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Effect of thyroxine on the isolated rat intestine

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EDITORIAL SYNOPSIS In the intact animal some observers have shown an increased absorption of sugars and fatty acid after treatment with thyroxin. The following paper, in which uptake studies on the small isolated intestine of rats made thyrotoxic are reported, shows that inhibition of absorption of water, glucose, and a non-utilizable amino-acid (α-amino isobutyric acid) can also result. The authors feel that the estimation for these differences may be because of differences of doses of thyroxine, methods of administration, and possibly a variable effect of different tissues.

Hyperthyroidism is well known clinically to bring about emaciation and gastrointestinal disorders. Recently it was shown in many animals, such as rats, guinea-pigs, rabbits, and dogs, and also in frogs, that there is a reduction in the gastric acid secretion when these animals were fed with desiccated thyroid (Nasset, Logan, Kelley, and Thomas, 1959; Goldsmith and Nasset, 1959; Nasset and Goldsmith, 1961). Accelerated uptake of glucose in the intact intestine of hyperthyroid rats has been reported (Althausen, 1949; Althausen and Stockholm, 1938), while it has been claimed that thyroxine increases glucose absorption from the perfused gut of *Rana esculenta* (Gellhorn and Northup, 1933).

The effect of thyroidal hormones on the absorption processes of the isolated intestine do not, however, appear to have attracted much attention. Halliday, Howard, and Munro (1962) have shown that glucose transfer across the isolated everted intestine of mice was inhibited when the animals were fed with 0·5% desiccated thyroid for 14 days (personal communication), but that there was no change in the water movement across the gut; Levin and Smyth (1963) have also observed that hyperthyroid rats show little change in their hexose transfer mechanism across the isolated intestine. Very recently one of us (Seshadri, in preparation) has obtained results to show that thyroxine can produce inhibitory effects on the oxygen consumption and phosphorylation of the intestinal segments of rats. The present work reports the effect of thyroxine on the absorption and movement of non-utilizable amino-acid, α-amino isobutyric acid, glucose, and water by the isolated rat intestine.

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MATERIALS AND METHODS

Female rats of about 200 g. body weight were given intraperitoneal injections of 100 μg. Na-thyroxine in physiological saline (0·9% NaCl) per 100 g. of body weight daily for three days. Control rats received only normal saline solution. Rats were killed by ether on the fourth day following 18 hours of starvation. The entire intestine was washed *in situ* with cold saline as soon as the viscera were opened. The ilial portion, from the posterior end of the duodenum up to the caecum was removed, everted, and divided into five equal portions. Three of the intestinal segments (I, II, and III) from the duodenal end were prepared following the technique of Wilson and Wiseman (1954) as modified by Parsons, Smyth, and Taylor (1958) for longer pieces of gut. Each segment was made into a sac, tying both ends with cotton threads after filling it with 1·0 ml. bicarbonate Ringer solution containing glucose (0·4%) and non-labelled α-amino isobutyric acid ($15{\cdot}4 \times 10^{-3}\mu$ moles/ml.).

The sacs were then suspended in conical flasks containing the same Ringer solution as mentioned above except that the α-amino isobutyric acid was marked with C^{14}. The flasks were then gassed with a 95% O_2 : 5% CO_2 mixture, for three minutes, sealed, and incubated for one hour with mild shaking in a water bath maintained at 37°C. Each segment was weighed when empty and after filling, at the beginning of the experiment, and when full and after emptying at the end of the experiment. The net water movement and tissue water uptake were determined as has been described by us earlier (Green, Seshadri, and Matty, 1962).

As the intestine was everted the mucosal side formed the outer layer immersed in the Ringer solution in the flask, while the serosal side became the lumen of the sac which initially contained non-labelled α-amino isobutyric acid Ringer solution. At the end of the experiment samples were taken both from the mucosal side (flask) and from the serosal side (lumen of the sac) for analysis.

For assaying α-amino isobutyric acid-C^{14}, 0·1 ml. of

mucosal, serosal, or standard solution was added to 3 ml. of ethyl alcohol and 10 ml. of a mixture containing 4 g. of 2·5 diphenyl oxozole and 0·5 g. of 2·1 naphthyl 5 phenyl oxozole litre of toluene. This was counted in a liquid scintillation counter for 100 seconds. Glucose was estimated by the glucose oxidase method (Huggett and Nixon, 1957; Kramer Jakobsen, 1960), using the commercial modification of Boehringer in the form of their Blutzucker Farb test.

RESULTS AND DISCUSSION

There is a significant loss in the body weight of rats following three days of thyroxine treatment. Control rats lost 0·9 ± 1·7 g., *i.e.* 4·4%, of their initial weight while the treated rats lost 16·4 ± 4·0 g., *i.e.* 7·8%, of their weight. This loss in the weight served as a criterion to determine the hyperthyroid condition. The isolated intestinal segments of the treated rats showed decreased water movement, α-amino isobutyric acid-C^{14} transport across the intestinal wall, and a decreased glucose uptake by the tissue.

Table I shows that the net water flux expressed as serosal gain in ml./total length of the segment/hr. decreases from segment I to III and that thyroxine produced an inhibition of flux in all the segments, the effect being more in the segments I and II. Though there is an apparent reduction in the mean tissue water uptake in all the segments of the treated intestine it is not within the significant level.

TABLE I

EFFECT OF THYROXINE ON WATER MOVEMENT AND UPTAKE OF ISOLATED RAT SMALL INTESTINE[1]

Segments	*Net Water Flux (ml./segment/hr.)*		*Tissue Water Uptake (ml./segment/hr.)*	
	Control (5)	*Treated (6)*	*Control (5)*	*Treated (6)*
I	0·64 ± 0·09	0·22 ± 0·05[2]	0·28 ± 0·05	0·25 ± 0·07
II	0·59 ± 0·12	0·18 ± 0·03[2]	0·38 ± 0·08	0·33 ± 0·05
III	0·48 ± 0·04	0·37 ± 0·17[2]	0·38 ± 0·07	0·36 ± 0·09

[1]Results are expressed as the mean ± S.E.
[2]$P < 0·001$; numbers within brackets are numbers of animals used.

Movement of α-amino isobutyric acid-C^{14} across the intestine wall is significantly reduced in segments I and II but not so in III (Table II).

TABLE II

EFFECT OF THYROXINE ON AIB-C^{14} MOVEMENT THROUGH RAT INTESTINE[1]

Segments	*Net α-Amino Isobutyric Acid-C^{14} Flux (μ mol × 10^{-3}/segment/hr.)*	
	Control (5)	*Treated (6)*
I	17·83 ± 1·9	10·54 ± 2·4[2]
II	21·24 ± 3·8	11·17 ± 1·5[2]
III	21·57 ± 2·1	20·10 ± 5·4

[1]Results are expressed as the mean ± S.E.
[2]$P < 0·001$; numbers within brackets represent number of animals used.

Intestinal glucose uptake as shown in Table III is inhibited in hyperthyroid rats both when expressed in terms of concentration or as total uptake per segment per hour. No change in the dry weight of these intestinal segments of both controls and treated has been observed, results being obtained by running a series of experiments in parallel with these reported under exactly similar conditions. Thus it is apparent that there is a real decrease in glucose utilization in the treated intestine and that reduced uptake does not come about because of a change in the tissue water content of the segments before incubation.

There is loss of glucose from the serosal side, either when represented in terms of concentration (Table III), or when calculated for the whole segment in all the segments both in the controls and treated; and the loss is even more conspicuous in the treated than in the control intestine. The explanation for this serosal transfer of glucose, especially in the treated animals, in spite of the decreased uptake of glucose is not clear.

These results lend support to the view that thyroxine can produce inhibitory effects on metabolic processes of the intestine of the rat. Experiments conducted elsewhere (Seshadri, in preparation) under similar conditions to the present ones have shown that thyroxine reduces the rate of respiration and phosphorylation of the corresponding three intestinal segments of the rat. However, Althausen and Stockholm (1938), performing experiments *in vivo*, observed increased absorption of sugars and oleic

TABLE III

EFFECT OF THYROXINE ON THE GLUCOSE MOVEMENT IN ISOLATED RAT INTESTINE[1] (MEAN ±S.E.)

Segment	*Glucose Concentration (μ mol./ml.)*						*Total Tissue Glucose Uptake (μ mol./segment/hr.)*	
	Initial		*Final*					
	Control	*Thyroxine*	*Mucosal*		*Serosal*		*Control*	*Treated*
			Control	*Thyroxine*	*Control*	*Thyroxine*		
I	23·00 ± 0·59	22·17 ± 0·43	10·41 ± 0·8	17·41 ± 0·80	17·67 ± 0·82	12·25 ± 1·08	95·5 ± 6·8	63·1 ± 12·1
II	23·04 ± 0·29	22·92 ± 0·28	12·10 ± 0·79	14·88 ± 0·34	11·86 ± 0·49	13·07 ± 0·40	127·7 ± 9·1	85·7 ± 9·1
III	22·94 ± 0·28	22·43 ± 0·59	13·58 ± 1·17	15·28 ± 0·58	10·26 ± 0·33	9·72 ± 0·83	102·9 ± 10·8	86·7 ± 5·6

[1]Results are expressed as the mean ± S.E.

acid by the digestive tracts of rats injected with 0·1 mg./100 g. body weight/day of thyroxine for 12 days and a reduction in thyroidectomized rats. Abnormally high concentrations of blood sugar are well known in Grave's disease with patients showing normal tolerance curves after intravenous administration of hexoses. This increased blood sugar is therefore held to be due to accelerated absorption of sugars from the intestine. Houssay (1946) has pointed out, however, that increased sugar absorption rates are not specific for hyperthyroidism in spite of this occurring in experimentally induced and clinical hyperthyroidism.

Our experiments, and those of Halliday *et al.* (1962) appear to be somewhat at variance with studies on whole animals, but as has been pointed out by the latter authors, it may be that the induced deficiency of the intestinal cellular mechanisms are offset *in vivo* by such factors as increased gastrointestinal motility and blood flow. It is fairly well established that thyroxine stimulates motor activity in the stomach and intestine, thus accelerating the movement of food through the gut.

The dose level of thyroxine (100 μg./100 g. body weight) used by us, although not high when compared with the dosage used by many other previous workers in order to obtain an experimental hyperthyroid condition, may be a near toxic one. Certainly the effects reported here must lead one to this assumption of toxicity, for Tata, Ernster, and Lindberg (1962), have recently shown that the basal metabolic rate of rats is raised by the administration of only 10-25 μg. thyroxine/100 g. body weight every fourth day. Furthermore Barker (1962) has pointed out that various observers working on different isolated tissues have found no change of oxygen consumption using methods which seem to be as reliable as any reported. Thus there exists a probable specificity of action of thyroid hormones for different organs. Some thyroxine-treated tissues, such as liver, diaphragm, and heart, show increased oxygen consumption, others, such as spleen, mammal brain, and thymus, show no change while yet a further group comprising intestine and toad kidney (Barker, 1962) show decreased oxygen consumption. It is more than probable that the method of hormone treatment, dose level, type and state of tissue are all conditions that determine the oxygen consumption response. Levin and Smyth (1963), using approximately the same dose of thyroxine as ourselves, but who injected intraperitoneally daily for 12 to 16 days, found increase in intestine weight, greater uptake of glucose from the mucosal fluid, and increased rate of metabolism of the glucose by the isolated rat intestine. We have not been able to observe increase in intestinal weight or an enhanced uptake of glucose in our rats. An explanation of this descrepancy may be that at this possibly near-toxic dose the hormone can either enhance or inhibit metabolism depending on external or internal factors such as temperature, age, or sex.

The inhibition of α-amino isobutyric acid-C^{14} and also that of isotopic sodium transport (unpublished observations) in the hyperthyroid rat intestine is in conformity with inhibition of oxygen and glucose uptake and is not unexpected in view of the dependence of transport mechanisms on energy-producing systems. The inhibition of the net flux of water may be regarded as decreased passive permeability through the intestine brought about as a consequence of decreased active transport processes.

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