time of occurrence of the "digitalis effect" in the duck hearts was dependent upon the amount of digitoxin added. Thus, standards (Table I) were obtained. Such standards were found to be necessary because of the significant but relatively fixed loss of digitoxin in the extraction of such large quantities of urine.

For the assay of urinary excretion of digitoxin, control urine samples (200 ml) were obtained from 4 young males and one female subject (average age 32 years). These were extracted and tested on the duck hearts as described above and found to produce no effect. Each subject then was given 1.2 mg of digitoxin by mouth within a period of 6 hours. Urine collections subsequently were obtained. The daily volumes were measured, and then 2 separate 200 ml samples of each were extracted and assayed on 6-8 duck hearts. The average time of occurrence of the "digitalis effect" of the 2 samples was compared to the standard values. In this manner the amount of digitoxin originally present in a 200 ml quantity of urine could be approximated. The total daily excretion then was calculated by multiplying the amount of digitoxin in the 200 ml quantity of urine, by the total daily urine volume/200.

Results. Each of the 5 subjects (Table II) excreted greatly varying amounts of digitoxin in his urine after the oral ingestion of 1.2 mg of the drug. During the first 24 hours after ingestion an average of approximately 87 μg (Range: 44-144) or 7% of the administered dose could be detected in the urine. The second day's urine of each subject contained somewhat less digitoxin (average: 55 μg). The third day's urine of 4 subjects tested contained appreciably less digitoxin (average: 21 μg). These results indicate that approximately 14% of the amount of digitoxin administered to these 5 subjects could be recovered in the urine collected the first 3 days after its administration.

Summary. The quantitative detection of orally administered digitoxin in the urine of subjects receiving the drug has been accomplished. Approximately 14% of the given dose is excreted in the urine of normal subjects during the first 72 hours after its oral ingestion.

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Adrenotrophic Activity of Human Urine.* (17471)

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The ability to determine adrenocorticotrophic hormone in body fluids of man would be a valuable aid in extending our knowledge of pituitary-adrenal physiology. Although there have been previous reports dealing with the presence of adrenotrophin in urine, the results (summarized in Table I) are conflicting. Part of this confusion arises from the failure of some of the investigators to use hypophysectomized test animals, an omission which unfortunately invalidates their conclusions. The remaining confusion apparently cannot be so explained. For example, Jones and Bucher,[3] using a specific assay method, found no adrenotrophic activity in 200 to 440 ml of urine of normal persons, while Reiss and associates[5] and Williamson[6] have reported the

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1 deBoissezon, P., *Bull. d'histol. appliq. a la physiol.*, 1936, **13**, 129.

TABLE I.
Tests for Adrenotrophic Activity in Human Urine.

Reference	Source	Preparation	Total vol. per animal, ml	Assay method	Adrenotrophic activity
DeBoissezon[1] (1936)	Pregnancy	Untreated	16	Incr. in adrenal wt and cholesterol in intact guinea pig	Yes
Paschkis, Rakoff, and Cantarow[2] (1942)	Normal	70-86% ethyl alcohol precipitate	Not stated	Incr. in adrenal wt in intact 2-day-old chicks and 4-day-old rats	Yes
Jones and Bucher[3] (1943)	Normal	Fractional ammonium sulfate precipitates	200–440	Increase in adrenal wt of hypophysectomized rat	No
Blumenthal[4] (1945)	Pregnancy, normal female	Untreated, fresh and preserved	4–16	Incr. mitosis in adrenals of intact guinea pig	Yes
Reiss, Peglar, and Golla[5] (1946)	Pregnancy	1. Untreated 2. Benzoic acid adsorbed 3. Picric acid ppt.	Not stated Not stated 25	Disappearance of sudanophobe zone in adrenal of hypophysectomized rat	1. No 2. No 3. Yes
Williamson[6] (1946)	Normal female	1. Untreated 2. Dialyzed 3. Dialyzed, acetone precipitated 4. Dialyzed pervaporated and acetone precipitated	4 4 120–460 30	Incr. in adrenal wt in intact rats Ascorbic acid depletion of adrenals of hypophysectomized rat	1. Yes 2. Yes 3. Yes 4. Yes
Cooke, Graetzer, and Reiss[7] (1948)	Normal	Permutit adsorbed	250	Ascorbic acid depletion of adrenals of hypophysectomized rat	Yes

presence of adrenotrophic activity in about 25 ml of urine of normal and pregnant humans. Since our work was completed, Cooke and associates[7] have reported positive reactions for adrenotrophin in concentrates representing 250 ml of normal urine, prepared by an adsorption technic.

Because of these conflicting results and because of some claims that the presence of adrenotrophin in body fluids in Cushing's syndrome is of etiologic significance, we have reinvestigated this problem using several different methods of concentrating the urine, and have placed particular emphasis on the study of urine from such pathologic states as may conceivably be associated with extremes of adrenotrophic activity. Although in general, our results have been negative, we are reporting them briefly, since they may be of some interest and of some use to others interested in the clinical-laboratory aspects of pituitary-adrenal disorders.

Material and methods. Adrenotrophic activity was determined by the ascorbic acid depletion method described by Sayers, Sayers

2 Paschkis, K. E., Rakoff, A. E., and Cantarow, A., *Endocrinology*, 1942, **30**, 523.

3 Jones, G. E. S., and Bucher, N. L. R., *Endocrinology*, 1943, **32**, 46.

4 Blumenthal, H. T., *J. Lab. and Clin. Med.*, 1945, **30**, 428.

5 Reiss, Max, Peglar, Harold, and Golla, Y. M. L., *Nature*, 1946, **157**, 413.

6 Williamson, M. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 191.

7 Cooke, D. S., Graetzer, E., and Reiss, M., *J. Endocrinol.*, 1948, **5**, lxxxix.

and Woodbury[8] modified by: (1) the use of hypophysectomized male rats of the Wistar strain rather than the Sprague-Dawley, and (2) the injection of test material into the jugular vein or peritoneum, rather than the tail vein. In most instances 5 rats were used for each sample of urine. Ascorbic acid concentration of the rat adrenal glands was determined according to the procedure described by Roe and Kuether,[9] adapted for use with tissue. The solutions were read in a Coleman, Jr. spectrophotometer at 510 λ and the adrenotrophic activity was expressed in terms of the reduction of ascorbic acid concentration (milligrams per 100 g fresh tissue) in the right gland, removed one hour after injection, as compared to the left gland, removed prior to the administration of the urine preparation. This calculation is subsequently designated as Left—Right, or L—R.

Urine was obtained from a variety of normal and abnormal subjects and prepared by one of the following methods: (1) fresh unconcentrated urine, or fresh dialyzed urine; (2) urine dialyzed and pervaporated to one-fifth volume, after which it was precipitated by means of acetone (Williamson,[6]); (3) ultrafiltration of urine through a collodion membrane at 60 to 80 lb pressure, after which the membrane was dissolved and the residue was extracted with 0.1 N sodium hydroxide and neutralized (Gorbman[10]); and (4) urine precipitated by 4 volumes of acetone at pH 3.5 and the precipitate extracted with 0.1 N sodium hydroxide, neutralized and dialyzed (Frank and associates[11]).

In order to obtain control values for comparison with experimental values, solutions containing no adrenotrophic activity (0.9% saline solution; distilled water) were administered to 25 hypophysectomized rats in volumes of 1 to 10 ml according to the procedure described previously. The mean concentration of ascorbic acid in the right gland was found to be higher than the left by 4 ± 3.2 mg, or a mean L—R of −4 ± 3.2. These results are much more variable than those reported by Sayers and associates[8] which indicate no difference in ascorbic acid concentration between left and right glands.

Ordinarily the urine or urinary concentrate was administered to 5 rats, but owing to toxicity not all the animals survived each experiment. Only those results in which 2 or more animals survived so that a mean value could be determined are included. Because of the variability of the animals, already mentioned, no mean L—R value less than 25 mg was considered significantly different from the controls, this value representing the minimal level for a test of significance between means. That toxicity of the concentrate did not impair the action of adrenotrophin was assured by dissolving pure adrenotrophin in the concentrate and observing the response as compared with the same amount of hormone in saline. Under these conditions, no difference in the L—R values was observed.

Results. Fresh, unconcentrated or dialyzed urine. Williamson[6] reported that 4 ml of fresh urine possessed adrenotrophic activity as measured by the increase in adrenal weight in the intact rat. Urines obtained from 3 normal persons, one patient with Cushing's syndrome and one patient with somatic and sexual precocity were inactive when tested in hypophysectomized rats in amounts of from 2 to 10 ml of urine per rat. Thus, the results obtained with intact rats would appear to be nonspecific responses.

Dialysis and pervaporation method of Williamson. Fresh mixed female urine prepared by dialysis, pervaporation and acetone precipitation was reported by Williamson to induce an L—R value of 145 mg when administered in an amount of 30 ml equivalent of urine. This method was repeated using from 14 to 88 ml equivalent of urine with entirely negative results.

Ultrafiltration method. The method described by Gorbman[10] for the recovery of urinary gonadotrophin was tested to determine whether adrenotrophin, if present, could also

[8] Sayers, M. A., Sayers, George, and Woodbury, L. A., *Endocrinology*, 1948, **42**, 379.

[9] Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.

[10] Gorbman, Aubrey, *Endocrinology*, 1945, **37**, 177.

[11] Frank, R. T., Salmon, U. J., and Friedman, Reuben, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

TABLE II.
Assays for Adrenotrophic Activity in the Urine of Various Endocrinopathies.

No. of patients	Diagnosis	No. of assays	Adrenotrophic activity		
			Positive (L—R >25 mg)	Doubtful (L—R = 20-25 mg)	Negative (L—R <20 mg)
4	Cushing's syndrome, Adrenal cortical hyperplasia	9	1	3	5
2	Hypopituitarism	2	0	1	1
1	Addison's disease	1	0	0	1
2	Sexual and somatic precocity	2	1	0	1
1	Natural menopause	1	1	0	0
3	Surgical menopause	3	1	0	2
1	Ovarian dwarfism	1	0	0	1
1	Pregnancy 5 mo.	1	0	1	0
1	Pituitary tumor	1	1	0	0

be recovered. Concentrates equivalent to 15% of the total 24-hour specimen, obtained from menopausal and pregnant women, states in which urinary adrenotrophin had been previously detected, gave negative results.

Acetone precipitates of urine. The precipitation of urine by means of acetone and the extraction of the precipitate yield fairly good recoveries of urinary gonadotrophin (Frank and associates[11]). This method was investigated extensively for adrenotrophic activity. Twenty-four-hour urine specimens were collected from 7 normal subjects (4 females and 3 males) and 10 tests were carried out. Concentrates ranging from 2.5 to 23% of the total 24-hour specimen were injected per rat. Concentrates equivalent to 2.5 to 10% of the total specimen gave negative results. However, the equivalent of from 10 to 23% of the specimen per rat yielded positive L—R values (more than 25 mg) in 2 instances, borderline responses (L—R of 20 to 25 mg) in 2 instances, and negative responses in 2 instances. Thus, while far from consistent, these results indicate at least that an amount equivalent to 10% or more of the 24-hour specimen was necessary to induce positive responses.

Since adrenotrophic activity could not be demonstrated consistently in normal urine, tests were performed on urines which might conceivably be richer in adrenotrophin, that is, in certain endocrine disorders. Among the endocrinopathies which might be associated with extremes of adrenotrophic activity, we tested the urines of patients with Cushing's syndrome, hypopituitarism, Addison's disease, in the menopause, and with a few miscellaneous disorders (pituitary tumor, sexual and somatic precocity and ovarian dwarfism). All tests were performed with amounts of urine representing 10% or more of the entire 24-hour specimen. The results of these assays are summarized in Table II.

Comment. The attempts to demonstrate adrenotrophic activity in human urine of both normal and pathologic states have been generally unsuccessful. We have been unable to confirm the presence of adrenotrophin with consistency, although occasional specimens have indeed given positive results. While it may be possible that adrenotrophin is either not excreted in the urine or excreted in biologically inactive form, the few occasional positive results encountered by us as well as by other investigators would seem to overrule this possibility. Another possibility is that adrenotrophin is excreted in biologically

active form but as a degraded part of the protein molecule. Such a possibility might explain why most of the methods used, designed to concentrate the urinary protein, do not yield adrenotrophic activity with consistency.

Assuming that adrenotrophin is excreted in human urine, the problem of its detection might be resolved in two ways. The first approach would be to increase greatly the sensitivity of the bioassay procedure so as to permit the use of fresh urine. Were it possible to obtain positive results with a few milliliters of fresh urine, it would then be relatively simple to work out systematically a technic for the concentration and purification of the hormone from urine. However, since the present Sayers' assay procedure is already extremely sensitive (in our hands a minimum of 0.8 μg of pure adrenotrophin was capable of inducing significant L—R values) it seems quite unlikely that any new assay method sufficiently sensitive to permit the use of fresh urine will be soon available.

The alternative approach would be to continue the empirical attempts to purify and concentrate the urine in the hope that a method will be found to permit the administration of large volumes of urine without toxicity to the test animals or appreciable loss of adrenotrophic activity. As such, the method described by Cooke and associates may represent a solution.

Summary. Using a specific and sensitive assay procedure, we have tested the urine of normal persons and of patients having a variety of disease states for its adrenotrophic activity. Tests using both fresh urine and urine concentrates prepared in three different ways were carried out. Contrary to previous reports, no adrenotrophic activity could be consistently demonstrated.

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Plasma Levels of Radioactive Iodine (I^{131}) in Human Tracer Studies. (17472)

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The purpose of the studies reported here was to examine the post ingestion plasma radioiodine curves in euthyroid, hypothyroid, and hyperthyroid subjects as a first step toward analysis of the various factors regulating the levels of such plasma iodine curves.

Methods. Patients were selected after previous clinical and laboratory study had established the presence of a euthyroid, hypothyroid or hypothyroid state. All were in an untreated state. They were given 100 microcuries of radioiodine by mouth before breakfast. The dosage was calculated on the basis of the shipping tag from Oakridge Laboratories. No carrier iodine was added. Each dose was given with 50 cc of distilled water and followed by two 50 cc portions of water to wash the container and mouths of the subjects. Blood specimens were drawn at 30 minutes, 1, 3, 6, 12, 24, and 48 hours; the blood was oxalated and centrifuged at 2500 rpm for 10 minutes. Then 0.2 cc of plasma was pipetted into shallow cups containing one drop of phenolphthalein, 0.2 cc of 0.75 N NaOH and 0.2 cc of detergent (Orvus). Duplicate samples were dried under an infra-red lamp and counted with a bell type Geiger Müller tube (Cyclotron Specialties Co., Model 410-A) and counter (Technical Associates Model GS4). Duplicate specimens of reagents alone, exclusive of plasma, were checked for radio-contamination The counts were corrected for decay with reference to the day of ingestion. Eleven cases of untreated myxedema and 14 non-myxedematous cases (5 hyperthyroid and 9 euthyroid) were so studied.

Results. When the data for the entire 25