eunochoid males(6). It has also been demonstrated that testosterone increases mitotic activity in the female thyroid gland(11). The effects of androgens on the basal metabolic rate of polycythemic mice are currently being investigated.

Testosterone has been shown to increase the organ weights of several non-endocrine organs, most notably the kidney(12). The epithelial cell cytoplasm is most affected, but the mechanism responsible for this change is not known. Since much evidence points to the kidney as a site of erythropoietin production(13,14), it is conceivable that erythropoietin production by the kidney might also be increased by direct action upon the renal parenchymal cell.

Testosterone might potentiate the effect of small amounts of endogenous erythropoietin elaborated in the polycythemic mouse if the small amount of iron incorporation in the unstimulated polycythemic mouse is a consequence of the continued elaboration of very slight amounts of erythropoietin. Such an effect could be a direct one, or might alternatively be indirect, by antagonization of the already demonstrated inhibitory action of estrogens on erythropoiesis(15).

Finally, testosterone might have a direct erythropoietin-like action upon the stem cells, inducing differentiation into the erythrocytic compartment. These various possibilities are currently under study.

Summary. Testosterone has been demonstrated to stimulate erythropoiesis in the polycythemic mouse. A response can be observed following a single injection of 0.5 mg of testosterone. One mg is as effective as larger single doses. A greater erythropoietic response was observed with 10 daily injections. Possible mechanisms of action of testosterone on erythropoiesis are discussed.

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Specificity of Immunofluorescence in Experimental and Human Renal Diseases.* (29714)

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Immunofluorescent staining for gamma globulin and complement and its components has come into wide use for the localization of immune processes in tissues and especially in the kidney. The objection has been made, that positive immune staining for gamma globulin and even for complement and its components may be due to a mere transuda-

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tion of gamma globulin and complement into inflamed and necrotic areas with an increased vascular permeability. Even the fact that albumin cannot be demonstrated with immuno-fluorescent antisera in such areas has been countered by the objection that albumin due to its smaller molecular size and its greater solubility may not be trapped there or be washed out during the rinsing of the tissues. To exclude merely trapped gamma globulin and complement as a cause of positive immune staining in renal diseases, a series of experiments were carried out.

Methods and materials. Female Sprague-Dawley rats weighing approximately 150 g were fed Purina rat pellets and water *ad lib.* The animals were kept in individual metabolism cages and 24 hour urine collections were examined for proteinuria semi-quantitatively with 20% sulfo-salicylic acid, unless electrophoretic determinations of the urinary protein pattern were carried out by the method of Collens, Meyers and Lange(1).

One and five-tenths mg per 100 g of rat of the aminonucleoside of puromycin were injected daily subcutaneously for production of the nephrotic syndrome.

Antirat kidney rabbit serum was produced as previously described(2). One i.v. injection of 10 mg per 100 g of rat weight of the gamma globulin fraction of the rabbit antirat kidney serum was used for production of the experimental nephritis.

Antihuman gamma globulin was produced by injection of 75 mg of human gamma globulin in saline with complete Freund's adjuvant in divided doses into the foot pads, intramuscularly and intradermally into white New Zealand rabbits of both sexes weighing between 2 and 2.5 kg. These injections were repeated 3 times at weekly intervals and the animals were bled 2 weeks after the last injection. Antirabbit and antirat gamma globulin sera were produced in an identical fashion or obtained commercially† using goats as producers of antirabbit gamma globulin sera.

The gamma globulin fractions were isolated by a previously described modification (3) of the methods of Saifer *et al*(4), and Gordon *et al*(5), using 6,9-Diamino-2-ethoxyacridine (Rivanol) for isolation of the gamma globulin. This fraction was then labeled with fluorescein isothiocyanate according to the method of Riggs(6).

The third component of complement, β_{1a} and β_{1c}, was isolated according to the method of Müller-Eberhard(7), and antisera produced by injecting 2 mg of the isolated material in complete Freund's adjuvant intradermally into the neck region of the rabbit. The rabbit was bled after 10 days. Again the gamma globulin was isolated as described above and labeled with fluorescein isothiocyanate.

Small sectors of the rat kidney were immediately frozen upon sacrifice in an isopentane-dry ice mixture, transferred to the cryostat and sectioned at —20°C at a 3 μ microtome setting.

Human biopsies were immediately imbedded in a marginal piece of fresh rat liver and the entire "sandwich" frozen and sectioned as described above. All sections were air dried and then stained with the respective labeled antibodies for one hour in a moist chamber and washed for half hour in slowly running buffered saline.

Results. An aminonucleoside nephrotic syndrome was produced in 6 rats. Proteinuria appeared between the 7th and 10th day after injection. Immediately upon the occurrence of the proteinuria considerable amounts of gamma globulin could be discovered in the urine of these rats by paper electrophoresis. Values between 3% and 5% of the total proteins were the rules. When these animals were sacrificed 4, 6, 7, 8, 9 and 12 days after onset of proteinuria and cryostat sections of their kidneys were prepared, rat gamma globulin could not be demonstrated on any of the glomeruli with a fluorescein isothiocyanate labeled rabbit antirat gamma globulin serum. Considerable gamma globulinuria was present in all animals at time of sacrifice. Similarly, staining for the third component of complement β_{1a} was completely negative in all rats.

Twenty-one rats were made nephritic by i.v. injection of 10 mg per 100 g of rat of the gamma globulin fraction of antirat kidney rabbit serum. Severe proteinuria appeared

† Microbiological Associates, Washington, D. C.

within 12 hours. Again, considerable amounts of gamma globulin were present in the urine of these rats as determined by paper electrophoresis. Three animals at a time were sacrificed 3, 5, 7, 11, 23 and 30 days after the injection and kidney sections were stained with fluorescein labeled anti rabbit gamma globulin goat serum. Intensive staining of all glomeruli was noted. Complement could also be demonstrated fixed on the basement membrane of the rat glomeruli caused by the interaction of the rat glomerular basement membrane and the localized antirat kidney rabbit serum as antibody. In contrast, *no* rat gamma globulin was found on the glomeruli after 2, 3 and 5 days. After 7 days, however, rat gamma globulin could be demonstrated in varying amounts initially only on small segments of the glomerular basement membrane but within 24 hours diffusely staining the entire basement membrane. The third component of complement was also found in greater amounts than before. Apparently the rats were now forming antibodies against the localizing part of injected rabbit protein and a complement fixing immune reaction between the localized foreign protein and the rat's own gamma globulin took place. Identical results were obtained on the 11th, 23rd and 30th day after injection of the nephrotoxic serum.

The kidney biopsy of a patient with multiple myeloma, severe Bence-Jones proteinuria and marked albuminuria and gamma globulinuria again did not show any immune fluorescence when stained with antisera for human gamma globulin and human complement.

It thus becomes apparent that in spite of severe renal damage in 2 types of experimental glomerulonephritis with marked gamma globulinuria, gamma globulin is not demonstrable in the kidney as long as it is not fixed there by immune processes. Only when the rat's gamma globulin starts to interact with an antigen on the kidney, positive staining occurs. Similarly, the mere passage of gamma globulin in a patient with multiple myeloma did not lead to any localization on the glomeruli.

Summary. Severe proteinuria with considerable gamma globulin content was produced in rats by aminonucleoside injections. Sections of their kidneys at intervals between 4 and 12 days after onset of proteinuria did not show immunofluorescent staining for gamma globulin and complement. Rats made nephritic by injection of anti-rat kidney rabbit serum showed immediate morphologic damage and proteinuria with considerable gamma globulin content. Rabbit gamma globulin can be demonstrated immediately on the glomeruli while rat gamma globulin does not appear before the seventh day of disease. A kidney biopsy of a patient with multiple myeloma and advanced renal damage did not show immune staining for gamma globulin and complement, in spite of a severe proteinuria with large amounts of albumin, Bence-Jones protein and gamma globulin in the urine. Positive immune staining for gamma globulin or complement and its components in renal diseases can therefore not be attributed to trapping or unspecific transudation in the damaged kidney.

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