1. Guyton, A. C., Crowell, J. W., *Fed. Proc. Supp.*, 1961, No. 9.

2. Fleisher, M. S., Loeb, L., *Arch. Int. Med.*, 1909, 3278-91.

3. Christian, H. A., Smith, R. M., Walker, I. C., *ibid.*, 1911, v8, 468.

4. Brunson, J. G., Gamble, G. N., Thomas, L., *Am. J. Path.*, 1955, v31, 489.

5. Glaser, R. J., Thomas, W. A., Morse, S. I., Darnell, J. E., Jr., *J. Exp. Med.*, 1956, v103, 183.

6. Szakace, J. E., Cannon, A. L., *Am. J. Clin. Path.*, 1958, v30, 425.

7. Nahas, G. G., Brunson, J. G., King, W. M., Cavert, H. M., *Am. J. Path.*, 1958, v34, 717.

8. Thomas, L., *J. Exp. Med.*, 1956, v104, 865.

9. Hinshaw, L. B., Gilbert, R. P., Kuida, H., Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 684.

10. Schweinburg, F. B., Fine, J., *J. Exp. Med.*, 1960, v112, 793.

11. Walton, R. P., Richardson, J. A., Thompson, W. L., *Am. J. Physiol.*, 1959, v197, 223.

12. Palmerio, C., Ming, S. C., Frank, E. D., Fine, J., *J. Exp. Med.*, 1962, v115, 609.

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Recovery of Thiamine Injected into the Rat Cecum.* (27334)

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Thiamine is synthesized by the microbial flora of the simple stomach animal and evidence has been presented that this biosynthesized thiamine cannot be absorbed from the large intestine of the rat(1,2,3). It is possible that lack of absorbability is due to the form of the newly synthesized thiamine; for example, it has been noted that thiamine is held within the bacterial cell(4). Recently Wostmann and Knight(3) have shown that thiamine synthesized in the large intestine is present largely in a form that remains in the centrifugal supernatant of the cecal contents and, therefore, is probably extracellular. This thiamine was not utilized by the host animal. It was of interest then to see if thiamine in water solution, injected directly into the rat cecum, could be absorbed.

Materials and methods. Two separate experiments were conducted. In the first, 3 groups of 6 male weanling rats (Holtzman) were fed a purified diet devoid of thiamine for 2 weeks. One animal died in Groups 1 and 2, leaving 5 rats in these groups. Coprophagy was prevented in all rats by plastic tail cups(5) and rats were housed individually in raised wire bottom cages. In Group 3 (Table I) 50 mg of procaine penicillin was added per kilo of diet. On the first experimental day all rats were anesthetized with ether and the cecum exposed through a midline abdominal incision. Using a tuberculin syringe with a No. 26 needle introduced through the wall of the cecum, Group 1 received 0.2 ml of water and Groups 2 and 3 received 140 μg thiamine hydrochloride in 0.2 ml water. On the eighth day the operative procedure was repeated, but this time 0.1 ml was injected into the cecum; Group 1 receiving water and groups 2 and 3, 82 μg thiamine hydrochloride. Feces were collected daily and total thiamine determined by the thiochrome method as previously described (1). A control series was run with 3 groups of 10 rats each. The rats were fed the purified diet devoid of thiamine for 2 weeks. Coprophagy was prevented in all rats. On the first experimental day all rats were anesthetized with ether and the cecum exposed through a mid-line abdominal incision. This was a sham operation for only 0.2 ml of water was injected into the cecum. After the incision had been closed the 3 groups were given, by stomach tube, 1 ml of water, 7 μg thiamine hydrochloride, 14 μg thiamine hydrochloride. This procedure, including the sham operation, was repeated on the eighth experimental day.

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TABLE I. Recovery of Thiamine Injected into the Cecum.

Group No.	Thiamine injected, μg/rat (single dose)	No. of rats	Wt of feces, g/wk/rat	Fecal thiamine, μg/wk/rat	Non-recovered thiamine, μg/wk/rat
			Weanling rats (avg wt 66 g)		
1 1st wk	0 (.2 ml H_2O)	5	5.5	35	
2	164 "	5	6.3	169	30*
3†	164 "	6	5.3	171	28
1 2nd wk	0 (.2 ml H_2O)	5	2.8	27	
2	82 "	5	3.9	81	28
3†	82 "	6	3.4	88	29
			Young adult rats (avg wt 186 g)		
1	0 (no injection)	6	3.9	19	
2	0 (.1 ml H_2O)	6	5.9	24	
3	144 "	6	10.1	140	27

* 164 — (169 — 35) = 30.
† 50 mg of procaine penicillin per kilo of diet.

In the second experiment, 3 groups of 6 young adult male rats (Holtzman) weighing approximately 185 g were fed a purified diet, free of thiamine, for 4 weeks. Coprophagy was prevented in all rats as in the previous experiment. No surgical manipulation was carried out with the first group. At the end of the third week Group 2 received 0.2 ml water injected into the cecum and Group 3 received 144 μg thiamine hydrochloride in 0.2 ml water in the same manner (Table I). Feces were collected daily during the fourth week and analyzed for total thiamine as in the previous experiment. A control series for this experiment was run with 3 groups of 10 rats each. The rats were of approximately the same average weight (180 g) as the experimental group. Coprophagy was prevented and after receiving the thiamine deficient diet for 3 weeks, 0.2 ml of water was injected into the cecum. The 3 groups then received by stomach tube 1 ml of water, 70 μg thiamine hydrochloride or 140 μg thiamine hydrochloride.

Results and discussion. Growth results for the 2 experiments are shown in Fig. 1 and 2. From previously reported responses to graded dosages of thiamine(1), it can be estimated for the first experiment with weanling rats that not more than 1 μg thiamine per day or 7 μg per week were absorbed from the injected dosages. This is borne out by the magnitude of response seen in the controls (Fig. 2, Exp. I A). Comparable data for the calculation of dose-response are not available for the older animals used in the second experiment. The control series (Fig. 2, Exp. II A) does show that a relatively large dose of thiamine by stomach tube (70 μg) gave a good response. Doses of 7 or 14 μg by stomach tube were found in a separate experiment to be insufficient to elicit a response in the larger rats. Therefore the older rats were definitely not as sensitive as the weanling rats in measuring the possible extent of absorption from the cecum. Since there is normally some regurgitation of cecal contents into the lower segment of the ileum, one might expect some slight absorption from that region of the tract. Recovery of injected thiamine from the feces shows an average loss in all 5 observations (combining both experiments) of 27 μg (range 20.7-30.4) for the 7-day collections following injection. If this amount of thiamine was absorbed it would amount to 4 μg per day, which obviously was not the case for this quantity would have provided for good growth, at least in the younger rats. It must be concluded that thiamine injected into the cecum is not absorbed from the cecum or the colon. The non-recovered thiamine was independent of the dose administered and probably represents the amount metabolized by intestinal microflora. This loss was approximately equal to the total daily output in the feces. In other words, fecal thiamine in the rat may represent only about one-half of the total thiamine synthesized in the large intestine.

Penicillin had no effect upon growth re-

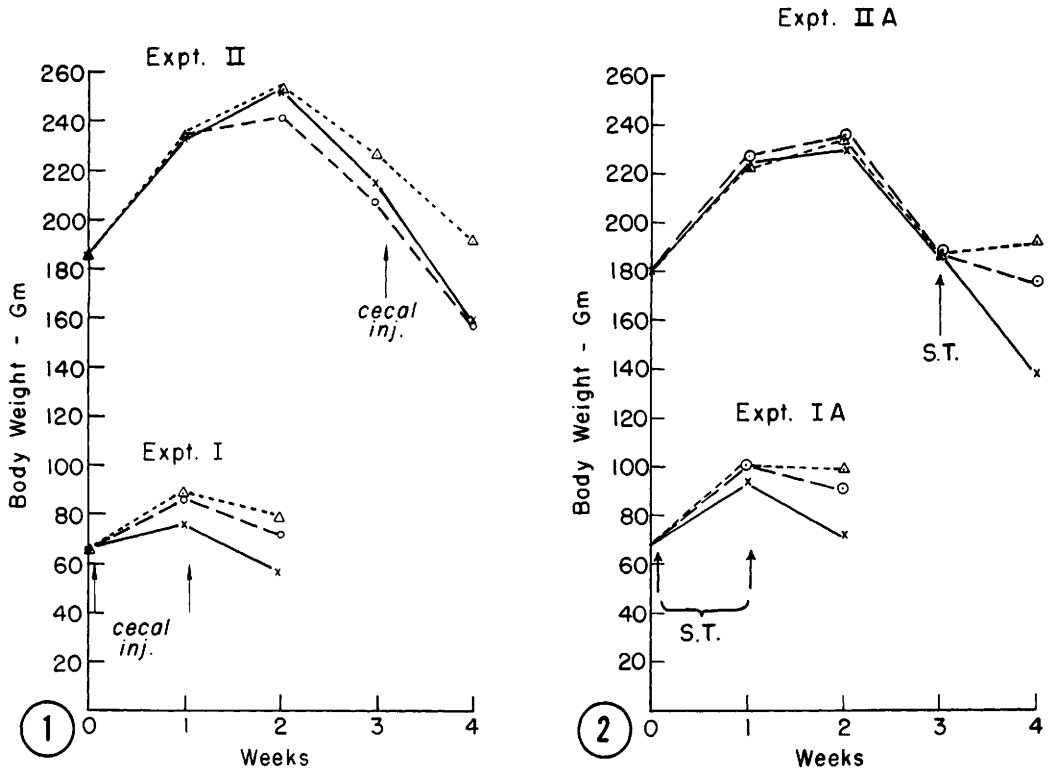

FIG. 1. Effect of thiamine injection into the cecum on growth response of rats. Arrows indicate time of inj. Exp. I, first inj. 164 μg, second inj. 82 μg thiamine hydrochloride. ×——× H_2O inj.; ○– – –○ thiamine inj.; △- - - -△ thiamine inj. plus penicillin in diet. Exp. II. ×——× control, no inj.; ○– – –○ H_2O inj.; △- - - -△ 144 μg thiamine hydrochloride inj.

FIG. 2. Control studies with sham operation and water inj. into the cecum. Arrows indicate time of administration of thiamine hydrochloride by stomach tube. Exp. I A, ×——× 1.0 ml H_2O by S.T.; ○– – –○ 7 μg thiamine HCl by S.T.; △- - - -△ 14 μg thiamine HCl by S.T. Exp. II A, ×——× 1.0 ml H_2O by S.T.; ○– – –○ 70 μg thiamine HCl by S.T.; △- - - -△ 140 μg thiamine HCl by S.T.

sponse or amount of injected thiamine recovered from the feces. Therefore, this antibiotic did not alter absorbability nor did it affect the rate of destruction of thiamine in the large intestine.

Summary. Rats receiving a thiamine-free diet and with coprophagy prevented were administered thiamine by injection directly into the cecum. Lack of growth response indicated little or no absorption of the injected thiamine from the large intestine. Recovery from feces of the injected thiamine showed a loss equivalent to approximately 4 μg per day. It has been concluded that this represents the rate of destruction of thiamine in the large intestine. The inclusion of penicillin in the diet did not alter the absorbability nor did it affect the rate of destruction of thiamine in the large intestine.

1. Barnes, R. H., Kwong, E., Delany, K., Fiala, G., *J. Nutrition,* 1960, v71, 149.

2. Mameesh, M. S., Webb, R. E., Norton, H. W., Johnson, B. C., *ibid.,* 1959, v69, 81.

3. Wostmann, B. S., Knight, P. L., *ibid.,* 1961, v74, 103.

4. Abdel-Salaam, A., Leong, P. C., *Biochem. J.,* 1938, v32, 958.

5. Barnes, R. H., Fiala, G., McGehee, B., Brown, A., *J. Nutrition,* 1957, v63, 489.

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