4. Experiments in which corn oil was fed to rats showed that the 'fatty supernatant' did not rapidly equilibrate with the dietary fat.

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Effect of Carbohydrates on the Intestinal Synthesis of Thiamine in Rats

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The intestinal synthesis of thiamine depends on dietary constituents, which affect both the type and number of intestinal micro-organisms (Kuno, 1953; Johansson, 1956; Balakrishnan, Baliga & Rajagopalan, 1957; Mameesh & Johnson, 1958; Nath & Meghal, 1960).

Banerji (1941) observed that the presence of much protein in the basal diet prevented the bradycardia and loss of weight usually associated with thiamine deficiency. Banerji & Yudkin (1942) reported that rats grow and thrive well in the absence of thiamine, provided that they do not have to metabolize dietary carbohydrate. According to Banerji & Yudkin (1942) and Yudkin (1951) the signs of thiamine deficiency are due to the toxic products of disturbed carbohydrate metabolism. As little as 10% of glucose in the diets led to polyneuritis and death (Yudkin, 1951), and sorbitol has been shown to be thiamine-sparing (Morgan & Yudkin, 1957; Jones & Baumann, 1958).

When potato starch is added to the thiamine-free basal diet, rats can grow well and this phenomenon was described by Fridericia, Freudenthal, Gudjohnson, Johanssen & Schaubye (1927) as

'refection'. According to them the end products of fermentation lowered the pH of the intestinal contents and this appeared to promote the growth of micro-organisms which synthesize vitamins of the B group. Nagase & Fugita (1956) reported that cellulose helps in the intestinal synthesis of thiamine in man.

Therefore, we have studied the effect of different carbohydrates on the faecal coliform count and daily excretion of thiamine in the urine and faeces of rats. Hydrolysed 'glucose cycloacetoacetate', which has been shown to contain glucose (1:2-dienol) and has a strong reducing action even in acid medium (Nath & Bhattathiry, 1956), not only helps in prevention of experimental diabetes (Nath & Behki, 1957*a, b*; Nath & Bhattathiry, 1959) and experimental hyperlipaemia induced by high concentrations of saturated fats or cholesterol in the diet (Nath & Saikia, 1959*a, b*), but also stimulates the intestinal biosynthesis of thiamine in rats kept on a thiamine-free diet (Nath & Meghal, 1960). Therefore we also studied the effect of hydrolysed 'glucose cycloacetoacetate' on the thiamine deficiency induced by adding either glucose or sucrose to a thiamine-free basal diet.

EXPERIMENTAL

Forty-eight male weanling albino rats (30–40 g. each) were divided into eight groups and placed individually in wire-bottomed cages. Coprophagy was not prevented. A carbohydrate-free basal diet, deficient in thiamine (Morgan & Yudkin, 1957), was used and the additional carbohydrates were given by replacing the same weight of casein. The composition of the thiamine-free diets given to the different groups of animals is shown in Table 1. Each rat received the respective diet *ad lib.* and daily dietary intake was noted.

The animals were given the appropriate diets for 6 weeks. After the first 4 days in each week, urine and faeces were collected for the remaining 3 days of the week and the thiamine content was determined by the method of Mawson & Thompson (1948). Weekly weights of animals were also recorded.

During the second, fourth and sixth weeks the faecal bacterial count was measured in three rats from each group. The faeces of each rat were collected aseptically on the last 3 days of the week in weighed sterile test tubes and a uniform suspension of about 0.1 g. of faeces with 10 ml. of 0.9% NaCl soln. was made under aseptic conditions. Further dilutions were made with the saline and the coliform colonies in the faeces were counted by the plate method in Mackonkey's agar medium (Balakrishnan & Rajagopalan, 1952).

At the end of the sixth week the animals were weighed and killed. The thiamine contents of liver, kidney, brain and thigh muscle were determined by the method of Kratzing & Slater (1950).

The thiamine content of honey estimated by the thiochrome method of Harris & Wang (1941) was 6.4 $\mu\text{g.}/100\text{ g.}$ 'Glucose cycloacetoacetate' was prepared by condensing glucose with ethyl acetoacetate as described by West (1927) and hydrolysed according to the method of Nath & Sahu (1953). The aqueous extract of hydrolysed material, after repeated washing with ether, was diluted with water so that it contained the equivalent of 80 mg. of 'glucose cycloacetoacetate'/ml.

RESULTS

Daily dietary intake. The daily dietary intake of rats belonging to groups 1 (controls), 4, 6 and 8 gradually increased, and the dietary intake of those

belonging to groups 2, 3 and 5 decreased (Table 2). The daily thiamine intake from honey (group 2) was 0.29–0.32 $\mu\text{g.}/\text{rat.}$

Urinary and faecal excretion of thiamine. From the values in Table 3, it can be seen that the weekly urinary and faecal excretion of thiamine of groups 1 (controls), 4, 6 and 8 was higher than that of all other groups, thereby giving an indication of increased rate of thiamine synthesis throughout the entire experimental period; the rats in group 2 showed a slight increase up to the fourth week in the excretion of thiamine in urine and faeces. After the fourth week it decreased. As the rats in groups 3 and 5 died after the fourth week owing to thiamine deficiency, their faecal and urinary excretion of thiamine could be estimated only during the first 4 weeks and it was found to be decreased. The rats in groups 3 and 5 showed loss of appetite and polyneuritic symptoms. The pectin-

Table 1. *Composition of thiamine-free diets*

The diet for each group of rats consisted of vitamin-free casein 70%, salt mixture no. 185 (McCullum & Davis, 1915) 5%, groundnut oil 15% and the addition (10%) indicated below. Each rat also received daily: riboflavin 80 $\mu\text{g.}$, nicotinic acid 500 $\mu\text{g.}$, pyridoxine hydrochloride 40 $\mu\text{g.}$, calcium pantothenate 250 $\mu\text{g.}$, biotin 0.2 $\mu\text{g.}$, *p*-aminobenzoic acid 250 $\mu\text{g.}$, folic acid 10 $\mu\text{g.}$, choline chloride 5 mg., inositol 2 mg., α -tocopherol acetate 30 $\mu\text{g.}$, vitamin K 50 $\mu\text{g.}$, vitamin B₁₂ 0.2 $\mu\text{g.}$. Vitamins A and D were given twice weekly in the form of Adexolin [Glaxo Laboratories (India) Ltd. Bombay] 84 mg.

Group	Addition
1 (Control)	Casein
2	Honey
3	Glucose
4*	Glucose
5	Sucrose
6*	Sucrose
7*	Cellulose
8	Gum acacia (pectin)

* These groups also received hydrolysed 'glucose cycloacetoacetate' (80 mg./rat/day).

Table 2. *Average daily dietary intake (g.) of rats in each group*

For group diet see Table 1.

Group	Week no.					
	1	2	3	4	5	6
1	3.5	3.8	4.2	4.5	5.0	5.0
2	3.10	4.6	3.7	3.2	4.0	4.2
3	4.0	4.2	3.8	3.9	—	—
4	3.9	4.8	5.1	5.2	5.1	4.8
5	4.8	3.8	3.9	4.2	—	—
6	3.6	3.8	3.9	4.2	4.6	4.5
7	3.6	4.6	5.7	5.2	5.0	5.0
8	4.5	5.0	5.5	5.7	5.7	5.8

Table 3. *Effect of carbohydrates on weekly urinary and faecal excretion of thiamine by rats*Values are expressed in $\mu\text{g./rat/24 hr. mean} \pm \text{s.d.}$ For group diet see Table 1.

Group	Urinary excretion in week					
	1	2	3	4	5	6
1	0.78 $\pm$ 0.02	0.85 $\pm$ 0.05	0.98 $\pm$ 0.06	1.63 $\pm$ 0.08	2.33 $\pm$ 0.05	2.35 $\pm$ 0.06
2	0.81 $\pm$ 0.04	0.89 $\pm$ 0.06	0.93 $\pm$ 0.03	1.21 $\pm$ 0.01	0.88 $\pm$ 0.02	0.88 $\pm$ 0.06
3	0.55 $\pm$ 0.06	0.43 $\pm$ 0.03	0.35 $\pm$ 0.06	0.28 $\pm$ 0.07	—	—
4	0.63 $\pm$ 0.03	0.85 $\pm$ 0.05	1.66 $\pm$ 0.03	1.88 $\pm$ 0.05	2.08 $\pm$ 0.06	2.13 $\pm$ 0.04
5	0.61 $\pm$ 0.05	0.63 $\pm$ 0.02	0.31 $\pm$ 0.07	0.20 $\pm$ 0.03	—	—
6	0.85 $\pm$ 0.07	0.99 $\pm$ 0.01	1.35 $\pm$ 0.02	1.83 $\pm$ 0.06	2.16 $\pm$ 0.03	2.18 $\pm$ 0.04
7	0.85 $\pm$ 0.03	0.96 $\pm$ 0.03	1.21 $\pm$ 0.04	0.85 $\pm$ 0.05	0.55 $\pm$ 0.03	0.55 $\pm$ 0.03
8	0.83 $\pm$ 0.04	0.93 $\pm$ 0.05	1.61 $\pm$ 0.05	1.81 $\pm$ 0.03	1.95 $\pm$ 0.02	2.20 $\pm$ 0.03

Group	Faecal excretion in week					
	1	2	3	4	5	6
1	1.21 $\pm$ 0.09	1.35 $\pm$ 0.03	1.36 $\pm$ 0.05	1.81 $\pm$ 0.06	2.39 $\pm$ 0.03	2.43 $\pm$ 0.01
2	1.01 $\pm$ 0.03	1.21 $\pm$ 0.02	1.26 $\pm$ 0.03	1.00 $\pm$ 0.02	0.89 $\pm$ 0.03	1.00 $\pm$ 0.06
3	0.33 $\pm$ 0.06	0.51 $\pm$ 0.03	0.37 $\pm$ 0.05	0.35 $\pm$ 0.06	—	—
4	1.18 $\pm$ 0.01	1.35 $\pm$ 0.09	1.89 $\pm$ 0.09	2.09 $\pm$ 0.04	2.10 $\pm$ 0.28	2.12 $\pm$ 0.18
5	0.88 $\pm$ 0.06	0.75 $\pm$ 0.06	0.55 $\pm$ 0.08	0.21 $\pm$ 0.03	—	—
6	1.21 $\pm$ 0.06	1.35 $\pm$ 0.18	1.89 $\pm$ 0.09	2.12 $\pm$ 0.12	2.28 $\pm$ 0.11	2.30 $\pm$ 0.18
7	0.86 $\pm$ 0.02	1.05 $\pm$ 0.07	1.12 $\pm$ 0.03	1.00 $\pm$ 0.02	0.89 $\pm$ 0.03	1.00 $\pm$ 0.06
8	1.23 $\pm$ 0.06	1.35 $\pm$ 0.07	1.65 $\pm$ 0.09	1.75 $\pm$ 0.06	2.11 $\pm$ 0.09	2.06 $\pm$ 0.02

Table 4. *Faecal coliform count of groups of three rats*

Average values are expressed in 1000/g. of wet faeces. For group diet see Table 1.

Group	Week no.		
	2	4	6
1	60.0	80.0	103.0
2	62.0	25.0	8.0
3	16.0	5.3	—
4	57.3	95.8	99.0
5	30.0	10.8	—
6	49.0	88.0	102.0
7	50.0	32.0	12.0
8	82.0	95.0	108.0

fed group (group 8), however, showed a constant increase in the urinary and faecal excretion of thiamine; the cellulose-fed group (group 7) showed a slight increase in the urinary and faecal excretion of thiamine during the first 4 weeks, after which it decreased.

Faecal coliform bacteria. The faecal coliform organisms were increased in groups 4, 6 and 8 and their number was similar to that of the controls (Table 4).

Examination of the relative coliform counts in the faeces of rats of the eight groups brings out the following points: (1) The coliform organisms were suppressed by the ingestion of glucose and sucrose throughout the experiment and the animals belonging to these groups died of thiamine deficiency in the fifth week. (2) Hydrolysed 'glucose cycloacetate' had the capacity to overcome

the harmful effect of glucose and sucrose on the intestinal flora and this beneficial effect is seen in groups 4 and 6 as the coliform count in the faeces of these groups increased gradually. (3) The coliform count in groups 2 and 7 was nearly the same as in the control group during the second week, after which it showed a gradual decrease. (4) The pectin-fed group (group 8) showed a constant increase in the faecal coliform count during the experiment, thereby indicating the increase in the intestinal synthesis of thiamine.

Weekly weight of animals. The animals of groups 1 (controls), 4, 6 and 8 showed a gradual increase in weight during the experiment; but glucose- and sucrose-fed rats showed a loss of weight and ultimately died of thiamine deficiency. Honey- and cellulose-fed rats showed no significant change in body weights (Table 5).

Tissue storage of thiamine. The content of thiamine in different tissues gives an indication of the state of health of the rats and the efficiency of the dietary factors in contributing to the body reserve. A comparison of the thiamine contents of liver, brain, kidney and muscle of eight groups (Table 6) shows that rats belonging to the groups 1 (controls), 4, 6 and 8 had greater storage of thiamine than did other groups.

DISCUSSION

The results of this experiment support the view that the animals receiving only 10% of glucose or sucrose in their thiamine-free diet suffer from

Table 5. *Effect of carbohydrates on the weekly weights of rats*Mean values in g. $\pm$ S.E.M. For group diet see Table 1.

Group	Initial wt.	Week					
		1	2	3	4	5	6
1	36 $\pm$ 2.1	42 $\pm$ 2.8	48 $\pm$ 1.6	59 $\pm$ 2.7	62 $\pm$ 1.9	64 $\pm$ 2.0	69 $\pm$ 5.6
2	39 $\pm$ 3.1	42 $\pm$ 2.2	50 $\pm$ 3.1	48 $\pm$ 2.6	46 $\pm$ 3.7	43 $\pm$ 5.1	44 $\pm$ 3.8
3	35 $\pm$ 3.2	36 $\pm$ 2.8	39 $\pm$ 4.2	39 $\pm$ 2.3	38 $\pm$ 3.1	—	—
4	38 $\pm$ 4.5	44 $\pm$ 2.8	56 $\pm$ 5.2	59 $\pm$ 3.7	62 $\pm$ 2.6	71 $\pm$ 4.7	72 $\pm$ 5.2
5	30 $\pm$ 2.2	31 $\pm$ 3.8	34 $\pm$ 2.2	32 $\pm$ 1.6	30 $\pm$ 2.5	—	—
6	37 $\pm$ 1.8	40 $\pm$ 2.1	52 $\pm$ 2.7	56 $\pm$ 3.1	66 $\pm$ 2.5	70 $\pm$ 3.9	74 $\pm$ 4.2
7	32 $\pm$ 2.2	39 $\pm$ 1.8	40 $\pm$ 3.8	42 $\pm$ 1.8	38 $\pm$ 1.9	36 $\pm$ 1.2	36 $\pm$ 1.5
8	32 $\pm$ 1.5	39 $\pm$ 1.2	48 $\pm$ 2.8	64 $\pm$ 3.2	66 $\pm$ 2.3	68 $\pm$ 3.2	68 $\pm$ 1.7

Table 6. *Effect of carbohydrates on tissue thiamine content after feeding for six weeks*Mean values in μ g./100 g. $\pm$ S.E.M. For group diet see Table 1.

Group	Liver	Brain	Kidney	Muscle
1	64.1 $\pm$ 0.096*	86.4 $\pm$ 1.20*	42.2 $\pm$ 1.88*	24.5 $\pm$ 1.88*
2	29.8 $\pm$ 0.64	34.3 $\pm$ 1.24	16.6 $\pm$ 0.52	14.2 $\pm$ 1.06
3	—	—	—	—
4	68.7 $\pm$ 0.97*	81.7 $\pm$ 0.82*	49.7 $\pm$ 0.68*	23.4 $\pm$ 0.89*
5	—	—	—	—
6	62.3 $\pm$ 0.81*	69.9 $\pm$ 2.43*	31.8 $\pm$ 1.41*	21.6 $\pm$ 1.53*
7	16.7 $\pm$ 1.43	22.6 $\pm$ 0.85	17.4 $\pm$ 0.89	10.6 $\pm$ 0.65
8	60.4 $\pm$ 0.78*	73.8 $\pm$ 3.61*	52.4 $\pm$ 2.8*	25.2 $\pm$ 1.06*

* Difference from group 2 values are significant at $P < 0.01$.

thiamine deficiency (Yudkin, 1951), as their thiamine excretion in urine and faeces is very much reduced as compared with other groups. The coliform organisms in the faeces of these groups were very much reduced, showing that these two substances might have a suppressing effect on the intestinal flora. In such experiments, coprophagy plays an important role. In our experiment, coprophagy was allowed. Morgan & Yudkin (1959) showed that sorbitol was effective in preventing thiamine deficiency in rats only when coprophagy was allowed. They further showed that, when coprophagy was prevented in sorbitol-fed rats, the rats suffered from thiamine deficiency. These workers also observed that the thiamine-deficient rats fed on glucose excreted in the faeces an amount of thiamine that was too small to prolong the life of such rats even for a short time. The hydrolysed 'glucose cycloacetate', which enhances the biosynthesis of thiamine (Nath & Meghal, 1960), overcame the harmful effect of glucose or sucrose when it was fed along with a thiamine-free basal diet. In the honey-fed group there was also a decrease in urinary and faecal excretion of thiamine but the animals of this groups did not suffer from thiamine deficiency. Honey contains about 80% (w/w) of reducing sugars (Loesecke, 1949) and therefore the rats of this group would have been expected to show the signs of thiamine

deficiency; this may have been prevented by the presence of thiamine or thiamine-sparing substances in the honey.

When potato starch is fed, rats can grow well in the absence of thiamine (Fredericia *et al.* 1927). Barnes, Kwong, Delany & Fiala (1960) did not confirm this; they showed that the inclusion of dextrin as carbohydrate source in diets deficient in thiamine permitted enhanced growth but the effect of dextrin was completely abolished when coprophagy was prevented. In our experiment, the effect of cellulose can be compared with that of dextrin because the cellulose-fed rats showed no signs of thiamine deficiency. Nagase & Fugita (1956) have shown that cellulose helps in the intestinal synthesis of thiamine in man. The present work shows that cellulose did not have the harmful effect of glucose or sucrose.

The pectin-fed group showed a constant rise in the urinary and faecal excretion of thiamine, thereby indicating the enhancing effect of pectin in the biosynthesis of thiamine. The pectin is formed of a branched chain of D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. The pectin undergoes hydrolysis in the process of digestion and the substances mentioned above are liberated; this may have a favourable effect on the intestinal flora, thus increasing the faecal thiamine that is ultimately available to rats after coprophagy.

SUMMARY

1. Eight groups of rats were given thiamine-free diet containing 70% of protein, with additional protein (10%) or carbohydrate (10%), e.g. honey, glucose, sucrose, cellulose or pectin. The amount of hydrolysed 'glucose cycloacetoacetate' given in combination with glucose or sucrose was 80 mg./rat daily.

2. Rats receiving either glucose or sucrose only suffered from thiamine deficiency and died during the experiment.

3. Hydrolysed 'glucose cycloacetoacetate' overcame the harmful effect of glucose or sucrose and the rats of these two groups showed an increase in urinary and faecal thiamine excretion, maintaining a constant increase in weight during the experiment. The tissue storage of thiamine in these rats was also increased.

4. Rats fed on honey and cellulose showed no significant change in urinary and faecal excretion of thiamine.

5. Urinary and faecal excretion of thiamine was increased in pectin-fed rats and their tissue thiamine content was also greater than that of honey- or cellulose-fed groups.

6. The faecal coliform count in control groups fed with hydrolysed 'glucose cycloacetoacetate' or with pectin was higher than in honey- and cellulose-fed groups.

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