solution described above to dryness the lysozyme was found to be insoluble in water. This may be taken as an indication of denaturation. While a marked serological change follows denaturation it does not necessarily destroy antigenicity. More study of the antigenic properties of lysozyme seems indicated.

The presence of lysozyme in secretions and tissues[7] suggests a possible precipitating effect on insulin *in vivo*. The solubility of the complex in serum is sufficiently great, however, to make this unlikely.

Summary. Crystalline egg lysozyme precipitates insulin. The complex produces a prompt lowering of the blood-sugar level in rabbits. Precipitates formed by mixing insulin with lysozyme which has been heated, or treated photodynamically so that 90 to 100% of its lytic activity on *M. lysodeikticus* is destroyed, but about 60% of its precipitating power for insulin is left, produce a hypoglycemic response which, up to 7 hours after injection, is intermediate between that resulting from the injection of insulin in solution and that following administration of protamine-zinc-insulin.

I wish to record my appreciation to Mr. E. K. Wolfe of the Biochemical Control Department of Sharp and Dohme, Inc., Glenolden, Pa., for performing the animal experiments.

[7] Fleming, A., and Allison, V. D., *Brit. J. Exp. Path.*, 1922, **3**, 252.

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Renal Excretion of Digitoxin in Man Following Oral Administration.* (17470)

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The amount of digitoxin excreted in the urine of human subjects receiving the drug has not been determined, primarily because of previous inability to measure minute amounts of any type of digitalis glycoside in biological media. However, the discovered sensitivity of the embryonic duck heart preparation[1] to minute amounts of digitoxin when present in Tyrode's solution and human serum offered the possibility that this method also could be used for the quantitative detection of digitoxin excreted in the urine of patients receiving the drug. The preliminary results of this study are reported herein.

Methods. A human urine sample of 200 ml was boiled down to approximately 50 ml on a hot plate and thereafter more slowly evaporated until the volume was reduced to

TABLE I.
Relationship Between Initial Concentration of Digitoxin in Human Urine (200 ml) and Time of Occurrence of "Digitalis Effect" in Final Extract.

Amt. dig. added (μg)	No. of extracts assayed	Avg time, "dig. effect" (min.)	S.E. of mean (min.)
1	5	—	—
2	12	47	2.28
3	10	35	1.82
4	11	27	1.45
6	9	22	1.14
8	10	17	0.73
10	5	14	0.35
12	6	11	0.20

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5 ml. Enough diatomaceous earth (5-7 g) was added to this concentrated urine sample to form a friable powder which was dried at 75-80°C *in vacuo* (7-10 in.) for 16 hours.

The dry powder was transferred to a Waring Blender, 35 ml of $CHCl_3$ (U.S.P. grade) added, and the mixture stirred for one minute. The mixture was transferred to an Erlenmeyer flask; then the blender was rinsed with 15 ml of $CHCl_3$, which also was poured into the Erlenmeyer flask. The flask was stoppered with a cork covered with aluminum foil, and the mixture shaken for an hour, then filtered. The residue was washed 3 times with 15 ml portions of $CHCl_3$, pressing out excess solvent each time, and the combined filtrate and washings evaporated on the hot plate to a volume of 1 ml. This last quantity was quantitatively transferred to a 25 ml Erlenmeyer flask and evaporated to dryness by heating in the vacuum oven for one half hour. The dry residue was taken up in one ml of 95% ethanol by shaking for one hour, the alcoholic solution decanted to a clean Erlenmeyer flask, the original flask washed with one ml of alcohol, and the solution together with the ethanol wash dried in a vacuum oven for 30 minutes. Five ml of $CHCl_3$ were added to the dry residue, boiled down to one ml, allowed to stand for 30 minutes, decanted into a fresh Erlenmeyer flask, the original flask washed with $CHCl_3$, and the combined solvent evaporated to complete dryness in vacuum oven. Fifty ml of Tyrode's solution were added to the final residue and the flask shaken for one hour. The isolated embryonic duck heart was found to beat in this final solution as regularly and as normally as it did in pure Tyrode's solution.

Known amounts (1-12 μg) of pure digitoxin (Sandoz) then were added to 200 ml quantities of human urine and the urines were extracted as above. It was found that the final extract produced a "digitalis effect"[2] if two or more micrograms of digitoxin were added to the original 200 ml of urine. Moreover, the

TABLE II.
Urinary Excretion of Digitoxin in Normal Human Subjects.

		First 24 hr			Second 24 hr			Third 24 hr		
Age	U.V. (ml)	Time of "dig. effect" (min.)†	Tot. amt. dig. exer. (μg)	U.V. (ml)	Time of "dig. effect" (min.)†	Tot. amt. dig. exer. (μg)	U.V. (ml)	Time of "dig. effect" (min.)†	Tot. amt. dig. exer. (μg)
39	1450	21(6)	44	1500	19(8)	60	1700	13(10)	85
31	1720	14(10)	86	2250	24(6)	68	940	19(8)	38
35	2000	20(6)	60	1210	14(10)	60	970	26(4)	19
30	2470	10(12)	144	1050	23(6)	30	520	46(2)	5
24*	1700	12(12)	102	1930	20(6)	58	760	23(6)	23
Avg 32	1868	15	87	1588	20	55	978	26	34

* Female.
† Figures in parentheses represent calculated amount of digitoxin (μg) in original 200 ml quantity of urine.

[1] Bine, R., Jr., and Friedman, M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 487.

[2] Friedman, M., and Bine, R., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 533.

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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† Deceased.

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