 female healthy young monkeys (Macacus radiata), each weighing approximately 4.5 lb., were used for the study. The monkeys were housed in individual cages with raised screen bottoms so that the excreta would fall out of the cage and the monkeys will not have access to it.

The investigation consisted of four stages or periods of feeding. During the entire experiment, daily individual food intake and body-weight records were maintained. Blood samples from the femoral vein were periodically withdrawn during every stage of feeding and analysed for plasma prothrombin by the Quick method (1988).

Stage I.—The animals were fed a synthetic diet, the composition of which is indicated in the Table. The diet was prepared daily by mixing with water (75 ml. water were added to 100 g. diet), and made into ‘chapaties’ and autoclaved at 15-lb. pressure for 15 minutes. The diet and water were given ad libitum. Once a week each animal was given 33,000 I. U. of vitamin A, 3,300 units of vitamin D₃ (Vanitin, synthetic vitamin A acetate, USP, in vegetable oil, Roche), 50 mg. tocopheryl acetate and 620 mg. of 60 per cent linoleic acid in 2 ml. glycerol by smearing on the ‘chapaties’. The animals were fed this diet for 50 days.

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Stage 2.—Since the diet used in stage 1 contained a high level of starch (75 per cent) which could promote bacterial flora, the possibility of intestinal synthesis of vitamin K at this stage was considered. If the intestinally synthesized vitamin K was absorbed directly from the lower intestines, adequate amounts of vitamin K could become available to the monkey. Hence, in the second stage of the experiment which lasted for 30 days, 54.5 per cent of the starch in the diet was replaced by sugar.

Stage 3.—Even in stage 2 the possibility of intestinal synthesis could not be ruled out. So, it was thought of interest, to actively inhibit whatever bacterial synthesis of vitamin K was taking place in the intestines on the high-sugar-casein diet, and follow up the changes in the plasma prothrombins. The animals were divided into two groups. The first group consisted of 6 monkeys: 5 male and 1 female. In the second group there were 3 monkeys: 1 male and 2 female. Both groups were continued on the high-sugar-casein diet, but the first group was given 1 per cent sulfasuxidine (succinyl-sulfathiazole) mixed in the diet before cooking. The animals were fed for 20 days on these two diets.

Stage 4.—In this stage, the group of monkeys receiving sulfasuxidine was now given aureomycin, in addition, since the latter is a wide-spectrum bacteriostat. Aureomycin in water was given to the monkeys for 48 days by a stomach tube at the rate of 260 mg. a day, two hours after food.

Vitamin K assay of the synthetic diet.—Having failed to produce hypoprothrombinemia in the monkeys on a vitamin K-free diet even with the inclusion of sulfasuxidine and aureomycin in the diet, it was thought that the vitamin K which could be present as an impurity in the dietary components used, perhaps satisfied the vitamin K requirements of the young monkeys. The vitamin K activity of the high-sugar-casein diet was, therefore, measured by the chick assay and found to be 0.08 microgram menadione (K₃) per gramme of the (dry) diet.

RESULTS AND DISCUSSION.

During stage 1 (first feeding period) of the experiment the average daily (dry) food intake was 64 ± 3.5 g. which is estimated to provide 236 calories per monkey per day. The monkeys lost some weight during the first 20 days of feeding, but later they recovered and maintained their weight.

The plasma prothrombin times in the beginning of the feeding period were 23 ± 0.6 seconds. There was little change in the prothrombin levels during the following several weeks. On the 60th day of feeding the high-starch-casein diet the plasma prothrombin times were 22 ± 0.4 seconds. Thus, the plasma prothrombin levels remained essentially the same.

In stage 2 (the second feeding period) the monkeys ate the high-sugar-casein diet avidly for 30 days. After 10 days on this diet a female monkey was found dead; however, death was not due to hypoprothrombinemia. Autopsy revealed no gross pathology except a little hemorrhage in the stomach wall. The rest of the monkeys maintained normal plasma prothrombin levels.
Vitamin K Requirement of the Monkey.

TABLE.
Composition of the diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein¹</td>
<td>12</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.3</td>
</tr>
<tr>
<td>Starch</td>
<td>74.5</td>
</tr>
<tr>
<td>Sugar</td>
<td>3</td>
</tr>
<tr>
<td>Glycerol</td>
<td>3</td>
</tr>
<tr>
<td>Vitaminized glucose²</td>
<td>3.2</td>
</tr>
<tr>
<td>Mineral mixture³</td>
<td>4</td>
</tr>
</tbody>
</table>

1. Fat-free, vitamin free—British Drug House.
2. 100 g. of vitaminized glucose contained thiamine HCl and riboflavin 42 mg. each; pyridoxine HCl, 63 mg., calcium pantothenate, 210 mg., nicotinic acid, 84 mg., folic acid 17 mg., inositol, 12 mg., biotin, 1-2 mg., crystalline vitamin B₁₂, 1-6 mg., ascorbic acid, 400 mg., and choline chloride, 2.8 g.
3. 100 g. of mineral mixture contained sodium chloride 10.8 g., potassium citrate 23.7 g., potassium monophosphate 7.7 g., calcium diphosphate 35.8 g., calcium carbonate 16.3 g., magnesium carbonate 4.1 g., ferric citrate 1.6 g., copper sulfate 18 mg., manganous sulfate 124 mg., potassium aluminium sulfate 9 mg., potassium iodide 4.4 mg., cobalt chloride 9 mg., zinc carbonate 4.4 mg., and sodium fluoride 0.08 mg.

During the subsequent 20-day feeding period (of stage 3) when 1 per cent sulfasuxidine was included in the high-sugar-casein diet of 6 monkeys no significant change was observed in their plasma prothrombin levels. It was possible that sulfasuxidine was not an effective bacteriostat as far as the intestinal synthesis of vitamin K was concerned.

During stage 4 (the fourth feeding period) also, which lasted for 48 days with 250 mg. of aureomycin per monkey a day, the plasma prothrombin levels were not affected. Thus, even by feeding a synthetic diet to which no vitamin K was added and by inhibiting the intestinal bacterial activity with sulfasuxidine and aureomycin it was not possible to produce vitamin K deficiency in the monkey during a 148-day experiment. The only exogenous source of vitamin K for the monkey under the present experimental conditions could be from the dietary ingredients used which could contain vitamin K as an impurity. By the chick assay the synthetic diet was found to have vitamin K₃ (menadione) activity of 0.06 microgram per gramme of the diet. From the food intake data, we may compute an intake of approximately 4 microgram of vitamin K₃ per monkey per day. Thus, 4 microgram of vitamin K₃ a day was apparently adequate to maintain normal plasma prothrombin levels in the young monkeys. The possibility that even with the rigorous attempts to suppress intestinal synthesis followed in this study, some intestinal synthesis of vitamin K was taking place cannot be ruled out. Studies are currently under way to investigate this possibility.

Summary.

The requirement of vitamin K was investigated on the monkey (Macacus radiata). Seven male and 3 female young monkeys were individually fed ad lib. a synthetic diet (12 per cent vitamin-free casein, 0.8 per cent l-cystine, 74.5 per cent...
starch, 3 per cent sugar, 3 per cent glycerol, 3.2 per cent glucose, 4 per cent minerals, adequate amounts of methyl-linoleate and all vitamins except vitamin K). During a 50-day feeding period plasma prothrombin times (23 seconds) showed no change. Coprophagy was not observed. With a view to reduce possible intestinal synthesis, the diet was then modified to contain 20 per cent starch, and 57.5 per cent sugar, and fed for 30 days. Again, plasma prothrombin times remained normal. Six monkeys were then continued for 20 more days on the same high-sugar diet with 1 per cent sulfasuxidine, and 48 days thereafter they were, in addition, fed aureomycin (250 mg. each/day) to further inhibit intestinal synthesis of vitamin K. However, these antibiotics also did not influence the plasma prothrombin levels. Chick assay revealed a vitamin K₃ activity of 0.06 microgram/g. of the (dry) diet. Thus, the vitamin K₃ requirement of the growing monkey does not seem to exceed 0.06 microgram/g. of the diet, or is approximately 4 microgram vitamin K₃ per day.

REFERENCES.


