4. Miller, W. L., and Dulin, W. E., *Science*, 1956, v123, 584.

5. Dulin, W. E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 115.

6. Folin, O., and Wu, H., *J. B. C.*, 1920, v41, 367.

7. LaBarre, J., and Reuse, J., *Arch. Neerl. Physiol.*, 1947, v28, 475.

Received July 24, 1956. P.S.E.B.M., 1956, v93.

Tubular Reabsorption of Protein in Experimentally Produced Proteinuria in Rats. (22687)

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It has long been recognized that cells lining the proximal convoluted tubule of the kidney of various animal species are capable of absorbing colloid substances from the tubule lumen(1-4); the process has been named *athrocytosis*. Dock(5) and Addis(6) were the first to suggest that under *normal* circumstances plasma proteins are to some extent filtered through the glomerulus of the kidney and reabsorbed by cells of the tubules. Because of the enormous volume of glomerular filtrate, a concentration of protein in the glomerular filtrate of only 15 mg 100 ml would result in excretion in the human of about 27 g of protein in 24 hours if the protein were not reabsorbed by the tubule cells. The work of many investigators has contributed to the concept of glomerular filtration and tubular reabsorption of plasma proteins in normal and abnormal conditions. These reports have recently been reviewed by Rather(7). Since this review, the quantitative aspects of tubular reabsorption of protein in the normal rat has been studied by Sellers, *et al.*(8) and the mechanism of tubular handling of reabsorbed protein has been investigated by Oliver and co-workers(9). Theoretically, a decrease in tubular reabsorption of plasma proteins filtered through normal glomerular basement membrane could account for the protein found in urine in most instances of proteinuria. Actually, however, in glomerulonephritis there is evidence of glomerular damage, and even in cases of idiopathic nephrotic syndrome, where only basement membrane thickening is found to suggest changes in glomerular permeability, calculations based on clearance studies in patients with massive proteinuria lead to the conclusion that glomerular permeability to plasma proteins is increased (10). This still leaves open the question of whether proteinuria occurs solely because filtration through abnormally permeable glomeruli exceeds the normal ability of tubules to reabsorb protein, or whether a decrease in tubular reabsorption of protein is an added factor in production of proteinuria.

The dye T-1824 (Evans Blue) binds firmly to serum proteins and has been used to measure renal clearance of albumin in humans(11) as well as tubular reabsorption of protein in rats(5,8,12). The amount of dye accumulating in droplet form in cells of the proximal convoluted tubule is assumed to be a measure of reabsorption from the tubule lumen of dye-labeled serum proteins. In the present report, dye-labeling of serum proteins has been used to study the role of tubular reabsorption of protein in 2 types of experimentally produced proteinuria.

Materials and methods. Female Osborne-Mendel rats of about 150 g body weight were used. Proteinuria due to uranium administration was produced by intravenous administration of 0.174 mg uranyl acetate containing 0.1 mg uranium, in 1.0 ml of 0.15 M saline. Proteinuria due to anti-kidney serum was induced by a single intravenous injection of rabbit anti-rat kidney serum. The nephrotoxic serum was obtained from rabbits immunized by repeated intraperitoneal injections of glomerular fraction of perfused rat kidney homogenate. To obtain urine for pro-

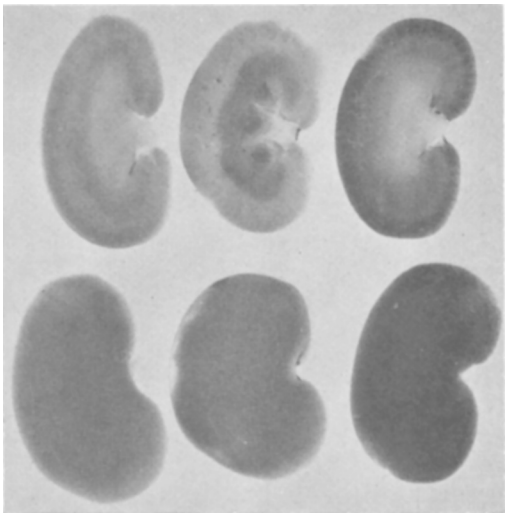

FIG. 1. Sagittal section and surface view of unfixed kidneys from control rat (left), rat with proteinuria produced by uranium inj. (center), and rat with proteinuria produced by inj. of nephrotoxic serum (right). Each rat received 2.5 mg T-1824 intrav. 24 hr before kidneys were removed.

tein determinations, rats were placed in individual metabolism cages with an adequate liquid diet containing casein hydrolysate in place of protein and urine was collected overnight. Proteinuria was measured by the method of Shevsky and Stafford(13). Two and one-half mg of T-1824 dye in 0.15 M saline were injected intravenously into a foot vein of rats followed by an overnight urine collection. Twenty-four hours after dye injection, the rats were anesthetized with ether, exsanguinated from the abdominal aorta, and both kidneys were decapsulated and removed. One kidney was sliced in half with a razor blade, placed in a Petri dish containing ice cold 0.15 M saline and examined in the gross and at various magnifications under a Leitz binocular dissecting microscope. The other kidney was fixed in 10% formalin and both frozen sections and hematoxylin and eosin and PAS stained sections were made. In another experiment, some animals were autopsied at daily intervals after antiserum injection and the kidneys were examined and weighed.

Results. Normal rats. After intravenous injection of 2.5 mg of T-1824 in normal rats, a normal straw or amber colored urine was obtained. Study under dissecting microscope of kidneys removed 24 hours after dye injection showed a pale green-tan or blue-green surface, and at higher magnifications none of the mosaic pattern of convoluted tubules was strikingly stained by the dye. On cut section (Fig. 1), the outer zone of the cortex was a faint but definite green, the inner zone was colorless and the medula was red. It was evident that relatively small amounts of the blue dye were present in the tubular cells.

Uranium proteinuria. It was observed in preliminary experiments, data from one of which are shown in Table I, that proteinuria was produced after a latent period of 2 or 3 days by 0.174 mg of uranyl acetate. This dose was chosen for 4 T-1824 experiments in 8 rats each. Forty-eight hours following injection of uranyl acetate, T-1824 dye was injected. Urine collected overnight was blue and contained protein. Twenty-four hours after T-1824 injection, the external surface of kidneys were a pale green color not unlike the T-1824 injected normal rat kidneys (Fig. 1), but at higher magnification the mosaic of tubules consisted of pale green colored tubules alternating with completely colorless tubules not observed in control rats. The cut section of kidneys showed a pale green outer cortex, containing somewhat less blue color than the T-1824 injected control kidneys, a colorless inner zone of cortex and the red medullary zone. Microscopic examination showed apparently normal glomeruli and changes in cells of proximal convoluted tubules consisting of marked necrosis and even desquamation of cells from the tubular basement membranes.

Nephrotoxic serum proteinuria. After intravenous injection of a standard dose of rabbit anti-rat kidney serum (twice the minimum amount required to produce proteinuria), the 150 g female rats developed immediate proteinuria. In most rats proteinuria persisted indefinitely, but in some rats it gradually subsided. Definite renal enlargement occurred in 2 or 3 days. T-1824 studies were made as early as 24 hours and at various longer periods after antiserum injection in 17 rats with chronic proteinuria due to nephrotoxic serum. The urine collected after T-1824 injection contained about 7 mg/hr of protein and was blue. When examined 24 hours after T-1824

TABLE I. Time of Onset of the Proteinuria Produced by Intravenous Injection of Uranium and Nephrotoxic Serum.

Material inj.	No. rats	Proteinuria Day 1	Day 2	Day 3	Day 4	Day 7
		mg/hr				
Saline (controls)	4	.3	.2	.1	.2	.2
.0178 mg uranyl acetate	2	.2	.1	.3	.3	.2
.178 mg " "	8	.3	.7	1.6	1.0	.5
1.78 mg " "	4	1.1	3.5*			
Nephrotoxic serum	4	11.0	5.1	4.0*		

* Sacrificed for autopsy examination.

injection the surfaces of kidneys were deep blue and under higher magnification one could see against the background of blue-stained tubules an occasional convoluted tubule which was an even more intense blue (Fig. 1). At highest magnification the blue could be seen to be in granular form, apparently in the tubule cells. The cut sections showed intense blue staining of outer and inner zones of the cortex; the medulla was red. Frozen sections of such kidneys after formalin fixation showed the blue granules or droplets in the cytoplasm of the cells lining the proximal convoluted tubules as has been described by other investigators in kidneys of normal rats 24 hours after intravenous injection of much larger amounts (25 mg) of T-1824(8,12). Varying degrees of basement membrane thickening and patchy lipid deposition in tubule cells were present.

It should be noted that the amount of T-1824 injected in our experiments was sufficiently small that it should have been completely bound to albumin even in chronically proteinuric rats with lowered plasma albumin levels. To avoid complication of the difference in plasma albumin levels between control and nephrotic rats, 2.5 mg of T-1824 were administered to 12 normal rats and followed within a few minutes in 6 rats by nephrotoxic antiserum. In those rats receiving nephrotoxic serum, there was deep blue staining of the cortex in 24 hours as described in the chronically proteinuric rats.

Discussion. Previous investigators have summarized the facts and the reasoning behind the assumption that the appearance of the T-1824 dye in the cytoplasm of proximal convoluted tubule cells represents dye-labeled protein which has been filtered through the glomerulus and reabsorbed from the tubule lumen by proximal convoluted tubule cells(5, 8,12). The T-1824 in serum and urine of proteinuric rats was all precipitated with the protein by protein precipitating agents in the experiments of Gilson(12), and Sellers, *et al.* (14), and in our own experiments.

In the present experiments, the injection of one-tenth of the amounts of T-1824 used in experiments of others with normal rats(12, 14) resulted in appearance of only a small amount of color in the cortex of normal rats after 24 hours. In rats with proteinuria resulting from anti-kidney serum administration, appearance of large amounts of T-1824 in the tubule cells probably indicates that dye-labeled protein entered the cells in larger amounts than in the T-1824 injected control rats. This is consistent with the hypothesis that an increase in glomerular permeability due to antiserum damage led to increased filtration of dye-labeled serum proteins through the glomerulus, followed by increased tubular reabsorption of dye-labeled protein. The greater than normal accumulation of blue dye in 24 hours in the tubule cells of rats which received T-1824 prior to nephrotoxic serum administration seems to us to eliminate the possibility that the accumulation of dye in nephrotic rats is due to the presence of a larger amount of unbound dye in nephrotic rats than in controls. An alternative explanation for our finding is that damage to the tubule cells interfered with the mechanisms for disposing of the reabsorbed dye so that an increase in the dye in tubules occurred without an increase in tubular reabsorption of protein. Consideration of the findings in the kidneys of rats with uranium damaged tubules makes this possibility seem unlikely.

The renal enlargement which occurs in rats receiving anti-kidney serum presumably is related to the increased reabsorption and perhaps degradation of filtered protein; this is suggested by the observation of similar enlargement when proteinuria was produced by repeated infusions of protein in normal rats (15).

The rats injected with 0.1 mg uranium intravenously developed proteinuria only after 48 to 72 hours. Uranium is transported in combination with serum albumin and bicarbonate(16). Since almost all the injected uranium leaves the blood stream, and concentration in the kidney is greatest in the first few hours(17), it is difficult to account for the delay in onset of proteinuria. Tubular reabsorption of uranium from the tubule lumen would account for the well known localization of uranium produced renal damage in the proximal convoluted tubule(17). It is also consistent with the concept that the proteinuria produced in uranium injected rats is due primarily to decreased tubular reabsorption of protein by damaged tubule cells since no increase, and in fact a probable decrease, in T-1824 accumulation by tubule cells is found. Since no glomerular damage is detected in the presence of obvious localized tubular necrosis in such rats, it is tempting to consider that the protein appearing in urine is normally filtered plasma protein which is no longer reabsorbed by damaged tubule cells. One cannot, however, rule out the possibility that an increase in glomerular permeability has occurred in addition to decreased tubular reabsorption of protein.

The findings indicative of increased reabsorption of dye-labeled protein by the tubule cells of the kidneys of rats injected with anti-rat kidney serum are in accord with similar observations made by Spector(18) using radioactive iodine as the tag for serum protein. It should be noted that the rats receiving antikidney serum develop hypoproteinemia and lipemia and have renal lesions which are similar to those observed in many cases of the nephrotic syndrome in man(19).

Summary. An attempt was made by labeling plasma proteins with the blue dye T-1824 to estimate the amount of protein filtered and reabsorbed by the kidney under several conditions. A single intravenous injection of 2.5 mg of T-1824 into normal rats did not cause the urine to be blue and the kidneys examined 24 hours after the injection exhibited only faint blue staining of the proximal convoluted tubules of the cortex. When proteinuria was produced by a single small injection of uranyl acetate, the injection of T-1824 was followed by blue staining of the urine. However, the cortices of the kidneys contained less blue dye than those of control animals, indicating, presumably, that there was less protein reabsorption than normal by the damaged tubular cells. Injection of the blue dye into nephrotic rats with proteinuria due to administration of nephrotoxic anti-rat kidney serum was followed by blue staining of the urine and intense blue staining of the cortices of the kidneys. This presumably indicated that protein reabsorption by the tubular cells was greater than normal, and that the proteinuria was a result of increased glomerular permeability and not of decreased tubular reabsorption of normally filtered protein.

1. Suzuki, T., *Zur Morphologie der Nierenskretion,* 1912, Jena, Fischer.

2. Gerard, P., *Comp. Rend. Soc. Biol.,* 1933, v115, 199.

3. Lambert, P., *ibid.,* 1933, v114, 1370.

4. Smetana, H., and Johnson, F. R., *Am. J. Path.,* 1942, v109, 1029.

5. Dock, W., *N. Eng. J. Med.,* 1952, v227, 663.

6. Addis, T., *Tr. Assn. Am. Phys.,* 1942, v57, 106.

7. Rather, L., *Medicine,* 1952, v31, 357.

8. Sellers, A. L., Griggs, N., Marmorston, J., and Goodman, H. C., *J. Exp. Med.,* 1954, v100, 1.

9. Oliver, J., MacDowell, M., Lee, Y. C., Moses, M. J., Kretchmer, N., Dickerman, H. W., and Cherot, F., *J. Exp. Med.,* 1954, v99, 589.

10. Chinard, F. P., Lauson, H. D., Eder, H. A., Greif, R. L., and Hiller, A., *J. Clin. Inv.,* 1954, v33, 621.

11. Chinard, F. P., Lauson, H. D., and Eder, H. A., *ibid.,* 1952, vv31, 895.

12. Gilson, S. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 608.

13. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry; Methods,* Baltimore, 1932, 682.

14. Sellers, A. L., Smith, S., Marmorston, J., and Goodman, H. C., *J. Exp. Med.,* 1952, v96, 643.

15. Baxter, J. H., and Cotzias, G. C., *J. Exp. Med.,*

1949, v89, 643.

16. Barron, E. S. G., *Toxicology of Uranium, National Nuclear Energy Series,* 1951, vIV-23, p41, McGraw Hill, New York.

17. Newman, W. F., *Pharmacology and Toxicology of Uranium Compounds, National Nuclear Energy Series,* 1951, vVI-1, p707, McGraw Hill, New York.

18. Spector, W. G., *J. Path. and Bact.,* 1954, v68, 187.

19. Heymann, W., and Lund, H. Z., *Pediatrics,* 1951, v7, 691.

Received July 26, 1956. P.S.E.B.M., 1956, v93.

Genic Control and Hormonal Reversal of Sex Differentiation in Xenopus.* (22688)

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It has long been realized that in the cytodifferentiation of germ cells not only genes and external environmental factors play important roles, but also certain conditions of their immediate milieu(1). The first figure presents diagrammatically the basic facts and their interpretation based on recent work. Germ cells, on arriving in the primitive gonadal cortex, become invested each by a *nurse or follicle cell.* If unopposed, the follicle eventually induces development of the germ cell into an egg. However, if at the critical stage of sex differentiation a germ cell becomes located in the medulla, another inductor system, the *interstitial cells,* upsets the influence of the follicle cells and leads to the spermatogenetic type of differentiation.

How can such an interpretation be integrated with the well known facts of genic control of sex ratios? The present experiments seem to shed some new light on this problem.

Material and methods. Xenopus laevis Daudin has been bred in our laboratory since 1949 without addition of new stock. The experimental larvae were placed in hormone solutions of given concentrations, the solutions being completely replaced every 24 hours. The temperature was kept constant at 20°C.

Experiments. Since in the adult organism follicles and interstitial cells produce estrogenic and androgenic steroids, it was necessary to investigate the possibility that in embryonic sex differentiation the same hormones might first play the role of inductor substances. Experiments with Xenopus gave very interesting results, although not in the sense of a confirmation of the tentative assumption. Small or large doses of androgens added to the aquarium water equally produce what first seemed to be *all premature male cultures.* Given throughout the larval period they yield at metamorphosis little toads that all have well developed dark arm and hand pads and immediately start embracing each other. However, on dissection it is found that only about half of the animals have testes, the other half have normal ovaries (Fig. 2). We shall later come back to this experiment but next must describe the effect of the opposite treatment: exposing the larvae to estrogenic hormones, usually *estradiol.* Here the entire offspring is completely feminized. The original experiments with Allison(2,3) have often been repeated and thousands of offspring have been produced that consisted of females only(4,5). Some recent data are presented in Fig. 3.

These two hormone effects are all the more remarkable, because they are in contrast with the results of parabiosis and gonad transplantation, as described by Chang(6). The simultaneous presence of testes and ovaries in a system with a common blood circulation always results in normal testes and greatly suppressed ovaries. Sometimes even testicu-

* Aided by a grant from the Rockefeller Foundation. The authors are also indebted to Ciba Pharmaceutical Products, Inc., for the generous supply of the steroid hormones.