4. Eik-Nes, K., *J. Clin. Endo. and Metab.*, 1957, v17, 502.

5. Peterson, R. E., Wyngaarden, J. B., Serafim, L. G., Brodie, B. B., Bunim, J. S., *J. Clin. Invest.*, 1955, v34, 1779.

6. Hume, D. M., *Pathophysiologia Diencephalica* (Curri, S. B., Martini, L., Eds., Springer-Verlag, Vienna, Austria, 1958).

7. Egdahl, R. H., *Surgery*, 1959, v46, 9.

8. Osborn, W., Schoenberg, H. W., Murphy, J. J., Erdman, W. S., Young, D., *Surgical Forum*, 1961, v12, 485.

Received January 15, 1962. P.S.E.B.M., 1962, v109.

Thyroid Iodine Metabolism in the Alloxanized Rat.* (27388)

GEORGE S. SERIF AND KADIMAN SIHOTANG

Department of Biochemistry, School of Medicine, State University of South Dakota, Vermillion

The influence of thyroid status on the metabolism of carbohydrates in diabetes has been extensively studied(1). The effect of diabetes on thyroid metabolism, on the other hand, has received relatively little attention. The few studies which have been conducted in this area have been limited to the histochemical or cytological approach(2,3). The study reported here was designed to explore the thyroidal metabolism of iodine in the alloxan-diabetic rat using I^{131} as a metabolic tracer.

Experimental. Male Sprague-Dawley rats (135 ± 5 g) were maintained on a diet with a controlled iodine intake (0.0012 g of iodine/kg of diet) or on Nutrena dog pellets. Test groups received 4 intraperitoneal injections of alloxan monohydrate (15 mg, 20 mg, 20 mg, 20 mg) spaced at 3-day intervals. Control rats received intraperitoneal water (1 ml). Test and control rats were fasted 12 hours prior to each injection.

One test group received subcutaneous Lente insulin after the third alloxan injection at a dose rate of 3 units/day. Prior to I^{131} studies (16th day) this was increased to 3 units/12 hours.

Blood glucose values were checked after the final alloxan injection and at the end of experiment. Elevated blood glucose values, polydypsia and polyuria were considered to indicate that a diabetic state had been induced and maintained.

Three days after the final alloxan injection (16th day) all rats received 30 μc of carrier free Na I^{131} by intraperitoneal injection. The first measurements of thyroid I^{131} uptake were made 24 hours after injection of the radioactive tracer. Successive measurements of the thyroidal I^{131} content were made every 24 hours until the experiment was terminated.

Thyroid I^{131} uptake measurements were carried out using the procedure of Wolff(4) with the following minor modifications. Rats to be counted were not anesthetized but were immobilized by wrapping them in a light plastic cover and mounting them in a restraining cage designed to permit a constant geometry between the areas to be counted and the shielded, directional crystal-scintillation counter (Nuclear Chicago). The distance between the sensitive volume of the crystal and the areas to be counted was set at 2 inches to minimize minor variations in the position of the rats. Corrections for isotope decay and background were made in the normal manner. Correction for I^{131} content in nonthyroidal tissues was made using the epigastric procedure of Wolff(4).

The percentage of injected I^{131} which was concentrated by the thyroid gland in 24 hours in a given animal was approximated by comparing the 24-hour count to the count obtained from a 30 μc I^{131} standard mounted in a thin walled plastic tube and held above the scintillation counter in the approximate position of the rat thyroid. This standard, prepared on the day of I^{131} injection, was counted at the same time as the thyroid glands

* This investigation was supported by research grant from Division of Arthritis and Metab. Dis. U.S.P.H.S.

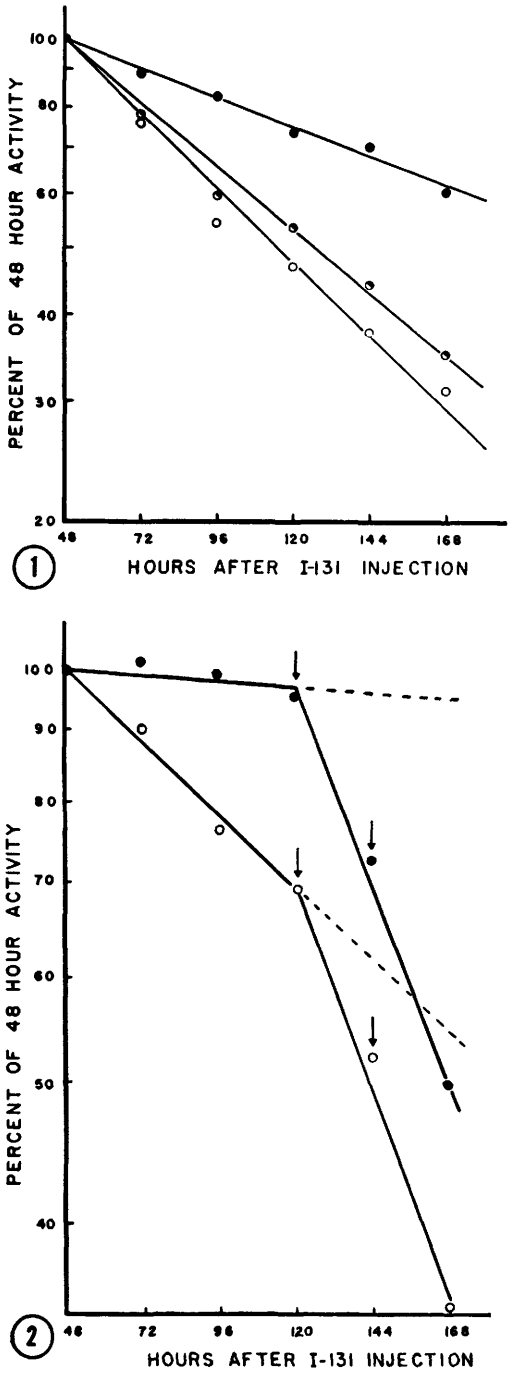

FIG. 1. Typical curves depicting release of I^{131}-labeled thyroid hormone from thyroid glands of alloxan-diabetic rats (●—●), alloxan-diabetic rats with insulin (◒—◒), and control rats (○—○). Statistical analysis of individual points on the curves using Student's 't' test shows that "control" and "diabetic" curves are significantly different at 95% confidence limit. Similarly the points on "diabetic plus insulin" curve are statistically different from those of "diabetic" curve at 95% confidence level after 72 hr. "Control" and "diabetic plus insulin" curves are not statistically different.

were counted, to eliminate the need for decay correction. Because of variations in back-scatter, absorption etc., between the standard and *in vivo* measurements, the percentage uptake figures are not absolute but do permit a relative comparison between test and control animals.

Results. Table I contains the values for 24-hour I^{131} uptakes in control rats, rats receiving alloxan, and rats receiving alloxan and insulin. A statistically significant difference exists between the rats of Group 2 and the rats of Group 1 and Group 3. Thus the iodide concentrating mechanism appears to be impaired in the diabetic animal but recovers upon treatment with insulin.

Fig. 1 demonstrates that the thyroid hormone is released much more slowly in the alloxanized rat than in the control animal. Treatment with insulin appears to have permitted return of the thyroid hormone release mechanism to normal.

Fig. 2 shows the influence of thyroid stimulating hormone (TSH) injections on thyroid hormone release in both control and alloxanized rats. Although both groups respond to TSH by increased release of thyroid hormone, the thyroids of the alloxanized animals respond more dramatically.

In original studies of this investigation a controlled iodine diet was used. Later studies indicated that such a diet was not necessary since only minor quantitative differences and no qualitative changes were observed when Nutrena dog pellets were substituted for this diet. Thus, for example, the rats of Fig. 2 were fed a controlled iodine diet while the rats of Fig. 1 received Nutrena pellets. This diet variation probably accounts for the

FIG. 2. Curves demonstrating influence of thyroid stimulating hormone on release of I^{131}-labeled thyroid hormone from thyroid glands of alloxan-diabetic rats (●—●), and control rats (○—○). Arrows indicate intraper. inj. of 1 International Unit of thyroid stimulating hormone per rat. Statistical analysis of individual points on these curves, using Student's 't' test shows that the curves are significantly different at 95% confidence level after 72 hr.

TABLE I. Twenty-Four Hour Uptakes of I^{131} of Rats with Varied Abilities to Control Carbohydrate Metabolism.

Group and treatment	No. of animals	Avg % uptake	p*
1. Controls	6	8.0	<.001
2. Alloxan diabetics	9	4.4	—
3. " " & insulin	5	7.3	<.001

* The probability "p" is derived from Student's "t" test and the expression $t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$.

Comparisons made are between Groups 1 and 2 and between Groups 2 and 3. Values obtained for "p" indicate that the observed differences are highly significant.

slightly different hormonal release rates for the control groups of Fig. 1 and 2. However, the qualitative effect, *i.e.*, depression of hormonal iodine release remains apparent regardless of diet.

Discussion. These data indicate that thyroid metabolism, as reflected by iodine uptake and hormonal iodine release, is depressed in the alloxan diabetic rat. These observations correlate with Telkka's finding of increased colloid and decreased cell height in thyroids of similarly treated animals(2). Additional studies in this laboratory indicate that the depression of thyroidal function is less pronounced with short term exposures to alloxan (1 to 2 injections). Whether this reduced effect is due to a less fully developed diabetic condition is not known.

The observation that the treatment of diabetic animals with exogeneous TSH initiates a rapid release of hormonal iodine, demonstrates clearly that the diabetic thyroid still possesses the capability of a response to TSH. This would suggest that the depressed thyroid capacity of the alloxan-diabetic rats may be due to a deficiency of endogenous TSH from the pituitary gland. This suggestion is in agreement with the histological findings of Desclaux who observed degranulation of the acidophilic cells and decreased basophilic cell counts in the pituitaries of alloxan-diabetic rats(5).

Decreased pituitary activity could be the outcome of stress of alloxan injection since stress has been stated to induce a deficit of TSH and alloxan has been called a stressor substance(2,6). The fact, however, that diabetic rats injected with insulin had been subjected to the same stress of alloxan injection without development of thyroid deficiencies would suggest that these deficiencies are more the result of the diabetic state than of the irritant action of alloxan.

Summary. Rats rendered diabetic by a prolonged series of alloxan injections suffer an impairment of ability to metabolize iodine, as manifested by reduced iodine trapping capacities and a subnormal thyroid hormone release rate. These effects are countered by insulin injections. Observed responses to thyroid stimulating hormone suggest that inadequate supplies of this hormone are being produced in the pituitaries of alloxan-diabetic rats.

1. Houssay, B. A., *Vitamins and Hormones,* 1946, v4, 187, Academic Press, New York.

2. Telkka, A., Kuuisisto, A. N., *Ann. Med. Exp. Biol. Fenniae* (Helsinki), 1954, v32, 266.

3. Desclaux, P., Soulairac, A., Brocheriou, J., *Compt. rend. soc. biol.,* 1948, v142, 783.

4. Wolff, J., *Endocrinology,* 1951, v48, 284.

5. Desclaux, P., Soulairac, A., Teyseyre, J., *Compt. rend. soc. biol.,* 1948, v142, 491.

6. Selye, H., Fifth Annual Report on Stress, M. D. Publication Inc., New York, 1955.

Received January 17, 1962. P.S.E.B.M., 1962, v109.