TABLE I.
Antistreptolysin Titers in Immunized Rabbits.

	Streptolysin O alone		Streptolysin O + cholesterol		Streptolysin O + antistreptolysin O	
Rabbit	A_1	A_2	B_1	B_2	C_1	C_2
Before immunization	<1	<1	<1	<1	<1	<1
After first course	60	50	50	20	<1	<1
After second course	50	16	30	20	1	2

respective groups. After 4 days this dosage was doubled and after a further 4 days, 4 times the original dosage was given. After an 18 day rest period a second series of injections were given and bleedings taken as before. This consisted of 3 injections at 4 day intervals of the original doses. Antistreptolysin titres of all the sera were determined by the method outlined by Todd. All values are recorded with reference to the standard serum.

Results. Table I shows the antistreptolysin titres of the rabbits before and after each course of injections. It will be seen that the titres of antistreptolysin produced by the inoculation of streptolysin-cholesterol mixtures do not differ significantly from those produced by streptolysin alone. The inoculation of streptolysin-antistreptolysin mixtures produced no change in the antistreptolysin titre whatever after the first course of injections and very little after the second.

Discussion. The results indicate that the antigenicity of streptolysin O is not impaired when its hemolytic activity is inhibited by cholesterol. The explanation offered is that some easily dissociated streptolysin-cholesterol complex is formed which loses its ability to lyse red cells but still functions as an antigen. The chemical grouping by which cholesterol combines with streptolysin is a matter for further study although there is evidence which implicates certain regions on the cholesterol molecule(8). Hewitt and Todd point out that sulfhydryl groups added in the form of cysteine did not effect the neutralization of streptolysin O by cholesterol. We have found that oxidized, inactive streptolysin O is neutralized in the same way as the active reduced form. The addition of -SH containing reducing agents to mixtures of oxidized, inactive streptolysin O and cholesterol failed to restore any hemolytic activity except to tubes containing less than a neutralizing dose of cholesterol.

Summary. 1. The observation of Hewitt and Todd that cholesterol neutralizes the hemolytic activity of streptolysin O is confirmed.

2. The antigenicity of streptolysin O remains unchanged in cholesterol-neutralized mixtures.

3. Under similar conditions streptolysin O neutralized by its specific antibody elicits virtually no immune response to streptolysin O.

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Bioassay of Desoxycorticosterone-like Material in Urine.* (17614)

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Many explanations have been offered for the retention of sodium which is characteristic of the various edematous states. Injury,

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ischemia(1), or congestion(2) of the kidney can reduce sodium excretion, while edema may also result from injury of capillaries or an alteration in hydrostatic-osmotic balance. The difficulty in explaining fully the presence and extent of edema by measurements of renal function, colloid osmotic pressure and venous pressure, has led to the suggestion that hormonal effects on the kidney might be important. Evidence of increased adrenal function in the edema of heart failure and of preeclampsia has been reported, but its relation to sodium retention is not clear(3,4,). In this communication, a method for estimation of sodium-retaining activity of urinary extracts is described, and some preliminary studies in edematous patients are reported.

Bio-assay method. Female adrenalectomized rats weighing 150 g are maintained for 3-4 days after operation on stock diet and given physiological saline to drink. On the day before the test, a sodium-free diet and distilled water are substituted, and each rat is given 5 cc of physiological saline intraperitoneally, which is repeated each evening during the test period. On the morning of the test, each rat is given 5 cc of distilled water intraperitoneally. Water intoxication is not seen, and the assay is more sensitive than when physiological saline is given. Urine is collected for 5 hours in individual metabolism cages. Urinary volume is recorded and total sodium excretion is measured with a flame photometer.

The first day serves as a control for subsequent test materials, which are injected 30 minutes before the beginning of the collection period. Rats with a sodium output below 30 μ eq on the control day are eliminated. If a rat is cold or lethargic at the end of a test, or if urinary output falls below 0.3 cc, the rat is discarded. The ratio of sodium excretion on the control day to that on the test day is used as a measure of activity of injected material. Repeated control tests on the second or third day in 5 groups of rats have given ratios of 0.7 to 1.2, averaging 1.0. Individual variations of response make it desirable to use groups of at least 5, and preferably 10 rats. Factors are averaged logarithmically.

This method follows the principles of Dorfman's(5) method, which we found inconvenient because of certain problems of supply, variable specific activity, and measurement of radioactive sodium, as well as because of variations from day to day due to the uncontrolled sodium intake.

Response to Crystalline Hormones. When a single rat group is given increasing doses of DOCA on succeeding days (Table I), a consistently increasing retention of sodium is elicited. However, the response to a standard dose of DOC or DOCA, as shown, varies between different rat groups. It is evident from the data that in order to obtain an accurate assay, a known dose of DOCA would have to be given to each group of rats.† Roughly, a factor greater than 2 may be expected with dosage of the order of 5 γ per rat. The factor of sodium retention increases with dosage, but a dosage of about 25 γ produces a maximum response. Estradiol, progesterone and testosterone have been assayed by this technic. In order to obtain a response comparable to the response to small doses of DOCA, the dose of these sex hormones must be 25 to 50 times greater. The order of effectiveness of these compounds (Table I) differs from that shown by Thorn and Engel(6) in male dogs.

Extraction and vehicles. Urine is acidified (pH 1.0) with HCl and extracted with chloroform. The extract is dried over sodium sulfate and evaporated to dryness *in vacuo* at 40°C. The residue is dissolved in ethyl alcohol, taken again to dryness, and redissolved in the proper volume for injection. The choice

1. Merrill, A. J., *Am. J. Med.*, 1949, v6, 357.
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3. Parrish, A. E., *J. Clin. Invest.*, 1949, v28, 45.
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5. Dorfman, R. I., Potts, A. M., and Feil, M. L., *Endocrinol.*, 1947, v41, 464.

† A. G. Spencer (personal communication) has independently developed a similar method of assay of DOCA, using adrenalectomized mice, in which the control of each group with a known dose of DOCA has minimized the errors due to variation between groups.

6. Thorn, G. W., and Engel, L., *J. Exp. Med.*, 1938, v68, 299.

TABLE I.
Effect of Certain Steroids on Urinary Sodium Excretion of Adrenalectomized Rats.

Material injected	Dose, γ	Rats, No.	Sodium retention factor
Controls	—	5 groups of 5 rats	0.7-1.2 (mean 1.0)
Desoxycorticosterone	0.2	11	1.2
	0.5	4	1.0
	1	8	0.8
	1	5	0.5
	10	6	5.6
	25	5	4.5
Desoxycorticosterone acetate	2	9	1.7 a
	5	9	3.0 a
	10	9	3.1 b
	10	5	2.0 c
	25	9	4.3 b
	25	5	3.0 c
	50	9	4.2 a
	100	9	3.9 b
Estradiol	10	9	1.0 d
	50	9	1.3 d
	250	9	1.5 d
Progesterone	10	9	1.4 e
	50	9	1.6 e
	250	9	2.5 e
Testosterone	10	9	1.2 f
	50	9	1.5 f
	250	8	2.9 f

Factors followed by the same letter were obtained from the same group of rats.

of vehicle for injection is important. Peanut and sesame oils are poor solvents, and may retain enough chloroform to cause renal damage. Glycerol and propylene glycol are viscous and poor solvents, which in mixtures with alcohol cause sodium retention in doses used. Carbowax 1500 and 4000 cause renal injury and sodium retention. Carbitol did not lead to sodium retention, but at autopsy the kidneys were swollen and mottled. Ethanol and polyethylene glycol are effective solvents and cause no sodium retention. Ethanol has been selected for routine use because it is a less viscous, better solvent, and because a similar dose of DOCA produced a greater sodium retention under the experimental conditions when dissolved in ethanol than when dissolved in polyethylene glycol.

Urine extracts. Urine is collected as a 24-hour specimen to avoid diurnal variations(7), suggested by case 12 Table II. The dose is expressed as the number of minutes of urinary output represented by the extract administered to each rat. In 12 determinations on normal individuals and pools, the average factor is 1.25, with a range of 0.7 to 1.6 (Table II). In 10 determinations on nonedematous patients in the hospital, the average factor is 1.4, with a range of 0.7 to 2.1. An interesting feature is the failure of the factor to increase with increasing dosage. A maximum effect seems to be reached at about the 20-minute dose. In several instances, the decreasing response to increasing dosage suggests the presence of an interfering factor. Of the 5 cases with congestive heart failure, 4 cases gave results well above the normal range at the 20-minute dose, while the fifth case gave an unusually high result at a 40-minute dose. The extract from one of these patients after a mercury-induced diuresis was much less active. The patient with constrictive pericarditis showed no increase above the normal level. The extracts from 3 patients with

7. Romanoff, L. P., Plager, J., and Pincus, G., *Endocrinol.*, 1949, v45, 10.

TABLE II.
Effect of Extracts of Human Urine on Urinary Sodium Excretion of Adrenalectomized Rats.

Group Case No. (Sex)	Description	Time dose (min.)	No. of rats	Sodium retention factor	Patient's urinary sodium (m.eq./24 hr)
Normals					
Pools		20	5	1.3*	
		20	5	0.7	
		20	5	1.4	
		120	4	1.3	
1 (M)		20	9	1.2	164
		40	10	1.2	
		120	6	0.9	
2 (M)		5	4	1.3	185
		20	9	1.6	
		40	10	1.5	
		120	8	1.1	
3 (F)	Immediately pre-catamenia	20	9	1.4	103
Cardiacs					
1 (M)	Arteriosclerotic heart disease in failure	20	5	1.7	11.5
		40	4	3.1	
2 (M)	Syphilitic aortic insufficiency in failure	20	4	2.1	2.1
3 (F)	Mitral stenosis, fibrillation, in failure	20	14	3.4	1.4
4 (M)	Tachycardia with decompensation	20	6	3.4	10
4 (M)	After mercurial diuresis	20	4	1.3	113
16 (M)	Arteriosclerotic heart disease, fibrillation, in failure	20	10	7.1	9 (Serum 127)
5 (F)	Constrictive pericarditis with edema	20	4	1.3	8
Nephrotic					
6 (M)	Edema-free	20	6	1.0	65
		60	5	1.3	
		120	6	0.6	
7 (M)	Moderate edema	20	15	1.6	1
		60	6	0.7	
		120	5	1.2	
8 (F)	Severe anasarca	20	9	6.5	0-1 (~~Serum 124~~)
Miscellaneous					
9 (M)	Acute glomerular nephritis, during diuresis	20	15	1.0	44
		60	4	1.2	
10 (F)	Probable lupus	20	10	0.7	189
		120	7	0.8	
11 (F)	Thyrotoxicosis	20	8	1.6	177
		110	9	1.7	
12 (M)	Hemophilia—daytime	20	8	1.6	76
12 (M)	nighttime	20	9	1.3	71
13 (F)	Rheumatoid arthritis (pre-treatment)	20	10	1.9	57
13 (F)	After 3 days Δ-5-pregnenolone	20	8	2.7	70
14 (F)	One kidney. No edema. Midcycle	20	9	2.1	176
15 (M)	Addison's disease, receiving DOCA	20	8	1.7	103
		120	9	1.0	

* This extract administered in oil.

nephrosis varied from normal to nearly the highest activity observed in an order corresponding to the severity of their edema.

Discussion. It is obvious that the results with urine extracts cannot be interpreted at this stage. Fractionation of the extracts must be attempted in an effort to identify or characterize the active principle which appears to be present. By such a procedure it may also be possible to explain or eliminate the presently paradoxical time-dose response observed. The preliminary observations reported indicate the presence of sodium-retaining factors in the urine of some cardiac and

nephrotic patients in concentration greater than that observed in normal controls or in patients with other forms of disease.

Summary. An assay for desoxycorticosterone-like activity in biological fluids is presented, having a sensitivity in the order of 10 γ of DOCA. The critical nature of solvent vehicle choice and of time-dosage of urinary extracts is presented and discussed. Urinary extracts from some edematous patients with heart failure and nephrosis show a sodium-retaining activity greater than that observed in normals and in non-edematous controls.

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Autotransplantation of Adrenal Gland in the Spleen and Mesentery of Rats. (17615)

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Ovarian transplants in the spleen survive and serve as an excellent tool in elucidating the role of the liver in gonadal hormone metabolism(1). Transplants of the adrenal gland in the spleen have hitherto been unsuccessful(2,3).* This paper reports the successful autotransplantation of the adrenal gland in the spleen and mesentery of the rat, with evidence that such a transplant permits normal maturation of the animal.

Experiments. In one experiment, each of 3 male and 3 female immature rats of the Long-Evans strain, averaging 62 g in weight, was subjected to 2 operations. In the first procedure, either a half or the entire left adrenal gland was transplanted into the spleen, except in 2 animals, where the ileocolic mesentery was employed. The second procedure consisted of right adrenalectomy. In the 3 females, this was done 8 days after the first operation, and the animals drank normal saline instead of water for the subsequent 8 days. Right adrenalectomy was performed in the 3 males 16 days after the initial transplantation, and tap water was the only fluid provided. Food was supplied *ad libitum* throughout the experiment. After 108 days, the 6 animals were sacrificed. They now averaged 240 g in weight and appeared normally developed when autopsied. In 5 of these 6 animals, the transplants varied from 3 to 5 mm in diameter, and were sharply outlined. On microscopic examination, they were composed of cells of the adrenal cortex. One of the 4 intrasplenic transplants could not be traced; of the other 3, a typical example is shown in Fig. 1A, B. Both of the ileocolic mesenteric transplants were successful; one of these shown in Fig. 2A, B.

In another experiment, 6 Long-Evans, adult, female rats were bilaterally adrenalectomized, and at the same time one of the adrenals was transplanted into the spleen. Two of the animals were sacrificed after 50 days and the remainder after 75 days. There was an average weight gain of 30 g and all of the transplants were successful. The transplants after 50 days were 4 mm in diameter and from 2-4 mm after 75 days. In every case they were composed of cells of the adrenal

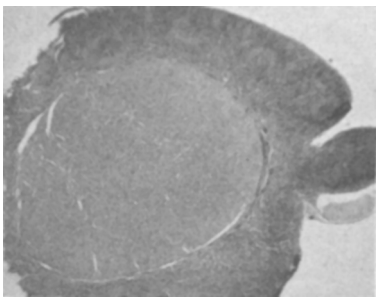

FIG. 1 A.
(S-3044 10× approximately) A 108-day-old transplant is well outlined in the splenic pulp.

1. Biskind, M. S., and Shelesnyak, M. C., *Endocrinology*, 1942, v30, 819.
2. Oldberg, E., *Am. J. Physiol.*, 1929, v91, 275.
3. Grieco, F., *Arch. Ital. di Anat.*, 1932, v3, 589.

* Addendum: An apology is offered to E. O. Butcher for oversight of his report in *Endocrinology*, 1948, v43, 30.