TABLE I.
Fecal Thiamine Excretion of Rats with Varying Thiamine Intakes.

Days on test	Group	No. of rats	Avg wt, g	Avg 24 hr fecal output, g	Total thiamine, μg/g feces	Cocarboxylase % of total thiamine
1st day	Control period	8	46	0.28	12.9	—
10th	Thiamine low	8	79	0.19	22.2	—
	5 μg thiamine	8	68	0.47	7.2	—
	50 " "	8	82	0.54	24.0	—
23rd	Thiamine low	8	81	0.21	12.9	—
	5 μg thiamine	8	115	0.51	11.3	—
	50 " "	8	136	0.65	21.3	—
31st	Thiamine low	7	67	0.11	15.1	80
	5 μg thiamine	7	132	0.51	12.6	69
	50 " "	7	176	0.83	16.6	41
42nd	Thiamine low	2	77	0.10	17.5	—

declined in weight and one of the group had succumbed to thiamine deficiency. These rats excreted 80% of their total thiamine in the form of cocarboxylase as contrasted with 69% for the animals receiving 5 μg of thiamine and 41% for the rats receiving 50 μg of thiamine daily.

After 42 days on test 6 of the animals in the original group had succumbed. The 2 remaining rats although evidencing signs of severe thiamine deficiency still excreted an appreciable quantity of total thiamine in their stools.

The non-availability of fecal thiamine was further demonstrated by the feeding of feces to thiamine-depleted animals. Five rats, placed at weaning on the thiamine low diet, were maintained on the regimen until a plateau in weight had been reached (25 days). Each animal was then dosed by stomach tube with a suspension of feces from a like group of thiamine-deficient rats. The feces were fed in a quantity sufficient to supply 5 μg of thiamine daily (as determined by the thiochrome method). The feeding of the thiamine containing feces was without effect upon the progress of the deficiency. However, when 5 μg of thiamine was administered daily spectacular weight gains were observed.

Summary. Rats maintained on a thiamine-deficient diet excreted essentially the same concentration of thiamine in their stools as did animals receiving 5 or 50 μg of thiamine daily. The output of feces was greatly reduced in the thiamine low group. During the later stages of the depletion the thiamine was present largely in the form of cocarboxylase.

The fecal thiamine of the deficient rats was not available when administered curatively by the oral route to animals which had plateaued in weight on a thiamine-low diet.

We are indebted to Miss Frances Lewis and Mr. Ling Chen for their technical assistance.

15066

Fecal Riboflavin of Rats Receiving Varying Intakes of Riboflavin.

HARRY G. OBERMEYER, ELIZABETH WURTZ, AND GLADYS A. EMERSON.
From the Merck Institute for Therapeutic Research and the Research Laboratories of Merck & Co., Inc., Rahway, N.J.

The biosynthesis of vitamin B factors was suggested by Roscoe[1] who observed that coprophagous rats maintained on diets containing cornstarch lived and in some cases grew despite the absence of the vitamin B

[1] Roscoe, M. H., *Biochem. J.*, 1931, **25**, 2056.

complex from their rations. She postulated that the B vitamins were probably synthesized in the lumen of the gut. Guerrant and Dutcher,[2] in experiments in which vitamin B (thiamine) was supplied as an alcoholic extract of yeast, found that the feces from vitamin G (riboflavin) deficient rats supplied this factor. The stools of depleting rats did not decrease in riboflavin content as the deficiency progressed. The same investigators[3] later observed that the synthesis of riboflavin was influenced by the source of carbohydrate in the diet. Dextrinized cornstarch favored riboflavin production while sucrose did not. Mannering and co-workers[4] reported that, in general, those carbohydrates which decreased the amount of dietary riboflavin needed by the rat for growth were also responsible for the greatest quantities of riboflavin in the feces. Najjar *et al.*[5] in studies with humans reported the maintenance of a normal fecal excretion of riboflavin although the purified diet fed contained only small amounts of this vitamin.

The above experiments, although affording adequate evidence for the synthesis of riboflavin do not constitute proof for the absorption of the vitamin from the large intestine. Selye,[6] working with bilaterally nephrectomized rats, obtained results indicating the destruction of riboflavin in the cecum; however, physiological amounts of riboflavin could not have been detected with the techniques employed.

In the present experiments the fecal excretion of riboflavin has been followed in rats receiving varying levels of this vitamin. These experiments were designed to show the effect of riboflavin intake upon fecal riboflavin excretion.

Experimental. Male rats were placed at weaning on Diet 30, the composition of which is as follows: casein (Labco) 18%; dextrose, 68%; salt mix, U.S.P. No. 1, 4%; crisco, 8%; cod liver oil, 2%; and supplemented with 0.8 mg each of thiamine and pyridoxine; 10 mg nicotinamide, 5 mg calcium pantothenate and 100 mg of choline chloride per 100 g of diet.

Animals receiving riboflavin were dosed daily by stomach tube with 10 μg or 40 μg of this factor. The rats receiving riboflavin were segregated into 2 groups; the one was fed the basal ration *ad libitum* and with the other the caloric intake was restricted to 50% of that consumed by the corresponding *ad libitum* group. In a previous experiment an attempt was made to maintain riboflavin fed animals on the same caloric intake as the deficient group. All rats thus restricted succumbed after 3 to 7 days on test. It should be mentioned in this connection that the progress of the deficiency state attributable to riboflavin-avitaminosis is unique in that weight gains are minimal and the food consumption is correspondingly low.

Fecal riboflavin was determined by the fluorometric method at intervals as indicated in Table I. The concentration of riboflavin as expressed in micrograms per gram of feces was essentially the same for all groups and was therefore independent of the riboflavin intake. Furthermore, the riboflavin content of the feces remained constant in the depleting group (A), despite the development of the deficiency state. The rats, in both groups (C and E) in which the caloric intake had been restricted, excreted less total riboflavin than did the animals (B and D) receiving the same intake of the vitamin but with the basal ration supplied *ad libitum*. In those animals receiving riboflavin with food supplied *ad libitum* the total daily fecal excretion of riboflavin exceeded that of the deficient and restricted groups. Thus the output of riboflavin in the stools more nearly followed the food consumption than the riboflavin intake.

It is of interest to note that Silber[7] observed that the fecal pantothenic acid excretion of dogs maintained on a pantothenic acid

2 Guerrant, N. B., and Dutcher, R. A., *J. Biol. Chem.*, 1932, **98**, 225.

3 Guerrant, N. B., and Dutcher, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 796.

4 Mannering, G. J., Orsini, D., and Elvehjem, C. A., *J. Nutr.*, 1944, **28**, 141.

5 Najjar, V. A., Johns, G. A., Medairy, G. C., Fleischmann, G., and Holt, L. E., Jr., *J. Am. Med. Assn.*, 1944, **126**, 357.

6 Selye, H., *J. Nutr.*, 1943, **25**, 137.

7 Silber, R. H., *J. Nutr.*, 1944, **27**, 425.

TABLE I.
Fecal Riboflavin Excretion of Rats Receiving Varying Intakes of Riboflavin.

Days on test	Group	No. of rats	Avg wt, g	Avg 24 hr fecal output, g	Riboflavin, μg/g feces	Riboflavin total output, μg/rat/day
1st day	Control period	9	42	0.15	27	4.1
12th	A	9	57	0.24	58	13.9
	B	9	81	0.25	23	5.8
	C	9	68	0.21	34	7.1
	D	7	92	0.43	—	—
	E	9	72	0.15	46	6.9
19th	A	8	55	0.18	21	3.8
	B	9	91	0.37	25	9.3
	C	9	76	0.22	20	4.4
	D	7	117	0.43	24	10.3
	E	8	82	0.14	26	3.6
26th	A	7	59	0.22	28	6.2
	B	9	117	0.46	26	12.0
	C	9	91	0.32	28	9.0
	D	7	144	0.50	25	12.5
	E	8	99	0.29	29	8.4
33rd	A	7	58	0.14	31	4.3
	B	9	126	0.35	38	13.3
	C	9	100	0.22	31	6.8
	D	7	164	0.56	25	14.0
	E	8	111	0.20	28	5.6
40th	A	6	59	0.16	27	4.3
	B	9	136	0.32	23	7.4
	C	9	106	0.20	23	4.6
	D	7	183	0.50	40	20.0
	E	8	119	0.20	29	5.8

Key to groups:
A—Riboflavin deficient.
B—10 γ riboflavin daily.
C—10 γ riboflavin daily, caloric intake restricted to 50% *ad lib.*
D—40 γ riboflavin daily.
E—40 γ riboflavin daily, caloric intake restricted to 50% *ad lib.*

deficient diet was the same when expressed in micrograms per gram of feces for both pantothenic acid deficient and for pantothenic acid supplemented animals.

Summary. The concentration of riboflavin in the stools of young rats maintained on a riboflavin deficient diet or at intakes of 10 μg or 40μg of this vitamin per day remained constant throughout a test period of 40 days and was essentially the same for all groups.

The total output of riboflavin was dependent upon the quantity of feces excreted. Rats receiving riboflavin but with the caloric intakes restricted to the extent of 50% excreted less total riboflavin than did their *ad libitum* controls.

Addendum. After this paper had been accepted for publication, Schweigert *et al.*[8] reported that cecectomized rats on complete synthetic rations and synthetic rations containing limited amounts of the individual B vitamins grew at the same rate as their controls. They concluded that growing rats on sucrose-containing diets did not depend to any extent on the cecum for the absorption or synthesis of these known or required factors.

8 Schweigert, B. B., McIntire, J. M., Henderson, L. M., and Elvehjem, C. A., *Archiv. Biochem.*, 1945, **6**, 403.