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Metabolism in Normal and Thyroidectomized Rats as Influenced by Thyroxine and Thyroid Globulin Feeding.

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The evaluation of thyroid preparations is still in an inchoate state, because (1) the accepted method, based on iodine content, does not give an estimation of the physiologic activity, since the thyroxine component of standard products may vary considerably (up to 600% as found by Harrington and Randall[1]); (2) the methods of thyroxine assay available give more comparative than absolute values; (3) the physiologic action of thyroid substance or extract seems to depend partly on the chemical linkage between the thyroxine and other groups present. While the investigation of Thompson and co-workers[2] showed that the potency of thyroid substance is considerably reduced by hydrolysis, which would break up such linkages, Palmer *et al.*[3] concluded from their experiments on guinea pigs that the calorigenic action of thyroid substance is a direct function of the thyroxine contained, which, however, is present in the *l*-form and twice as potent as the commonly prepared racemic mixture.

In our attempt to find a reliable and fairly accurate method of bioassay, we used first normal and later thyroidectomized male rats, finding in the latter a far more suitable test animal. In this we are at variance with the work of Gaddum,[4] which is probably explainable by the difference in the age of the rats used.

A closed circuit apparatus was used, as described by Benedict.[5] The normal rats used were 80-100 days of age. Thyroidectomy was performed when the rats were 70-80 days old. In the latter case, at least 3 weeks were allowed for recovery. The rats were starved for 20 hours and allowed to remain for 30 minutes in the respiration chamber before testing. The temperature was kept constant at 30° throughout the experiments. The time necessary for the

1 Harrington, C. R., and Randall, S. S., *Quart. J. Pharm.*, 1929, **2**, 501.

2 Thompson, W. O., Thompson, P. K., Taylor, S. G., III, and Dickie, L. F., *J. Clin. Invest.*, 1937, **16**, 479.

3 Palmer, W. W., and Leland, J. P., *J. Clin. Invest.*, 1935, **14**, 619; Foster, G. L., Palmer, W. W., and Leland, J. P., *J. Biol. Chem.*, 1936, **115**, 467.

4 Gaddum, J. H., *J. Physiol.*, 1930, **68**, 383.

5 Benedict, F. G., *J. Nutrition*, 1930, **3**, 161.

consumption of a constant volume of oxygen was measured, and results calculated as mg O_2 per hour per kg weight. Readings were accepted only when the animal remained quiet but not asleep, and at least 2 series had to agree on the same day. Two consecutive tests several days apart had to agree before medication was given. Such rats that did not remain quiet enough to give uniform results or showed abnormalities (*e. g.* tetany) after operation were eliminated. The percentage of normal as well as thyroidectomized rats that had to be rejected in proper selection of animals was quite considerable. Each animal was used only once for a medication, to avoid the possible influence of progressing age. As standard medication we used crystalline *d,l*-thyroxine, dissolved in slightly alkalinized water. In comparison, a thyroid globulin preparation was used, containing 0.565% total iodine, 0.14% of which was present as thyroxine iodine, determined by the method of Leland and Foster.[6] Considering an estimated loss of 15% in the determination, the corrected value is 0.165%, equivalent to a content of 0.25% thyroxine. Both preparations were fed by stomach tube in accurate quantities in proportion to body weight on 3 consecutive days. Metabolism was determined on the fifth day, at which time the peak of the metabolic curve is reached. Preliminary tests leading to this technic will be reported later in a complete publication.

TABLE I.
Normal Rats Fed Thyroxine.

No. of rats	1	1	5	1	3
Dose, micrograms	31	28	25	15	10
% increase of metabolism	39	47	20-41	18	0-29

TABLE II.
Thyroidectomized Rats Fed Thyroxine.

No. of rats	1	1	1	1	3	2	2	6	6
Dose, micrograms	25	12.5	10	6	3	2	1	0.75	0.5
% increase in metabolism	46	46	45	42.5	35-39	27-36	27-35	25-36	5-12
Avg	46	46	45	42.5	37	32	31	28.8	9.6

TABLE III.
Thyroidectomized Rats Fed Thyroid Globulin.

No. of rats	5	6	9	6
Doses of thyroglobulin	100	75	50	40
Equivalent to thyroxine	0.25	0.19	0.125	0.1
% increase in metabolism	28-36	18-31	13-20	3-10
Avg increase	31	24	16	7

[6] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

Results: The normal rats used had an oxygen consumption varying from 1420 to 1670 mg O_2 per kg per hour, but for the individual rat the figure was constant within a limit of 8%. The O_2 consumption of thyroidectomized rats was 900 to 1200 mg per hour per kg. Medication with varying doses of thyroxine in micrograms per 10 g weight and given daily for 3 days, and corresponding medication with thyroid globulin, calculated on thyroxine content, gave the results presented in Tables I-III.

While it seems that in normal rats the dose of 25 γ thyroxine might fall within the range of best measurable response, the spread of figures is too wide for acceptable conclusions to be drawn. Thyroidectomized rats are about 30 times more sensitive. Although the number of experiments presented so far is limited, a tentative interpretation of results seems to be permissible. They indicate that the percentage metabolic increase plotted as a function of dosage follows a logarithmic curve. A minimum dose is required before any response obtains. Increase of dosage produces initially a rapid increment of metabolism, which approaches a more gradual linear function at 20 to 35% increase and finally becomes slower. For comparative measurements, an increase between 25 and 30% seems to be the most favorable range. Surmising that thyroxine is the sole stimulator of metabolism in the thyroid globulin, between 0.19 and 0.25 γ thyroxine given in this form appears to be as active as 0.75 γ crystalline thyroxine, which would mean that thyroxine in natural linkage is 3 to 4 times more potent than the racemic substance.

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Hemolysin in the Urine in Aplastic Anemia.

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Ample evidence is at hand to prove that a substance which is lytic for erythrocytes is present in the urine of human beings under normal conditions. Few studies are available of this phenomenon in patients with disorders of the blood in which hemolysis might be a factor. Accordingly, the urines of patients with certain representative types of blood dyscrasia have been investigated for their