mechanism to keep osmotic pressure within certain limits(8). The rise in globulin has been attributed to (a) antibody formation, (b) alteration of relative rates of production and utilization of albumin and globulin, and (c) traumatic shock(8,9,10). Alpha-1 and alpha-2 globulin concentration did not vary more than 3-4 mg ml throughout the experiment. Changes in concentration for both alpha-globulins occurred within the 4-5 month infection period (Table I). The beta-globulin fraction exhibited very little change. Lack of variation in this fraction at 3-4 months, when significant alterations were taking place, could be due to the fact that as the gamma-globulin rose in concentration it tended to mask the increase, if any, of the beta-fraction in electrophoretic patterns.

Summary and conclusions. 1. New serum components were not detected in infected animals. 2. Significant increases in gamma-globulin fraction were observed during 3-4 month infection period. 3. The rise observed in gamma-globulin coincided with highest agglutinin titers in sera of infected animals during 12 months experimental period. 4. Absorption studies are being carried out in an effort to determine if the gamma-globulin rise is due mainly to antibody formation.

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Influence of Urine Volume on Output of Corticoids by Normal Human Subjects.* (24447)

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It has been noticed that the output of adrenal cortical steroids appeared to be dependent upon urine volume. This does not imply that the cortex was activated by changes in water balance, but rather that alteration in filtration and/or reabsorption in the kidney with urine flow influenced steroid output. Lloyd (1) has presented data to demonstrate this relationship in Addisonian and normal patients. Marks and Leaf(2), Kalant(3) and Lazo-Wasem *et al.*(4) have confirmed the results in dog, rat, and guinea pig. This is a report of the influence of urine volume on urinary steroid output in normal subjects under various conditions of salt and water load. As a corollary, renal clearances of steroids were also determined.

Methods. Our tests were performed on 24 normal medical students. Three different procedures were adopted to provide short and long term evaluation of the problem. 1. *Long term study (day by day).* Students on a relatively constant diet and in good health, collected 24 hour urine specimens kept in the cold and preserved with small amount of glacial acetic acid. The subjects voluntarily restricted water to a minimum, or drank water to excess, or maintained a normal water intake throughout the 24 hours. In another series of tests the subjects took 40 mg of hydrocortisone/day in a divided dose while controlling water intake. Each sample was

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TABLE I. Excretion of Total Corticoids in 24-Hour Urine Sample in Relation to Volume of Urine with and without Added Cortisone. A recovery period of 10 days occurred between each phase of the tests.

Condition of subject	No. of tests	Urine vol, ml/day	Steroid excreted, mg/day
Water deprivation	5	773 ± 80*	8.1 ± 1.2*
Water intake:			
Normal	8	1270 ± 130	9.3 ± 2.4
High	9	2430 ± 360	12.7 ± 2.2
Intake (40 mg cortisone):			
Low	3	750 ± 210	14.0 ± 2.8
Normal	3	1400 ± 360	34.0 ± 4.6
High	3	2130 ± 93	45.0 ± 1.2

* Stand. dev.

assayed for corticoids by the method of Reddy as modified by Brown(5). The method determines conjugated and free 17-hydroxycorticoids which represent about 85% of the daily adrenal output. 2. *Short term study.* Control levels of urinary steroids were determined after which each man rapidly drank 1 l of 1.4% NaCl, 0.9% NaCl, 0.54% NaCl, or tap water. Urine samples were collected at successive 20-minute intervals for the following 2 hours. Other subjects were given water to drink and after excretion of water reached high levels (about 40 minutes) 2 units of ADH (Pitressin, Parke-Davis) were given in the muscle. Urine samples were collected at 20-minute intervals. All samples were analyzed for steroid as outlined above. 3. *Renal clearances of corticoids.* Urine was collected over a 30-minute period in well hydrated subjects with good urine flow rates. Heparinized blood samples were taken at midpoint of each period. Urine and plasma were extracted with chloroform to obtain "free" steroids and with butanol to obtain "bound"† corticoids. The method of Reddy(7) was used to precipitate proteins in plasma before extraction. Duplicate portions of plasma and urine samples were analyzed for creatinine to determine the glomerular filtration rate (GFR). The method of Hare(6) was used to obtain preformed creatinine and standard picric acid method was utilized for color development.

Results. Definite correlation was found between urine volumes and adrenal corticcal steroid output in urine (Table I). Although differences in steroid excretion between low and normal water intake and between normal and high water intake were not significant, the trend was obvious and the difference between low and high water intake figures were highly significant.

A similar relationship could be shown (Table II) on a short time basis. An increase in urine output resulted in increased adrenal steroid output in urine regardless of salt intake. In those cases in which the change in

TABLE II. Relationship between Urine Volume and Steroid Output in Normal Subjects Drinking One Liter of Indicated Solution. Water excretion expressed as ml/20 min. period and steroid as μg/20 min. period. Each value represents average of 5 subjects.

	Water		.54% NaCl		.90% NaCl		1.40% NaCl		Water & ADH	
Period	Urine vol	Steroid output	Urine vol	Steroid output	Urine vol	Steroid output	Urine vol	Steroid output	Urine vol	Steroid output
1*	18	92	16	171	23	113	91	109	12	123
			All fluids taken after first control period							
2	70	168	31	236	44	97	44	106	34	146
3	198	186	54	291	46	75	24	127	106	302
									ADH given	
4	164	180	19	196	34	83	18	124	32	95
5	66	143	13	152	21	78	19	114	10	81
6	18	88	15	160	20	52	14	100	13	119

* Each successive period is consecutive 20 min. period with no time delay between periods.

† In urine the steroid is "bound" largely to glucuronide and may be liberated by enzymatic hydrolysis. The exact binding in plasma is not clear but presumably may be to protein as well as to glucuronide.

TABLE III. Plasma and Urinary Levels of Conjugated and Free Steroids and Clearances of These Fractions in Normal Subjects. Urine collections were made over 30 min. periods with blood samples at the mid-point. Clearances are expressed in the usual way: UV/P=C.

Subject	Creatinine clearance, ml/min.	Free steroid			Conjugated steroid		
		Urine, μg/ml	Plasma, μg/%	Clearance, ml/min.	Urine, μg/ml	Plasma, μg/%	Clearance, ml/min.
APR	92	.72	118	.01	7.5	168	4.78
HRB	71	.99	46	1.1	10.8	118	5.18
	82	1.0	46	1.8	9.5	110	7.00
EWH	80	.7	30	1.5	1.56	64	1.6
	83	1.6	100	1.9	2.8	160	2.1
EHE	86	.2	23	1.2	3.8	65	6.1
EHE*	96	6.3	350	9.8			
GHR	82				4.8	118	4.70
AVA	77				8.6	288	4.10

* 15 min. after infusion of 50 mg hydrocortisone.

urine output was not so marked as with 0.54% NaCl, changes in steroid excretion were also not marked. The greatest change was in subjects given ADH. Large water volumes and corticoid excretions were obtained upon hydration. When ADH was administered, a sudden drop in excretion of both corticosteroids and water occurred within a 20-minute period. It would be impossible to state whether any alteration in corticoid excretion *per se* is due to ADH acting directly on the adrenal gland rather than the kidney. Some workers have suggested that ADH may activate the adrenal, but this possibility would appear unlikely for all urine samples were analyzed for sodium with the flame photometer and total excretion per period did not show significant variations.

As a corollary of the corticoid excretion studies reported above, renal clearances were determined in normal subjects. Clearance of creatinine was used to measure glomerular filtration rate. Excretions of adrenal steroids in urine were similar to those reported in Table II; plasma level of "bound" corticoids averaged 58 to 128 μg % and "free" steroid averaged 13 to 46 μg %. The plasma ratio of free/bound corticoids was about 1:4. These values are similar to those reported by Reddy(7). Urinary ratios have been previously reported by Brown(5). Renal clearance data are presented in Table III. The clearances were very consistent with clearance of "bound steroid" higher in all cases (averaging about 4 ml/min.). These values indicate a greater reabsorption of the free molecule and a greater excretion of the bound form which would be predicted. Both forms must be reabsorbed to a considerable extent. The usual ratio of free/bound corticoids in urine is about 1:8, which confirms the greater excretion of the bound form. With a high degree of reabsorption by the kidney tubule, it is not surprising that great variation in urine flows would produce variation in amount reabsorbed and therefore in excretion of steroids.

These data indicate that there is a considerable influence of urine volume upon excretion of steroid, and point up the fact that this must be taken into account in evaluation of the steroid output in patients with great variation in urine volume.

Summary. The influence of urine volume upon excretion of corticoids has been determined in normal human subjects. There was a direct correlation with increases in urine volume resulting in an increase in steroid output whether measurement was made on a long time basis (day by day), or a short time basis (minute by minute). Subjects tested under waterloads and salt loads and with ADH also demonstrated the same positive correlation. As a corollary, clearances of steroids were measured and found to be about 1 ml/min. for free corticoids and about 4 ml/min. for bound steroids.

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Partial Purification and Properties of Lupus Erythematosus Cell Promoting Factor. (24448)

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Patients with disseminated lupus erythematosus (DLE) have a substance in their serum which is thought responsible for formation of the characteristic "lupus erythematosus cells." Previous attempts at isolation of this substance have indicated that it is associated with the gamma globulin fraction (1). A useful method for chromatographic fractionation of proteins using cellulose exchangers has recently been developed(2). The technic is carried out under conditions which favor preservation of the structure and biologic activity of the proteins recovered in the fractions. The wide range of applicability of this method is reflected in the reports appearing in the literature(3). A previously reported preliminary study(4) indicated that an anionic cellulose exchanger may effect some separation of the L-E factor. The purpose of this communication is to report on some of the properties of a preparation containing L-E activity by use of a cationic cellulose exchanger.

Methods. Sera obtained from normal individuals and from patients with DLE were stored in frozen state until ready for use. All fractionation procedures were carried out in cold room at 4°C. Isolation of serum gamma globulins was done by electrophoretic convection in 0.15M phosphate buffer at pH 7.4. Nitrogen determinations were done by micro-Kjeldahl technic and phosphorus values were obtained by Schmidt-Thannhauser technic (5). The resulting products contained little or no contaminating beta globulin as determined by moving boundary and paper electrophoresis. However, when the products were examined by agar diffusion(6) using rabbit anti-human gamma globulin as the developing agent, as many as 7 distinct antigenic components could be identified. The gamma globulin isolated from patients with DLE gave strongly positive reactions in the L-E cell preparations(7) and in the DNA-protein coated latex agglutination test(8). Serum samples and gamma globulin fractions from normals and subjects with DLE were prepared for column chromatography by preliminary dialysis against 0.05M phosphate buffer at pH 5.0. A small amount of insoluble material, usually present at end of 24 hours, was removed by centrifugation. When whole serum was to be chromatographed, the dialyzed sample was diluted 1:10 with the starting buffer before placing it on the column. Gamma globulin samples were placed on the column without preliminary dilution. Approximately 15 g of cationic exchange cellulose, CM-cellulose-SF,† were placed in 3 changes of 0.05M phosphate buffer at pH 5 for 48 hours. The slurry was placed in a 20 x 200 mm glass column and packed until a standard height with a flat crown had been achieved. Under these conditions, the flow rate varied from 60 to 80 ml/hour. The pH and salt concentration of the eluting buffers was increased simultaneously. The initial pH was 5 and final pH 7; initial salt concentration was 0.05M and final concentration 0.15M. Salt concentration was increased by step-wise addition of sodium chloride. Six ml samples

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