of the treatment and dosage of hormone. Extensive changes were noted after 21 days of hormone administration.

Summary. Rabbits were exposed to 650 r of radiation or were injected with 10 mg cortisol for 3 days. Two methods were used for fractionation of thymus from control and treated animals. Method I was based on sequential extraction with water and saline. Nuclear and cytoplasmic fractions of lymphocytes were prepared by Method II. Minced tissue was stirred in sucrose-$CaCl_2$ and residual material homogenized in saline. Method I does not distinguish cytoplasmic and nuclear components. The weights of all fractions except Fraction A were lower in experimental animals when data are expressed as g/100 g thymus. A large portion of Fraction A was found to be serum proteins as determined by the large increase in albumin in electrophoretic patterns. RNA was lower in most fractions from experimental animals. Electrophoretic analysis of the S_1 cytoplasmic fraction from lymphocytes obtained by Method II, again shows a higher weight fraction of albumin in the extract from treated animals. Decreases in fractions attributed to nuclear material were noted in treated animals. Nuclear fractions were attributed largely to lymphocytes. Saline extracts and final residue show little change following irradiation, indicating these fractions are from radioresistant elements. Both fractions are more sensitive to hormone treatment.

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Immunochemical Studies on Kidney Hypertrophy of the Rat. (27881)

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It has been the contention that in conditions of unilateral nephrectomy the hypertrophy of the remaining kidney occurs with no increase in the number of glomeruli as made by counting studies(1,2). This hypertrophy begins almost immediately after nephrectomy and some correlation with an increase in mitotic activity in the remaining kidney has been reported(3,4).

We have observed that in newborn animals treated with rabbit anti-rat kidney serum rapid localization of anti-kidney antibodies was seen in all glomeruli, by means of the indirect fluorescent antibody technic. Therefore, the antibody can be used as a marker for glomeruli. When animals treated in this manner are allowed to grow to 6 weeks before being sacrificed the newly formed glomeruli that have become active subsequent to the treatment are unmarked and hence can not be stained for the presence of the marker antibody. Only those glomeruli that were available to the antibody at time of treatment can be stained(5). Since every glomerulus can be marked by the antibody the procedure would be a sensitive way of

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TABLE I. Rats Treated with Rabbit Anti-Rat Kidney Serum.

	Rat No.	Wt at treatment	Wt of kidney removed	Wt of rat at sacrifice 7 mo later	Wt of kidney at sacrifice
Unilaterally nephrectomized	1	310	1.34	612	3.60
	2	313	1.10	566	2.70
	3	360	1.54	603	3.75
	4	308	1.27	473	3.50
	5	309	1.10	485	3.00
	6	406	1.48	662	5.00
Not nephrectomized	7	342	—	554	1.72
					1.78
	8	350	—	590	1.97
					2.23
	9	305	—	575	2.16
					2.04

determining whether any increase of new functional glomeruli occurs in unilaterally nephrectomized rat.

Methods. Nine 300-400 gram adult rats were injected with 2 ml of anti-rat kidney (anti-RK) serum and after 24 hours, unilateral nephrectomy was performed on 6 of these animals. The 3 remaining rats were not nephrectomized and held as controls.

The 6 kidneys that were removed were weighed and sections tested by the indirect fluorescent antibody technic for the presence of the marker antibody. Untreated and non-nephrectomized rats were also kept over a period of 1 year to compare body weight and kidney weight during this period of growth.

Antibody staining procedure. The indirect staining procedure using fluorescent labeled horse anti-rabbit globulin has been described(6). The paired label technic for determining presence of new glomeruli was carried out as follows. Sections of the hypertrophic kidneys were treated with fluorescein labeled horse anti-rabbit reagent for 1 hour, washed and treated with rabbit anti-rat kidney serum for 1 hour. The sections were washed and treated with Rhodamine-labeled horse anti-rabbit globulin, washed and observed under the U.V. microscope. All washings were made in phosphate buffered saline pH 7.0 for 10 minutes with constant agitation in a shaker.

Histological sections were made of all animals treated with the anti-rat kidney serum. Portions of the kidney were fixed in 10% formalin, embedded in paraffin and stained with H & E to determine the general appearance of the involved kidney.

The other unfixed portions were mounted on tissue blocks for frozen section studies with the fluorescent antibody technic.

Unilateral nephrectomy was performed by making an incision in the flank from the costal border to the iliac crests. Pressure along side of the wound causes the kidney to protrude through the wound. The renal pedicle is then ligated and the kidney excised (7).

Results. Anti-rat kidney serum has been shown to localize highly in glomeruli basement membrane, and the antibody remains at this site as long as one year and perhaps longer(8). We have observed that in newborn animals when anti-RK serum is administered, all the glomeruli stain but when the animals are allowed to grow for 6 weeks the newly formed glomeruli are unstained(5). These newly formed glomeruli are located toward the cortex whereas those originally present are all located closer toward the medulla. In the studies reported here the antibody treated nephrectomized rats all showed increase in weight during the subsequent period of 7 months, as did the treated non-nephrectomized controls. The kidneys from both groups are compared in Table I. The non-treated and non-nephrectomized rats (Table II) show no abnormal enlargement or kidney size when compared to the treated nephrectomized group.

As may be seen in the untreated group (Table II) (allowed to survive 1 year) the

TABLE II. Normal Rats Allowed to Survive for Over 1 Year after Attaining Initial Weight of 300-400 g.

Rat No.	Wt of rat when sacrificed	Wt of each kidney
10	640	1.93 1.84
11	733	2.03 1.96
12	677	1.83 1.87
13	645	2.21 2.19
14	645	1.49 1.43
15	604	1.76 1.74

body weight is larger than in the non-nephrectomized group (allowed to survive 7 months) but the kidney weight ranges are quite similar.

The kidneys removed from the 6 animals 24 hours after treatment with anti-RK serum when assayed by the indirect fluorescent antibody method showed the presence of the marker antibody in the glomeruli. Rats treated with 2 ml of normal rabbit serum were also included; none of these showed any localization of normal globulin in the kidneys at 24 hours.

In the animals showing hypertrophy of the kidney, the sections were treated by the indirect technic and carefully observed for unstained glomeruli toward the cortex. Even in the animal where the single kidney had attained twice the normal weight of the untreated control rats, all the glomeruli appeared stained showing fixed antibody. In order better to visualize any unstained glomeruli, kidney sections made at various levels were treated with the paired label procedure as follows. Each section was treated with the fluorescein labeled horse anti-rabbit globulin to demonstrate the marked glomeruli (fluorescing green); the section was then treated with rabbit anti-rat kidney serum to mark all the glomeruli present. The washed section was then treated with Rhodamine-labeled horse anti-rabbit globulin. When this was done all the glomeruli stained showed a yellow fluorescence (mixture of green and red fluorescence) indicating the presence of both fluorescent labels on the glomeruli. No glomerulus with red fluorescence was observed. The presence of any new glomeruli would be indicated by the red fluorescence.† It is apparent that while none of the antiserum treated animals can be considered to be normal the information obtained by this procedure is in agreement with the earlier findings of Arataki(1) and Saphir(2).

All normal serum treated animals showed no localization of rabbit globulin in kidneys at any time.

The histology of all the kidneys of the rabbit anti-RK serum treated rats, both nephrectomized and non-nephrectomized were compared, 7 months after antiserum treatment. In general all the kidneys showed the end stages of the effect of glomerulonephritis, *i.e.*, the acute lesions have disappeared and scarring and thickening of the basement membrane was present. The glomeruli even at this late date appeared not to be fully recovered and in some there was protein in the tubules indicating that proteinuria was present. In the nephrectomized animal glomerular size was greater in the remaining enlarged kidney when compared with the treated but non-nephrectomized rats.

Summary. Rabbit anti-rat kidney globulin was used as a marker of all glomeruli in the normal rat kidney. Adult animals were injected with anti-rat kidney serum and unilaterally nephrectomized to determine whether the remaining kidney would show the presence of new glomerular development in the enlarged cortical areas under these conditions. When rats treated in this manner were allowed to survive for 7 months hypertrophy of the remaining kidney occurred. All the glomeruli in the remaining kidney contained marker antibody as observed by the fluorescent antibody technic. This indicates that no new functional glomeruli are formed during the period when a compensatory hypertrophy in the remaining kidney is occurring.

† This technic was tested in the newborn rat system previously(5), red (new) glomeruli and yellow (original) glomeruli were easily contrasted.

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Changes in Serologic Reactivity of Endotoxin Induced by Fraction IV_1 (Cohn) of Normal Human Serum.* (27882)

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Incubation of bacterial endotoxins in human serum or defibrinated plasma has been shown to result in repression of many of the biological properties of endotoxin(1). In our laboratory, the changes following such incubation have been measured by quantitative analysis of the diminution of ability of the lipopolysaccharide to precipitate its homologous, standardized antibody(2), and by loss of pyrogenicity(3). When defibrinated plasma was fractionated with ethanol according to the method of Cohn *et al.*(4), incubation of endotoxin in 2 of the resulting fractions, IV_1, III_0, was shown to result in the above changes(3). In this communication are presented data showing that the alteration of endotoxin, (such that it is rendered less precipitable by homologous antiserum following incubation in Fraction IV_1) does not involve release of the di-deoxyhexose antigenic determinant sugar, tyvelose(5), *per se,* but that the loss in precipitability is associated with changes in the immunodiffusion pattern of endotoxin.

Materials and methods. Endotoxin. Salmonella typhosa 0-901 was grown in a 30-liter fermentor using the synthetic medium of Erlandson and MacKey(6). The cells were harvested by centrifugation and washed twice with ice cold 0.15 M NaCl. The endotoxin was extracted from the cells using the method of Boivin and Mesrobeanu(7), dried from the frozen state, and characterized as pyrogenic, Shwartzman reactive, lethal for mice, and precipitable with homologous antiserum. *O polysaccharide hapten.* This substance was prepared in this laboratory using the method of Staub and Combes (8). Its properties have been reported elsewhere(2). *Fraction IV_1.* The alpha globulin Fraction IV_1 prepared by Cohn's method 10 from normal human serum was generously supplied by Cutter Laboratories, Berkeley, Calif. *Incubation procedure:* Twenty-five milligrams of Fraction IV_1 were dissolved in 1.7 ml 0.15 M NaCl. Two ml of endotoxin solution at a concentration of 200 μg/ml in saline was added, the pH adjusted to 7.1 with 0.1 N NaOH and the volume of the mixture adjusted to 4 ml with 0.15 M NaCl. The mixture was then incubated in a 37°C water bath for 4 hr, aliquot samples removed immediately and tested for recoverable endotoxin as outlined below. The remaining portions of the incubation mixtures were then stored at −30°C until other tests could be performed. *Test for recoverable endotoxin:* The amount of precipitable endotoxin in a test sample was determined by adding an 0.8-ml aliquot of the incubation mixture to 1 ml of rabbit anti-*S. typhosa* serum (bacterial agglutination titer $>$1:20,000) and 1.2 ml of 0.15 M NaCl. Tubes were incubated for 2 hr at 37°C and 18 hr at 4°C. The nitrogen

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