Further Studies on Metabolism of Tissues from Thyroxine-Injected Rats.* (20396)

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Barker and Klitgaard(1) reported the effects of thyroidectomy and of thyroxine injection on the oxygen consumption of various tissues excised from rats. Their results showed that liver, kidney, gastric mucosa and several types of muscle (including cardiac, skeletal, and diaphragmatic and gastric smooth muscle) exhibited a depressed metabolic rate following thyroidectomy and increases to various extents after injection of a single large dose of thyroxine. In contrast, brain, spleen and testis were not altered by either hypo- or hyperthyroidism.

The present study was designed to investigate some of the implications of this work, such as possible effects on the female gonads as well as on accessory reproductive structures of both sexes and on other exocrine glands of the digestive tract.

Methods. Since many of the tissues included here were found not to be controlled by the thyroid, repeated injections of a smaller dose of hormone were employed to make certain that maximal increases in metabolic rate of the animal were obtained. Two mg of L-thyroxine† were injected subcutaneously per kilo of body weight per day for 4 days and the animal sacrificed on the fifth. In general, procedures were as outlined previously(1). Each animal was anesthetized with ether, and as much blood as possible withdrawn by syringe from the abdominal aorta. The desired tissues were then rapidly excised and chilled in ice-cold Ringer solution. In some cases, animals were sacrificed without anesthesia by guillotine in order to avoid possible effects of ether. In the preparation of tissues for oxygen consumption studies, connective tissue was removed as completely as practicable, in addition to all extraneous material such as the coagulating glands and seminal fluid from the seminal vesicles. Liver, kidney cortex, salivary glands, thymus and spleen were sliced according to Deutsch(2). The thinnest portions from each hemi-diaphragm were used. Uteri and seminal vesicles were opened lengthwise, the prostate was cut several times, and appropriate quantities of each were taken. Lymph nodes, ovaries and brain were cut several times along different planes with scissors and the resulting rough mince employed. Oxygen constituted the gas phase. Except in the case of ovaries and thymus, where only small quantities of tissue were available, duplicate vessels were set up, using Krebs-Ringer phosphate solution with 100 mg % of glucose. All results are shown in terms of wet weight.

Results and discussion. Table I presents the means and standard deviations of the tissues studied representing euthyroid, thyroidectomized and thyroxine-injected thyroidectomized animals.

Liver, kidney and diaphragm were included as positive controls, since these have previously been found to respond consistently to the level of thyroid function(1). The decreases of 15-30% following removal of the thyroid gland and increases of 50-75% resulting from thyroxine injection (Table II) agree with results from this and other laboratories and furnish assurance that the animals actually were in the metabolic conditions desired.

Salivary gland, completely exocrine, and pancreas, mixed exo- and endocrine, both displayed changes in oxygen consumption indicating dependence upon thyroxine: approximately 20% decreases after thyroidectomy and a 34% increase during thyroxine injection from salivary gland, 51% with pancreas.

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† Material obtained through the courtesy of Glaxo Laboratories, Smith, Kline and French Laboratories and Baxter Laboratories, Inc.

TABLE I. Metabolism of Various Tissues in Euthyroid, Thyroidectomized and Thyroxine-Injected Thyroidectomized Rats.

Tissue	Euthyroid — mm³ O_2/mg/hr		Thyroidectomized — mm³ O_2/mg/hr		Hyperthyroid — mm³ O_2/mg/hr	
	No.	Mean ± S.D.	No.	Mean ± S.D.	No.	Mean ± S.D.
Liver	12	1.26 ± .09	12	1.01 ± .07	12	1.64 ± .11
Kidney	12	3.72 ± .23	12	3.17 ± .13	12	4.68 ± .44
Diaphragm	12	.95 ± .08	11	.66 ± .07	11	1.14 ± .17
Salivary gland	8	2.31 ± .06	8	1.81 ± .08	8	2.43 ± .11
Pancreas	8	1.04 ± .04	7	.83 ± .07	8	1.25 ± .13
Lymph node	11	.87 ± .12	11	.85 ± .09	12	.92 ± .08
Thymus	12	1.09 ± .14	12	.99 ± .18	11	1.09 ± .15
Ovaries	6	1.14 ± .10	6	1.13 ± .11	6	1.04 ± .12
Uterus	6	.73 ± .09	6	.73 ± .10	6	.78 ± .06
Seminal vesicle	6	.77 ± .05	6	.75 ± .07	6	.75 ± .04
Prostate	6	1.52 ± .10	6	1.51 ± .16	6	1.54 ± .14
Brain	4	1.30	4	1.28	4	1.37
Spleen	4	1.42	4	1.35	4	1.48

TABLE II. Change in O_2 Consumption of Various Tissues Produced by Thyroidectomy and by Thyroxine Injection.

	Thyroidectomy change		Thyroxine inj. change	
	%	P	%	P
Liver	—19.8	<.01	+62.4	<.01
Kidney	—14.8	"	+47.6	"
Diaphragm	—30.6	"	+72.7	"
Salivary gland	—21.6	"	+34.3	"
Pancreas	—20.2	"	+50.6	"
Lymph nodes	— 2.3	>.1	+ 8.2	<.1, >.05
Thymus	— 9.2	"	+10.1	>.1
Ovaries	— .9	"	— 8.0	"
Uterus	.0		+ 6.8	"
Seminal vesicle	— 2.6	"	.0	
Prostate	— .7	"	+ 2.0	"
Brain	— 1.5		+ 7.0	
Spleen	— 4.9		+ 9.6	

These alterations are in keeping with similar ones exhibited by gastric mucosa, since Barker and Klitgaard(1) found a 28% decrease after thyroidectomy and a 60% peak increase in hyperthyroidism.

Lymph nodes (primarily cervical in origin) and thymus both constitute examples of reticulo-endothelial tissue. The former showed no significant variations, and the latter only a 10% change, of dubious significance considering the variations in individual values. In addition, such a change is far below the 35-75% alterations simultaneously produced in liver, kidney, diaphragm, salivary gland and pancreas.

Ovarian metabolism was not altered in the three states of thyroid function; thus they can be aligned with the male gonads, previously studied. A similar series of no metabolic responses was obtained with accessory sex structures of both sexes, including uterus, seminal vesicles and prostate. The "castration" effects of hypothyroidism on the female rat, involving prolonged diestrus(3) as well as increased sensitivity to administered estrogen (4), obviously cannot be explained on a purely metabolic basis.

Results are also shown on brain and spleen of a small number of animals in each status of thyroid function sacrificed without anesthesia. The data for brain metabolism are about 30% higher than those previously reported(1); this occurred because the brain mince previously was cleared of soluble materials by suspension in cold Krebs-Ringer solution and decantation. This process was omitted in the present series of experiments. Even with these different conditions, however, changes of only 1.5% and 7.0% from euthyroid values were produced by thyroidectomy and hyperthyroidism, respectively. In the case of spleen, the results were very close to those reported by Barker and Klitgaard.

A survey of Table II reveals a considerable number of tissues showing no metabolic relationship to thyroid hormone level, including lymph nodes, thymus, ovaries, uterus, seminal vesicles, prostate, brain, and spleen. Testis, studied previously, can be added. Using the t-test for statistical significance of the differences in Q_{O_2} between euthyroid and thyroidectomized and between thyroidectomized and

hyperthyroid, every one of these tissues has a P in excess of 0.1, indicating a very low level of confidence in any differences present. Examination of the entire series shows that several tissues exhibited increases of 7 to 10% during thyroxine injection; it is quite possible that accumulation of many more data for each one would eventually produce statistically valid differences. The group of tissues would still, however, stand in contrast to the others with much more marked effects. The conclusion seems valid that brain, lymphoid tissue, gonads and accessory reproductive structures are not responsive to thyroid hormonal control of metabolism, in contrast to several kinds of muscle and glands producing digestive secretions.

Preliminary results obtained on the distribution of radioactive thyroxine‡ following a single injection indicate that the lack of metabolic stimulation by the hormone, where this situation prevailed, cannot be attributed to a lack of exposure of these tissues to the hormone. With an impressive list accumulating of tissues not responding to thyroxine, the question of mechanism of action of this material becomes ever more intriguing. If the primary effect is one of uncoupling of oxidative and phosphorylative reactions or a direct involvement of the hormone as a member of an oxidation-reduction system(5), marked variations must occur in how these processes respond in different tissues.

Summary. Salivary gland and pancreas have been added to the list of tissues whose energy metabolism is controlled by the thyroid gland. In contrast, lymph nodes, thymus, ovaries, uterus, prostate and seminal vesicles have metabolic rates unaltered (within 10%) by thyroid status.

‡ Radioactive thyroxine synthesized by S. C. Wang, T. Winnick, S. Notrica, and R. J. Winzler from I^{131} allocated by the Atomic Energy Commission.

1. Barker, S. B., and Klitgaard, H. M., *Am. J. Physiol.*, 1952, v170, 81.

2. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Tissue Metabolism,* Burgess Pub. Co., Minneapolis, 1949, pp. 110-111.

3. Nelson, W. O., and Wheeler, H. E., *Fed. Proc.,* 1948, v7, 85.

4. Langham, W., and Gustavson, R. G., *Am. J. Physiol.*, 1947, v150, 760.

5. Barker, S. B., *Physiol. Rev.*, 1951, v31, 205.

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Effect of Cortisone on Diphtheria Intoxication and the Schick Test in Guinea Pigs.* (20397)

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A study of the effect of cortisone on diphtheria intoxication in guinea pigs was carried out as part of an attempt to assess the value of cortisone in infections. Diphtheria toxin, rather than *C. diphtheriae,* was used in order to exclude complications which might have resulted from the interference of cortisone with host resistance to invading microorganisms(1-4).

The observation that ACTH appeared to alleviate the effects of the neurotoxin elaborated by the black widow spider(5) suggested that cortisone might be of benefit in diphtheria intoxication.

Material and methods. Male and female guinea pigs weighing approximately 300 g were used. They were kept in separate cages, fed cabbage and rabbit pellets, and offered both tap water and 1% KCl solution *ad lib.* The last was used to prevent cortisone-induced hypokalemia. Animals were inspected daily. They were weighed initially and at the time of death or at the end of 10 days in the event of

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