CCXXIII. THE EFFECTS OF COPROPHAGY IN RATS DEPRIVED OF THE VITAMIN B COMPLEX.

By MARGARET HONORA ROSCOE¹.

From the Division of Nutrition, Lister Institute, London.

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INTRODUCTION.

THE presence of "vitamin B" in the excreta of birds and mammals has long been established. The antineuritic vitamin was found by Cooper [1914] in alcoholic extracts of faeces from hens and rabbits receiving normal diets, and Portier and Randoïn [1920] found it in the faeces, but not in the urine, of rabbits which had been kept on diets deficient in vitamin B. Its presence was attributed by these authors either to unabsorbed residues from the food or to synthesis by bacteria in the intestines.

Muckenfuss [1918], Gaglio [1919] and Van der Walle [1922] all found the antineuritic vitamin in adsorbates from the urine of animals fed on normal diets, but it was reduced in starving animals and absent after 3 weeks' deprivation of "vitamin B."

Later it was observed that animals on diets completely or partially deficient in "vitamin B" frequently consumed their own faeces, the result being, when suboptimal doses of the vitamin were being supplied, a greatly improved rate of growth. Thus Steenbock, Sell and Nelson [1923] observed that such rats grew normally with half as much "vitamin B" in the diet as was needed by the non-coprophagous rats. They considered that an additional supply of "vitamin B" was being obtained from the unabsorbed residue of the food, since in absence of "vitamin B" from the diet coprophagy did not prolong life.

Dutcher and Francis [1923], however, kept coprophagous rats on diets completely deficient in "vitamin B" for 5-7 months. They considered this prolongation of life to be due to the re-ingestion of excreted stores of "vitamin B," an explanation supported by the failure of rats to benefit by coprophagy if it was not started till after the first few weeks of vitamin depletion, when the stores of the vitamin were exhausted [Steenbock, Sell and Jones, 1923].

The limited capacity of animals for storing "vitamin B" made it improbable that their stores, even when wastage through the faeces was prevented, could be sufficient to protect against symptoms of "vitamin B" deficiency during the long periods of observation. It was suggested that only minute

¹ Beit Memorial Fellow.

quantities of the vitamin were used at a time, or that none was actually destroyed, the action being catalytic, but against these theories there is the certainty of loss in the portion of the faeces which is not consumed, and the probability of loss in the urine. The theory was therefore advanced that "vitamin B" was synthesised by bacteria in the alimentary canal of coprophagous rats and evacuated with the faeces, from which it was assimilated. This was the more likely as a number of bacteria have been shown to synthesise the vitamin B complex even when grown on synthetic media free of any traces of the vitamin [Schieblich, 1929, 1930, 1, 2].

In support of this theory Heller, McElroy and Garlock [1925] found a marked increase in spore-bearing organisms, which were able to synthesise "vitamin B," in the faeces of coprophagous rats. These had completely disappeared, however, after 3 weeks.

Bechdel *et al.* [1928] found that cows could be reared to maturity on "vitamin B"-deficient diets. Their rumens were shown to contain the vitamin, as the result, it was thought, of synthesis by a small unknown bacterium which was isolated. Coprophagy did not seem to be necessary in order that the vitamin complex should be obtained from the bacteria or their products.

In none of these researches was the vitamin B complex differentiated into its components. When rats were used the experiments dealt therefore with the whole complex; when the tests were based on the prevention or cure of polyneuritis in birds the vitamin B₁ was alone being considered.

Kennedy and Palmer [1928] showed that both vitamins B₁ and B₂ were supplied in the faeces and considered that a third factor was also present supplementing the "vitamin B"-deficient diet; this appears, however, to have been due to an inadequate supply of vitamin B₂.

Scheunert, Schieblich and Rodenkirchen [1929] studied the effect of coprophagy in rats receiving diets containing raw starch, but the greater number of their rats became refected [Fridericia *et al.*, 1927; Roscoe, 1927]. These workers did not apparently realise the difference between their results and those obtained by other workers on coprophagy in whose experiments refection had been prevented by using dextrin in the diet instead of starch [Roscoe, 1927].

Subsidiary reasons, which have been put forward to account for the improved condition of coprophagous animals, are (i) that the faeces act as roughage which is thought to prolong the existence of "vitamin B"-deficient animals [Heller, McElroy and Garlock, 1925], and (ii) that these rats do not suffer from the same disabling discomfort as those in which coprophagy is prevented by the use of cages with open wire floors [McCollum, Simmonds and Becker, 1925].

In this paper experiments are described involving an accurate investigation of coprophagy in rats deprived of the entire vitamin B complex, of vitamin B₁ alone or of vitamin B₂ alone. An attempt was also made to determine the origin of the B-vitamins which these rats acquired through eating their faeces, and to see whether this was similar to that of the vitamin B obtained by refected rats.

METHODS.

The rats were kept in the cages used in this laboratory for all work on the B-vitamins. These are made of perforated zinc with floors of galvanised wire netting of $\frac{1}{8}$ inch mesh. The floors of the cages, through which the faeces fall freely, are raised 2" above the peat bedding. In some of the experiments the rats were housed in glass jars 6" in diameter, on raised floors made from the same wire netting. Both the coprophagous rats and their controls were kept in these cages; no results could therefore be attributed to differing degrees of comfort enjoyed by the rats.

The peat was covered with a fine wire gauze which allowed the passage of the urine but not of the faeces, from this floor the faeces were collected daily. They were dried overnight at 37°, ground, in some cases weighed, and fed to the rats on the following day. In this form they were more readily consumed than when fresh.

The rats were fed on a basal diet complete except for B-vitamins. Vitamins A and D were supplied as a daily dose of cod-liver oil. The diet consisted of caseinogen 20 parts, rice starch 60, salt mixture 5, cotton-seed or arachis oil 15 parts. This diet was usually steamed for 3–4 hours before using so that the starch was rendered completely soluble.

When vitamins B₁ and B₂ were given these were provided as Peters's antineuritic concentrate from yeast (YE) [Peters, 1924; Kinnersley and Peters, 1925] and as an autoclaved yeast extract (F5A) [Roscoe, 1930], respectively.

No distinction has been made in this work between vitamins B₁ and B₄ [Reader, 1929, 1930, 1, 2] but it was known that the yeast concentrate used as source of vitamin B₁ having been made with acid alcohol contained vitamin B₄ [Reader, 1929]. The yeast extract used as source of vitamin B₂, having been autoclaved for 5 hours, would not contain any vitamin B₄. The rats were, therefore, either deficient in vitamins B₁ and B₄, or deficient only in vitamin B₂.

Young rats were weaned at the age of 3 weeks and received the basal diet and cod-liver oil as soon as they weighed 40 g. Litters 1,000, 2,000 and 4,000 then received their supplementary doses of faeces. Litters 45,000, 51,000 and 52,000 all received their doses of faeces and of vitamins B₁ or B₂ after 1 week on the basal diet alone.

EXPERIMENTAL.

Exp. 1. Growth and prolongation of life in rats deprived of the entire vitamin B complex.

Litters as in Table I.

In Table I are collected the data relating to coprophagous rats and their non-coprophagous litter-mates on a diet deficient in the whole vitamin B complex.

The results with rats receiving an uncooked diet will be considered under Exp. 5.

The coprophagous rats on the cooked diets all remained alive for much longer than their controls. Three developed neuritis after $9\frac{1}{2}$, $9\frac{1}{2}$ and 13 weeks respectively and four after 21, 22, 35 and 36 weeks. The rest were in good health when the experiments were terminated after 8 weeks (3 rats), 19 weeks (2 rats) and 9, 10, 15 and $16\frac{1}{2}$ weeks respectively.

For the greater part of the period of observation the rats did not grow

Table I. *Life histories of coprophagous rats and their non-coprophagous litter-mates on diets deficient in the entire vitamin B complex.*

(In brackets are given the average weekly weight increase.)			
Coprophagous		Rat No.	Non-coprophagous
Uncooked diet:			
1,005 ♂	Lost weight for 4 weeks. Died	1,009 ♂	Became refected after 1 week
1,004 ♂	Became refected after 1 week	1,010 ♀	Lost weight for 3 weeks. Dosed
1,006 ♂	" " " 2 weeks		
2,008 ♂	Lost weight for 4 weeks. Died	2,006 ♂	Lost weight for 3 weeks. Died
2,009 ♀	" " " 3 " Dosed	2,007 ♀	" " 4 " "
		2,010 ♂	" " 3 " Dosed
Cooked diets:			
1,001 ♂	Slow growth for 8 weeks (1 g.). Experiment stopped	1,007 ♂	Neuritis after 6 weeks
1,002 ♂	" " 8 " (3 g.). "	1,008 ♂	Lost weight for 4 weeks. Dosed
1,003 ♀	" " 8 " (2 g.). "		
2,001 ♂	<i>Grew (5 g.) for 16 weeks with one break of 4 weeks. Lost weight. Neuritis after 22 weeks</i>	2,002 ♀	Lost weight for 3 weeks. Died
2,011 ♂	Slow growth for 8 weeks (1 g.). <i>Grew for 9 weeks (6 g.). Stationary then lost weight. Neuritis after 21 weeks</i>	2,003 ♂	" " 5 " Dosed
2,004 ♀	<i>Grew (5.5 g.) for 16 weeks with one break of 3 weeks. Stationary then lost weight. Neuritis after 35 weeks</i>		
2,005 ♂	4 weeks (0 g.). <i>Grew (7 g.) for 13 weeks with one break of 3 weeks. Stationary then lost weight. Neuritis after 36 weeks.</i>		
4,004 ♀	Weight stationary for 9 weeks. Neuritis after $9\frac{1}{2}$ weeks	4,001 ♂	Lost weight for 5 weeks. Died
*4,006 ♂	" " 9 " $9\frac{1}{2}$ " "		
4,002 ♂	<i>Grew for 8 weeks (3 g.). Stationary for 5 weeks. Neuritis after 13 weeks</i>		
*4,005 ♂	<i>Grew for 11 weeks (2 g.). Then for 8 weeks (6 g.). Experiment stopped</i>		
4,003 ♀	" " 11 " (1.5 g.). <i>Then for 8 weeks (8 g.).</i>		
*4,007 ♀	" " 11 " (2 g.). <i>Then for 5½ weeks (6 g.).</i>		
51,384 ♂	" " 10 " (1 g.) Experiment stopped	51,383 ♂	Lost weight for 5 weeks. Neuritis
52,390 ♂	" " 6 " (2 g.). Lost weight for 3 weeks. Experiment stopped	52,389 ♀	" " 5 " Dosed
52,391 ♀	" " 6 " (2 g.). <i>Then for 9 weeks (5 g.). Experiment stopped</i>		

* These rats received faeces which had been sterilised.

The litter of the rats is indicated by the figures in the fourth and fifth places.

appreciably (average 2 g. per week) (see Fig. 1, curve C ii), but in 8 out of the total 16 there was a sudden increase in the rate of growth after varying periods of coprophagy (Table I, italics and Fig. 1, curve C iii). This growth was not very rapid (average 6 g. per week) but it was steady and continued till the animals weighed about 100–110 g., but never beyond this. Four of the rats were killed while still growing (rats 4,003, 4,005 and 4,007), two others stopped growing, lost weight and died of neuritis (rats 2,001 and 2,011) and the other two remained stationary in weight for several months and then lost weight and died of neuritis (rats 2,004 and 2,005).

No difference is apparent in the improvements due to coprophagy, between those rats which consumed their faeces from the start (litters 1,000, 2,000 and 4,000) and those which received a vitamin-free diet for 1 week first (litters 51,000 and 52,000).

From this experiment it appeared that coprophagous rats on cooked diets deprived of the entire vitamin B complex acquired, through the eating of their faeces, sufficient of the complex to prolong their lives for considerable periods. In all the cases investigated death finally occurred as a result of neuritis due to vitamin B₁ deficiency.

Fig. 1. *Exp. 3.* The growth of coprophagous rats on diets: (a) deprived of vitamin B₁, (b) deprived of vitamin B₂, (c) deprived of the entire vitamin B complex, compared with that of non-coprophagous controls on the same diets, and with normally growing rats.

The curves are in each case, until the vertical lines, averages for 3 rats, 2 males and 1 female.

Exp. 2. The quantities of faeces excreted and consumed by rats deprived of the vitamin B complex.

In these experiments (litters 2,000 and 4,000) two series of rats received a diet devoid of the vitamin B complex. Records were kept of the dry weights of faeces excreted and fed and of the amounts which they failed to eat (see Table II). The percentages of faeces consumed varied from 67 to 77 % in one litter, from 90 to 100 % in the other, the average consumption for any week never fell below 44 %. The growth, expressed as weekly weight increase, is not apparently related to the amount of faeces eaten during the whole time of the experiment.

In Table II are also shown the amounts of faeces eaten during two successive periods in the histories of 7 of the rats, in the first of which they were losing or stationary and in the second gaining in weight. For 3 rats where consumption was low in the first period (57–69 %) it was slightly raised in the second (71–73 %). For the others consumption was high in the first period (77–100 %) and was not increased in the second. It cannot therefore

be said that the growth was due to an increase in the amount of faeces consumed, but the figures do perhaps indicate that growth was only possible when more than about 70 % of the faeces excreted were being eaten.

After the first few weeks of coprophagy the dry weight of the faeces excreted became very high, rising to as much as 1.6 and 1.8 g. daily, whereas rats receiving the same diet adequately supplemented with B-vitamins, but in which coprophagy was prevented, excreted only 0.4–0.5 g. daily. This increase in amount is probably the result of the accumulation of undigestible products of metabolism which are continually re-ingested.

Table II. *Growth of rats and percentage of faeces consumed on a diet deficient in the vitamin B complex when coprophagy was allowed.*

The figures are given for the whole experiment, for several weeks when weight was being lost or was stationary, and for the immediately ensuing weeks when weight was increasing.

Rat No.	Whole experiment			Period of loss of weight			Period of gain of weight		
	Weeks	Av. weekly growth g.	% of faeces eaten	Weeks	Av. weekly growth g.	% of faeces eaten	Weeks	Av. weekly growth g.	% of faeces eaten
2,001 ♂	14	3.3	77	4	–2.5	77	4	4.5	74
2,004 ♀	16	4.0	71	3	–2.7	59	3	6.0	71
2,005 ♂	16	3.9	67	3	–3.3	69	3	9.3	72
2,011 ♂	16	3.7	69	5	–1.0	57	5	5.2	73
Average		3.7	71						
4,002 ♂	13	1.8	93	—	—	—	—	—	—
4,003 ♀	19	4.2	90	3	–1.0	95	3	10.1	90
4,004 ♀	9	0.6	97	—	—	—	—	—	—
4,005 ♂	19	4.0	98	3	1.7	100	3	6.6	96
4,006 ♂	9	0.0	100	—	—	—	—	—	—
4,007 ♀	19	2.0	98	5	0.4	100	5	5.2	93
Average		2.1	96						

Exp. 3. The effect of coprophagy in rats deprived of vitamins B₁ and B₂ respectively.

In this experiment (litters 45,000, 51,000, 52,000 and 66,000) the effect of coprophagy in rats deprived of either of vitamin B₁ (series (a)) or of vitamin B₂ (series (b)) only was investigated.

The experiment lasted 10 weeks. Table III shows the increase in weight during the first 5 and second 5 weeks respectively and the average weekly growth over the whole period for these rats and for their non-coprophagous litter-mates. Growth figures for rats from the same litters, deprived of the whole vitamin B complex (series (c)) are shown for comparison. In Fig. 1 are shown the average growth curves of these rats.

Series (a). The weight increase of the coprophagous rats receiving vitamin B₂ in their diet (as F5A) but deprived of vitamin B₁, approached the normal, being 64 g. (average) in the first 5 weeks, falling to 46 g. (average) in the second 5 weeks. Control litter-mates, prevented from coprophagy, grew for 2 weeks then lost weight and all developed neuritis by the 5th week (see Fig. 1, curves *a i* and *a ii*). The weight increase during the second 5 weeks

gave the more accurate measure of the vitamin B₁ obtained from the faeces owing to the exhaustion of reserve stores of vitamin B₁, which were sufficient to allow the growth of the controls for 2 weeks. In a further experiment with rats receiving a diet containing vitamin B₂, in which coprophagy did not start till the body weights were stationary, weight increase during the first 5 weeks of coprophagy was 55 g. (average).

These experiments indicate that enough vitamin B₁ was obtained from the faeces to prevent neuritis and support fairly good growth.

Series (b). The coprophagous rats receiving vitamin B₁ in their diet (as YE) but no vitamin B₂ increased in weight during the first 5 weeks by 34 g. (average) during the second 5 weeks by 41 g. (average). The control rats prevented from coprophagy, grew an average of 12 g. in the first 5 weeks, by

Table III. *Exp. 3. Growth of coprophagous rats and their non-coprophagous controls on diets (a) deprived of vitamin B₁, (b) deprived of vitamin B₂, (c) deprived of the entire vitamin B complex.*

Coprophagous rats				Non-coprophagous rats		
Rat No.	Weight increase (g.)			Rat No.	Weight increase (g.)	
	1st 5 weeks	2nd 5 weeks	Average weekly		1st 5 weeks	
(a) Diet complete except for vitamin B ₁ :						
45,338 ♂	68	51	12	45,333 ♂	+ 11, - 15 in 32 days.	Neuritis
45,339 ♂	68	56	12	45,334 ♂	+ 15, - 16 in 35 days.	Neuritis
45,335 ♀	57	30	9	45,340 ♀	+ 19, - 22 in 35 days.	Neuritis
Average	64	46	11		+ 15, - 18	
(b) Diet complete except for vitamin B ₂ :						
45,336 ♂	25	50	7.5	45,332 ♂	13	Dermatitis in 42 days
51,385 ♂	34	24	6	51,378 ♂	13	Dermatitis in 42 days
45,337 ♀	40	46	9	—	—	—
52,392 ♀	39	46	8.5	52,386 ♀	11	Dermatitis in 98 days
Average	34.5	41.5	8		12	
(c) Diet complete except for the vitamin B complex:						
51,384 ♂	5	4	1	51,383 ♂	+ 2, - 12 in 33 days.	Neuritis
52,390 ♂	12	- 5	1	52,388 ♂	+ 2, - 4	
52,391 ♀	11	(29)	2 (6)	52,389 ♀	+ 2, - 18 in 30 days.	Neuritis
Average	9	0	1		+ 2, - 11	

the 6th week 2 had developed dermatitis, the weight of the other remained stationary and it developed dermatitis after 14 weeks (see Fig. 1, curves b i and ii).

Thus when vitamin B₁ was being supplied in the diet an amount of vitamin B₂ sufficient to prevent dermatitis and allow subnormal growth appears to have been obtained by coprophagy.

Series (c). These rats, which were deprived of the entire vitamin B complex, increased in weight by only 9 g. (average) in the first 5 weeks of the experiment. This increase was much less than that observed in either series (a) or (b). During the next 5 weeks 2 rats continued to show no significant increase in weight though the other started to grow (see Fig. 1, curves c i, ii and iii).

Thus when no vitamin B₁-containing yeast concentrate (YE) or vitamin

B₂-containing yeast extract (F5A) was being supplied in the diet, less of each vitamin was obtained through coprophagy.

It was possible that the increased production of vitamins B₁ and B₂ respectively in the rats of series (a) and (b) was due to some factor common to the materials supplying vitamins B₂ (F5A) and B₁ (YE) respectively, or alternatively, the vitamin B₁ obtained from the faeces might have been increased when vitamin B₂ was present in the diet and the vitamin B₂ when vitamin B₁ was in the diet. To test these theories a further experiment was carried out.

Coprophagous rats (litter 66,000) were given the vitamin B₁ yeast concentrate (YE) autoclaved under the same conditions as the vitamin B₂ yeast extract (F5A) (5 hours at 120°, p_H 4.7). The concentrate now contained no vitamin B₁. These rats, however, grew as well as those receiving the unheated concentrate, 47 g. (average) weight increase in 5 weeks. It was not therefore vitamin B₁ that was stimulating the production of vitamin B₂ and it seems probable that there was some heat-stable factor present in both the preparations from yeast (YE and F5A) which stimulated the production of both vitamins B₁ and B₂ in coprophagous rats.

To summarise Exps. 1-3, the faeces of rats deprived of the vitamin B complex appeared to contain small amounts of vitamins B₁ and B₂. These amounts were in some cases increased so that growth of from 6 to 8 g. per week was allowed, either spontaneously, after varying periods of coprophagy (curve c iii, Fig. 1), or when some factor present in autoclaved yeast extracts, but not either vitamin B₁ or B₂, was being supplied in the diet (curves a ii and b ii, Fig. 1).

Exp. 4. The effect of feeding sterilised faeces to rats.

If the vitamin B complex obtained by coprophagous rats were present in the faeces as the result of synthesis by intestinal flora, the rôle of coprophagy might be explained in three ways: (1) the vitamin B complex might be acquired from the faeces, not having been accessible at the place of synthesis, (2) the faeces might re-infect the gut with the synthesising organism, or (3) they might contain some substance which stimulates the growth in the intestines of the synthesising organism. The effect of feeding sterilised faeces was investigated to test the second hypothesis. A group of rats was fed on faeces sterilised by heating in a moist state for 3 hours at 80° before drying. This degree of heating, which was not enough to destroy the B-vitamins, was found to kill the bacterial flora, cultures only very occasionally showing any growth.

The histories of these rats are shown in Table I, litter 4000. No difference was detected between the rats receiving sterilised faeces (marked with an asterisk) and their controls receiving faeces dried at 37° without previous treatment. The beneficial effect of coprophagy in rats deprived of the vitamin B complex is not therefore due to a constant re-infection of the gut with a vitamin B-producing organism.

Exp. 5. A comparison of the effects of coprophagy in rats on diets containing insoluble starch (uncooked) and on diets containing soluble starch (cooked).

In Table I are given the histories of 5 coprophagous rats on uncooked diets. Three of these rats thrived no better than their non-coprophagous litter-mates, death occurring as a result of vitamin B₁ deficiency after about 4 weeks. The other 2 rats, together with one of the controls, became refected.

This result is a great contrast to that obtained with rats which were fed with a cooked diet containing soluble starch, and which were always benefited to some extent by coprophagy. It should be noted again that the American workers on coprophagy all used dextrin as their carbohydrate and so avoided refaction.

The condition of refaction is obscure but it has been shown to depend on coprophagy. It can be transmitted from an affected rat to another by feeding the faeces, and it can only continue if the rats eat their own faeces. Fridericia and his co-workers concluded that micro-organisms in the faeces of refected rats were responsible for the transmission of refaction and probably also for its continuance, for transmission was prevented when the faeces were heated for 10 minutes at 100°. Schieblich and Rodenkirchen [1929, 1, 2] have indeed isolated a *Vibrio*-like organism from the faeces and gut of refected rats, which they think to be the responsible agent.

The state of refaction has many points in common with that of the coprophagous rats described in this paper. In both cases rats receiving no B-vitamins in the diet remained in health for considerable periods and even exhibited growth. This was much more marked in the refected rats, however. In both cases the state was induced and maintained in rats by the eating of their own faeces, though refected animals only ate a small quantity, the others a large proportion (see Exp. 2).

The two phenomena are however different. Refaction only occurred in rats receiving diets containing raw starch and ceased if the diet were cooked, whereas the prolongation of life and the growth observed in these experiments only occurred in rats receiving diets in which the starch had been rendered soluble by cooking.

It is however possible that the organism supposed to be responsible for refaction might be also functioning in the coprophagous non-refected rats, though unable to synthesise as much vitamin B complex when the diet was cooked. An experiment was therefore carried out in which the cooked diet of 5 growing coprophagous rats was changed to an uncooked diet.

These were rats 45,338 and 45,335 receiving vitamin B₂ but no vitamin B₁, rats 45,336 and 45,337 receiving vitamin B₁ but no vitamin B₂, and rat 52,391 receiving no vitamin B complex. After the first week on the new diet all lost weight steadily, their condition became worse and refaction did not develop.

The same organism did not therefore appear to be responsible for the possible synthesis of the B-vitamins in the two conditions. If synthesis by

micro-organisms in the gut is responsible for the supply of the vitamins to refected rats and to the unrefected coprophagous rats respectively, it appears that those causing refection flourish in a gut filled with raw starch, and the others in one containing soluble starch.

DISCUSSION.

The following conclusions may be drawn from these experiments as to the origin of the vitamin B complex which enables coprophagous rats on diets deficient in the complex, but containing soluble starch, to live and thrive.

It is improbable that the B-vitamins were obtained from the body stores excreted in the faeces, for firstly, there was wastage of the vitamins in the unconsumed faeces and even when faeces passed during the 1st week of vitamin B depletion were discarded, there was no lessening of the good effect of coprophagy (a result in contradiction to that obtained by Dutcher and Francis [1923]). Secondly, it frequently happened that whereas at the start of an experiment coprophagous rats only maintained their weights, after several weeks, when their body stores would be depleted, they started to grow, without any increase in consumption of faeces.

Synthesis of the vitamin B complex in these animals is therefore indicated, and this theory is supported by the fact that the amounts of the B-vitamins available for the coprophagous animal appeared to be increased by the presence in the diet of a factor, contained in autoclaved yeast extracts, but not one of the known B-vitamins (Exp. 3). This is difficult to explain except on the assumption that synthesis was being stimulated by this factor.

It seems probable that the synthesis was carried out by bacteria. This was not proved however, and an experiment designed to show whether the benefit of coprophagy was due to reinfection of the gut with bacteria gave negative results. Also it is difficult to explain by this theory the very steady and consistent growth obtained in the two series of animals (*a*) and (*b*) in Exp. 3, which were deficient in vitamins B₁ and B₂ respectively. For it is hard to suppose that the micro-organisms present in different animals would always produce the same amounts of these vitamins, or that they would be eliminated in the same amounts.

The exact rôle of coprophagy is not apparent. It may be that the vitamins were synthesised by bacteria in the intestines, but that they were not liberated here but only when the bacteria were digested higher up in the gut after re-ingestion of the faeces, or the faeces may contain some factor which stimulates the growth of bacteria.

No connection could be traced in these experiments between the process whereby these coprophagous rats, receiving diets containing soluble starch, obtained their B-vitamins and that whereby refected rats obtained their supplies. If both processes are based on a bacterial synthesis, it appears that different bacteria are involved in each.

SUMMARY.

1. A quantitative method of controlling the degree of coprophagy in rats, by collecting, drying and feeding the faeces is described.

2. The life of rats receiving diets deficient in the vitamin B complex or its constituents and containing soluble starch was prolonged considerably when they ate their faeces, and in some cases growth also took place. This was not prevented by a preliminary week of depletion during which coprophagy was not permitted.

3. From 44 to 100 % of their faeces was consumed by the individual rats. Growth did not occur when less than 70 % was being consumed, but it was not necessarily proportional to increased consumption.

4. The above facts are most easily explained by the theory that in coprophagous rats there is a synthesis of the B-vitamins, probably by bacteria in the lumen of the gut.

5. This synthesis was stimulated by the presence in the diet of a heat-stable factor, contained in autoclaved yeast extracts and differing from the recognised B-vitamins.

6. The rôle played by the re-ingested faeces is not evident. The efficacy of sterilised faeces showed that the action was not due to the re-infection of the gut with a synthesising organism. It may be that the B-vitamins are obtained directly from the faeces, or the faeces may contain a substance which stimulates the growth of synthesising bacteria already established in the gut.

7. When the rats were fed on uncooked diets containing raw starch, coprophagy did not benefit them unless refection occurred. Changing from a cooked to an uncooked diet resulted in the development of symptoms of deficiency of the B-vitamins.

8. Refection is a condition distinct from that observed in coprophagous rats fed on diets containing soluble starch. It only occurred in rats fed on diets containing raw starch. No connection between the two conditions could be traced.

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