These experiments indicate, therefore, that "slow" epinephrine will maintain the blood pressure during and following intestinal manipulation and will increase survival 300%. This confirms the encouraging clinical results reported in shock using vasoconstrictor drugs such as adrenalin,[7] ephedrine,[8] and neosynephrine.[9] Recent experiments of Best and Solandt[10] indicate that adrenalin and other vasoconstrictor drugs may be a valuable adjunct in the therapy of some forms of shock.

12001

Mechanism of Intestinal Absorption of Thiamin.

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We have reported[1] that the intestinal absorption of substances whose absorption is accelerated by phosphorylation is specifically stimulated by thyroxin. Banga Ochoa and Peters[2] have demonstrated that the phosphorylated form of thiamin, cocarboxylase, is active in pyruvic acid oxidation. Therefore it seemed of interest to ascertain whether thyroxin would accelerate the absorption of thiamin from the digestive tract.

Apparently a close relation exists between thyroxin and vitamin B because the requirement for vitamin B increases when metabolism is augmented. Cowgill[3] and his coworkers have shown that the need for the vitamin B complex is greatly increased when metabolism is accelerated experimentally by the administration of thyroxin. Drill[4] has demonstrated that thiamin injections offset the loss of weight in rats caused by feeding of thyroid substance. Furthermore, Peters and Rossiter[5] have found that hyperthyroidism causes a fall in tissue

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5 Peters, R. A., and Rossiter, R. J., *Biochem. J.*, 1939, **33**, 1140.

cocarboxylase and thiamin. Clinically, Parade[6] has shown that feeding of thyroid substance aids in alleviating the symptoms of vitamin B_1 deficiency and Frazier and Ravdin[7] have shortened the preoperative preparation of hyperthyroid patients by treating them with thiamin.

Experimental Procedure. Female rats weighing between 200 and 250 g were employed in these experiments. A group of these animals was rendered hyperthyroid by daily intraperitoneal injections of 0.1 mg thyroxin per 100 g of body weight for 12 days. The average increase of the basal metabolic rate of rats so treated, as determined in a closed circuit apparatus similar to that used by Benedict and Macleod,[8] was nearly 50%. All animals were fasted for 48 hours in order to clear their intestinal tracts of food residues. Since rats in the hyperthyroid state are adversely affected by prolonged fasts, each hyperthyroid animal was given a cube of sugar after 24 hours of fasting. Both normal and hyperthyroid animals were then given 100 μg thiamin chloride in 2 cc of water by stomach tube. Normally rats require only 10 μg of thiamin daily. At various intervals the animals were sacrificed and the amount of unabsorbed residue of thiamin in the digestive tracts was determined. In order to observe the effects of a still larger dose, 1 mg of thiamin was administered to normal and hyperthyroid rats in the manner described.

The thiamin was estimated by a simplification of the thiochrome method previously described by Borson.[9] Since the quantity of interfering substances is negligible, it was possible to proceed to the oxidation without preliminary adsorption onto Lloyd's reagent. From 0.2 to 1.0 ml aliquots were used, depending on the amount of thiamin present. Protection against destruction of thiamin in the residues was insured by bringing the solution to a pH of about 4.5 and adding toluol. Material preserved in this way retains thiamin indefinitely. The total volume of the washings was 200 ml. The original body weight before administration of thyroxin was chosen as a basis for calculation of the rate of absorption.

Results. The figures showing absorption of thiamin in normal and in hyperthyroid rats after administration of 100 μg are given in Table I. From a study of these figures it appears that in normal fasting rats an initial period of rapid absorption (56 μg per hour)

6 Parade, G. W., *Z. Vitaminforsch.*, 1938, **7**, 35.

7 Frazier, W. D., and Ravdin, I. S., *Surgery*, 1938, **4**, 680.

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TABLE I.
Absorption of Thiamin Chloride in Normal and in Hyperthyroid Rats after Administration of 100 μg per 100 g of Weight.*

Absorption time	Normal rats		Hyperthyroid rats	
	Amt absorbed, μg	Rate absorption per hr, μg	Amt absorbed, μg	Rate absorption per hr, μg
15 min	14 ± 4†	56.0	18 ± 2†	72.0
30 ''	15 ± 1	4.0		
1 hr	18 ± 3	5.3		
2 ''	21 ± 2	4.0	24 ± 2	3.5
3 ''	28 ± 2	5.1		
4 ''	30 ± 4	4.3		

*Each figure represents the average of 6 rats.
†Standard deviation.

is followed by a considerably diminished and almost constant rate of absorption (about 5 μg per hour). The hyperthyroid rats did not absorb a significantly larger amount of thiamin than did the normal animals. When 1 mg of thiamin was administered, the average absorption for a 1-hour period was 108 ± 18 μg per 100 g of body weight in 4 normal rats and 116 ± 25 μg in 4 hyperthyroid rats.

The method described estimates thiamin only. Preliminary hydrolysis with taka-diastase did not increase its recovery in either the normal or the hyperthyroid rats. It was concluded, therefore, that thiamin is not phosphorylated in the lumen of the gut before absorption.

Discussion and Conclusions. Our data show that injections of thyroxin do not increase the rate of intestinal absorption of thiamin chloride in rats as they do in the case of dextrose and other substances susceptible to phosphorylation. This observation would indicate that phosphorylation plays no dominant part in determining the rate of intestinal absorption of thiamin or that thyroxin has no stimulating effect on the rate of enzymic formation of the pyrophosphate ester linkage. The stimulating effect of thyroxin may be limited to the phosphorylating processes involving the mono-phosphoric acid ester, as in the transport of dextrose across the intestinal mucosa. The conclusion that phosphorylation plays no part in the intestinal absorption of thiamin is supported also by the work of Ochoa,[10] who found that *in vitro* intestinal tissue, in contrast to liver tissue, possesses little ability to phosphorylate thiamin.

Two observations made during the course of our experiments indicate that absorption of thiamin in the intestine probably takes place by means of simple diffusion. First, the rapid initial absorption

[10] Ochoa, S., *Biochem. J.*, 1939, **33**, 1262.

of thiamin is succeeded by a much slower rate of absorption, presumably after the diffusion equilibrium between the lumen of the gut and the intestinal mucosa has become established. Secondly, absorption of thiamin is roughly proportional to its concentration in the intestine, as was seen when the dose of thiamin was increased from 100 μg to 1 mg. On the other hand, if the absorption of a substance is aided by phosphorylation, as, for instance, is the case with dextrose, the rate of absorption remains constant and is within wide limits independent of the concentration of the substance which is being absorbed.

12002

Relationship Between Sugar Concentration and Glycogenetic Action of Insulin on Rat Diaphragm *in vitro*.

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Previous work has shown that the rate of glucose utilization by the peripheral tissues of both normal and depancreatized dogs depends on the blood sugar level.[1, 2] Insulin appears to act as a catalyst in this process, accelerating a reaction which proceeds at a slower rate in its absence. However, if the blood sugar be sufficiently raised the absence of insulin makes little difference.

The present work is a study of the relationship between the action of insulin and glucose concentration in respect to the storage of muscle glycogen. Using the technic of Gemmill[3] we have observed the relationship of sugar concentration to glycogen formation in rat diaphragm *in vitro*, with and without added insulin.

Methods. Normal fed rats weighing between 80 and 100 g were used for all experiments. The animals were killed by a blow on the head, the diaphragms were quickly removed, trimmed, divided into approximately equal halves, weighed while moist, and introduced into Warburg vessels. The medium used was Hastings'[4] Ringer

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4 Hastings, A. B., Muus, J., and Bessey, O., *J. Biol. Chem.*, 1939, **129**, 295.