nitrogen and uric acid, as well as for isotope concentration in these 2 fractions. 3. No significant difference could be found between the 2 subjects in the isotope distribution of the total nitrogen or uric acid. The percentage of $N^{15}$ excreted as uric acid was no greater in the gouty than in the normal subject. The significance of this finding in relation to the pathogenesis of gout is discussed.

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## Inhibition of Thyroid Function by Cortisone and ACTH in Hypophysectomized Rats.* (20020)

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There is experimental and clinical evidence that administration of cortisone or ACTH suppresses thyroid function(1-5). Similar findings have been obtained under experimental conditions which are known to be accompanied by increased secretion of cortical hormone, including injection of epinephrine(6) or formalin(7) and exposure to abnormal temperature(8). Some investigators believe that this inhibition of thyroid function is caused by suppression of secretion of TSH(1,2); however, differences between the inhibition produced by adrenal hormone and by lack of TSH have been pointed out(9,10).

The experiments reported in this paper were undertaken in order to investigate the possibility that the inhibiting action of cortical hormone is exerted at the thyroid rather than at the pituitary level. A similar investigation was reported by Woodbury *et al.*(11).

*Material and methods.* Hypophysectomized male rats of the Sprague-Dawley strain were divided into 4 groups. Group one served as control. Group 2 was injected for 5 days with either ACTH¶ or cortisone.¶ Group 3 received daily injections of a TSH preparation¶ for 5 days. Group 4 was treated with both TSH and either ACTH or cortisone. The hormone dosage is indicated in the tables. After 4 days of treatment all animals received 4 $\mu$c of $I^{131}$ (carrier free)¶ by intraperitoneal injection. Twenty-four hours later the rats were killed; the thyroid glands were dissected free of connective tissue and weighed on a Roller Smith torsion balance. The glands were then macerated in 5% NaOH; aliquots were plancheted and the radioactivity determined by means of a Geiger Counter.

*Results.* Results of experiments with ACTH are presented in Table I, those with cortisone in Table II. It is evident that the amount

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¶ The TSH preparations were generously supplied by Dr. Daniel McGinty of the Parke Davis Co., ACTH was obtained from Armour and Co. through the courtesy of Dr. E. E. Hays, and Cortisone from Merck and Co. through the courtesy of Dr. E. Alpert. The $I^{131}$ was obtained from the Atomic Energy Commission, Oak Ridge Laboratories.

TABLE I. Influence of ACTH on Uptake of $I^{131}$ in Hypophysectomized Rats.

| | No. of rats | % uptake of $I^{131}$ 24 hr after tracer, 4 $\mu$c: Total | % uptake of $I^{131}$ 24 hr after tracer, 4 $\mu$c: Per mg thyroid wt | Thyroid wt, mg |
|---|---|---|---|---|
| Exp. 1 | | | | |
| 1. .85% NaCl | 7 | 3.3 ± .53 | .51 | 7 |
| 2. ACTH 4 mg/day | 4 | 1.05 ± .19 | .1 | 11.5 |
| 3. TSH 1 " | 6 | 29.8 ± 2.8 | 2.53 ± .20 | 11.8 |
| 4. TSH 1 mg, ACTH 4 mg | 5 | 22.1 ± 1.17 | 2.07 ± .39 | 12 |
| Diff. 3-4 | | 7.7 p<.05 | .46 p<.3 | |
| Exp. 2 | | | | |
| 1. .85% NaCl | 10 | .83 ± .22 | .09 | 9.1 |
| 2. ACTH 4 mg/day | 10 | .67 ± .12 | .08 | 9.7 |
| 3. TSH 1 " | 8 | 19.8 ± .89 | 1.84 | 10.8 |
| 4. ACTH 4 mg, TSH 1 mg | 9 | 19.2 ± .64 | 1.73 | 11.5 |
| Diff. 3-4 | | .6 p<.2 | | |
| 5. TSH 2 mg/day | 10 | 27.7 ± 1.81 | 2.14 | 13.3 |
| 6. ACTH 4 mg, TSH 2 mg | 9 | 24.4 ± 3.19 | 1.80 | 13.7 |
| Diff. 5-6 | | 3.3 p<.4 | | |
| Exp. 3 | | | | |
| 1. .85% NaCl | 8 | .75 ± .23 | .08 | 10 |
| 2. ACTH 4 mg | 9 | .77 ± .16 | .08 | 10.2 |
| 3. TSH 1 " | 9 | 22.3 ± 1.57 | 1.77 ± .15 | 12.8 |
| 4. ACTH 4 mg, TSH 1 mg | 9 | 12 ± 1.34 | .95 ± .18 | 13 |
| Diff. 3-4 | | 10.3 p = <.01 | .82 p = <.01 | |

of TSH (1 mg or 2 mg) was adequate to increase the uptake of $I^{131}$ significantly. Simultaneous administration of ACTH (Table I) lowered the uptake of $I^{131}$; in 2 of 3 experiments the difference in uptake values between the TSH treated and the TSH-ACTH treated animals was statistically significant. Similarly, simultaneous treatment with TSH and cortisone significantly lowered the uptake of $I^{131}$ as compared with that of the rats injected with TSH alone (Table II).

*Discussion.* Observations in intact animals and in man have indicated that endogenous or exogenous cortical hormone suppresses thyroid function. The uptake of a tracer dose of $I^{131}$ by the thyroid gland is diminished following administration of cortisone in the intact rat(4), and in man(1,5). Similar results have been obtained by subjecting rats to various forms of stress(6-8,13,14,16). Migeon *et al.* have found cortisone treatment to diminish $I^{131}$ uptake by the thyroid in adrenalectomized but not in intact rats(12).

In humans, treatment with large doses of cortisone is often followed by clinical and metabolic manifestations of hypothyroidism, such as low BMR(1,2), high serum cholesterol (1,2), and low serum protein-bound iodine (5,15).

It has been assumed by some authors that this inhibition of thyroid function is the result of diminished production and/or release of TSH from the pituitary. This assumption is supported by the observation that administration of exogenous TSH simultaneously with cortisone counteracts the thyroid-depressing effect of the latter(1).

However, in experiments reported by Perry (9) and by Albert *et al.*(10), discharge of thyroidal $I^{131}$ was not influenced by cortisone or ACTH, whereas the uptake of $I^{131}$ was inhibited. Moreover, the increase in cell height due to TSH is not diminished by simultaneous cortisone medication(17). This response pattern differs from that produced by deficient secretion of TSH; in this state both uptake and release of iodine and the thyroid cell height are decreased.

The experiments reported in this paper, and similar work of Woodbury(11) demonstrate

TABLE II. Influence of Cortisone on Uptake of $I^{131}$ in Hypophysectomized Rats.

| | No. of rats | % uptake of $I^{131}$ 24 hr after tracer, 4 $\mu$c | | Thyroid wt, mg |
|---|---|---|---|---|
| | | Total | Per mg thyroid wt | |
| Exp. 4 | | | | |
| 1. Control | 9 | 1.16 ± .13 | .12 | 9.9 |
| 2. Cortisone 5 mg/day* | 8 | .40 ± .06 | .04 | 9.8 |
| 3. TSH 1 " | 8 | 11.42 ± .64 | .96 | 12.4 |
| 4. Cortisone 5 " * | 9 | 9.16 ± .28 | .88 | 10.4 |
| TSH 1 " | | | | |
| Diff. 3-4 | | 2.26 | .08 | |
| | | p = <.01 | p >.8 | |
| Exp. 5 | | | | |
| 1. Control | 9 | .69 ± .08 | .06 | 11.3 |
| 2. Cortisone 5 mg/day* | 10 | .96 ± .25 | .08 | 11.8 |
| 3. TSH 1 " | 10 | 19.92 ± .81 | 1.48 | 13.7 |
| 4. Cortisone 5 " * | 11 | 14.79 ± 1.08 | 1.17 | 13.5 |
| TSH 1 " | | | | |
| Diff. 3-4 | | 5.13 | .28 | |
| | | p = <.01 | p <.2 | |

* In 2 divided doses.

that uptake of $I^{131}$ by the TSH-stimulated thyroid is diminished by administration of cortisone or ACTH to hypophysectomized animals: this phenomenon, therefore, cannot be the result of diminished secretion of TSH; obviously endogenous or exogenous cortical hormone inhibits the thyroid-stimulating action of TSH, as measured by uptake of $I^{131}$, at extrapituitary levels, perhaps at the thyroid gland level.

In man, clinical and metabolic manifestations of hypothyroidism have been observed after prolonged treatment with cortisone(1,2, 5,15). It is obvious that in such cases not only the uptake of iodine, but also the release of thyroid hormone must be inhibited. The inhibition of $I^{131}$ uptake by cortical hormone, acting at the thyroid level, may be followed, after prolonged administration, by diminished secretion of thyroid hormone. It is possible, too, that the cortical hormone may exert a dual action, directly at extrapituitary levels, and later also through suppression of TSH production. The various reported observations could be reconciled by either of these hypothetical assumptions.

*Summary.* Administration of cortisone or ACTH decreases the TSH-induced uptake of $I^{131}$ by the thyroid of hypophysectomized rats. This indicates that the suppression of the iodine concentrating function of the thyroid is brought about at extrapituitary levels, and not by suppression of TSH secretion from the pituitary. Some discrepancies of reported effects of adrenal hormones upon thyroid function are discussed.

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## Nutrition of *Leptospira canicola*. III. Utilization of Vitamins and Amino Acids.* (20021)

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The present investigation is an extension of the authors' studies on the nutritional requirements of leptospira(1-4). A chemically-defined basal medium developed earlier was shown to be satisfactory for the cultivation of the spirochete when supplemented with dialyzed rabbit serum(3) or an electrophoretically-homogeneous rabbit serum albumin(4). Related studies on the utilization of amino acids and vitamins by pathogenic leptospira have been reported by other workers(5-11).

*Experimental.* The response of *Leptospira canicola*‡ to individual amino acids was determined by replacing the amino acid mixture in the previously described basal medium supplemented with rabbit serum albumin(4), with each of 22 amino acids in turn at 0, 10, 20, 40, 60, 80, 100, 150, 200 and 500 mg % concentrations. Seven replicate tubes were prepared at each concentration. The pH of the medium was 7.2-7.4. 0.3 ml of a suspension of *L. canicola*, prepared as described earlier(4), was added to each of 5 tubes with 2 tubes serving as controls. The final volume was 6 ml and the tubes were incubated for 7 days at 32°. Growth was measured as increase in optical density of the cell suspensions determined with the aid of a Klett-Summerson photoelectric colorimeter. In some experiments the inoculum was obtained directly from the 4th or 5th serial transfer of the organism grown on the medium (complete except for the omission of amino acids) in which growth had decreased progressively. The purpose was to determine whether or not the response of the spirochete had been affected by this treatment. Subsequently, an amino acid mixture simulating the composition of rabbit serum albumin was employed in an attempt to replace the albumin and the amino acid mixture of arbitrary composition used initially. The composition (Table I) of the electrophoretically-homogeneous rabbit albumin was determined by assaying for alanine by the method of Sauberlich and Baumann(12) and the 16 other amino acids by the procedures of Dunn *et al.*(13,14). All assays were performed in triplicate using 5 levels of sample and 15 levels of standard. The maximum deviation of the values at the

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