in the erythropoietic rate. On a per weight basis this dose is comparable to doses administered in human disease. Since the effect of testosterone increases with decreasing plethora, one might expect that even smaller doses would affect the non-plethoric mouse, although their effects would be diffcult to detect because they would be masked by the high basal rate of erythropoiesis.

The results reported above also indicate that as little as 0.25 mg of testosterone produces measurable increases in the erythropoietin titers of hypoxic mice. These studies make it unlikely that effects on erythropoiesis and erythropoietin formation are due only to some non-specific effect of extremely large doses of testosterone.

The above experiments also emphasize the value of mildly plethoric mice for the study of erythropoietic stimuli which are dependent on erythropoietin for their effect. Triiodothyronine(4), cobalt(8) and testosterone all have a more profound effect on the rate of erythropoiesis of mildly plethoric mice than on mice made more intensely plethoric.

While it may be premature to interpret the role of testosterone in the therapy of human anemias on the basis of these experiments in mice, the demonstration that doses of the hormone that are effective in the mouse are comparable to those used in the clinics, encourages us to investigate the effect of testosterone on erythropoietin levels in anemic patients.

Summary. Two daily injections of as little as 0.1 mg testosterone are capable of stimulating erythropoiesis in mildly plethoric mice. Twenty-five hundredths of a milligram of testosterone is sufficient to elevate the erythropoietin titers of hypoxic mice.

1. Fried, W., DeGowin, R., Gurney, C. W., Proc. Soc. Exp. Biol. and Med., 1964, v117, 839.
2. Janda, W., Fried, W., Gurney, C. W., ibid., in press.
3. Mirand, E. A., Gordon, A. S., Wenig, J., Nature, 1965, v206, 270.
4. Fried, W., Gurney, C. W., ibid., 1965, v206, 1160.
5. Gurney, C. W., Fried, W., J. Lab. Clin. Med., 1965, v65, 775.
6. Cotes, P. M., Bangham, D. R., Nature, 1961, v191, 1065.
7. DeGowin, R. L., Hofstra, D., Gurney, C. W., Proc. Soc. Exp. Biol. and Med., 1962, v110, 48.
8. Gurney, C. W., Fried, W., unpublished data.

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Urea Reabsorption by the Proximal Tubule of the Dog.* (30578)

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Both micropuncture and tissue analytical techniques have been used extensively to study urea reabsorption by the renal tubule of the rat(1-3). These procedures have made it possible to quantitate urea reabsorption in various portions of the rat renal tubule. However, in the dog, the percentage contribution of various portions of the nephron to urea reabsorption has not yet been documented. For this reason, simultaneous renal micropuncture and renal clearance techniques were used in the following experiments to study urea reabsorption by the proximal tubule of the dog.

Methods. Nine micropuncture experiments were performed on normal anti-diuretic dogs anesthetized with pentobarbital (30 mg/kg). The surgical procedure and the technique of renal micropuncture in this species have been described(4). After the urine flow has stabilized, serial urine samples of 15 minutes duration were obtained during the collection of samples of proximal tubular fluid. Blood was drawn at the mid-point of each collection period and concentrations of inulin and urea

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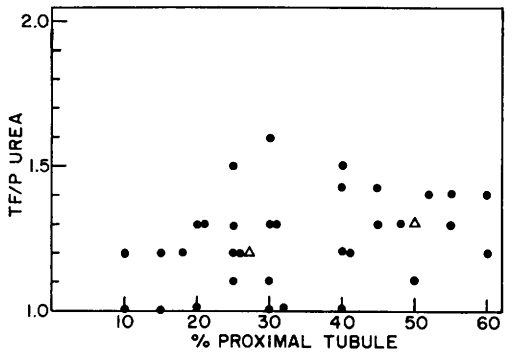

FIG. 1. TF/P concentration ratio in proximal tubular fluid of antidiuretic dogs. Open triangles represent mean values for the early (0-40% length) and late (40-60% length) proximal tubule.

were determined. Five to seven samples of tubular fluid (0.1 to 0.3 μl) were obtained from surface convolutions of the proximal tubule during each study. Plasma and urine concentrations of inulin were measured by the anthrone method of Fuhr and co-workers(5). The urea concentration in proximal tubular fluid was determined in duplicate by an ultra-micro modification of the colorimetric method by Chaney and Marbach(6). This method involves the urease-induced liberation of ammonia from urea and subsequent formation of a blue color by the reaction of ammonia with phenol reagent in the presence of hypochorite and nitroprusside. The urea concentration of 24 samples of plasma ultrafiltrate determined by this method averaged 99.2 (S.D. $\pm$ 6.1%) of simultaneous values obtained with the Conway microdiffusion method. Urea concentrations in plasma and urine were determined by the microdiffusion method of Conway(7).

Results. Urine flow from the experimental kidneys averaged 0.24 (S.D. $\pm$ 0.02) cc/min. For all 9 experiments, inulin and urea clearances averaged 36.2 (S.D. $\pm$ 6.7) and 19.9 (S.D. $\pm$ 4.1) cc/min, respectively. Plasma urea concentration averaged 4.7 (S.D. $\pm$ 0.44) mM. Tubular fluid/plasma (TF/P) concentration ratios of urea from 33 samples of proximal tubular fluid are shown in Fig. 1. It should be noted that the tubular fluid concentration of urea rose above that of plasma in the early portions of the proximal tubule. The TF/P urea ratios ranged between 1.0 and 1.5, averaging 1.2 (S.D. $\pm$ .18) within the first 40% of the proximal tubular length and 1.3 (S.D. $\pm$ 0.34) in the late proximal tubule (40-60% length).

Discussion. Renal micropuncture studies in antidiuretic rats(1) have shown that 50% of the filtered urea is reabsorbed within the first 60% of the proximal tubule, but that an equivalent amount of urea is subsequently added to the tubular fluid as it passes through the loop of Henle. The distal tubule and collecting ducts have been identified as the major determining sites of net urea reabsorption for the entire kidney. From these data, it has been suggested that urea diffuses into loop of Henle fluid as a result of the concentration gradient established by the high urea concentration in medullary interstitial fluid.

In the present studies, urea concentration in proximal tubular fluid was higher than that of plasma in almost every sample; the mean TF/P urea ratio in the late proximal tubule was 1.3 (S.D. $\pm$ 0.35). The results of previous micropuncture studies in the dog during similar experimental conditions have shown that the mean TF/P inulin ratio in this portion of the nephron is 2.3 (S.D. $\pm$ 0.18)(4). From these mean inulin and urea TF/P concentration ratios, one can calculate that approximately 56% of the filtered urea must have been reabsorbed within the first 60% of the proximal tubule. At the same time, it should be emphasized that net urea reabsorption by the entire kidney equalled only 55% of the filtered load of urea.

Two explanations could account for these findings. First, it is possible that the proximal tubule is the sole site of urea reabsorption in the dog, and that further urea reabsorption does not occur at more distal sites along the nephron. However, in view of the finding(8) that vasopressin markedly increases the rate at which intravenously administered urea-C^{14} equilibrates with medullary urea in the dog, it seems likely that a significant amount of urea must enter the renal medulla from tubular fluid at some site distal to the proximal tubule. For this reason, as in the rat, the occurrence of net urea addition to tubular fluid as it passes through the loop of Henle would seem a more likely

explanation for the present observations. Such an event would make it possible for additional urea reabsorption to occur in the distal tubules and collecting ducts and thus provide a satisfactory explanation for the finding of a urea clearance ratio in the late proximal tubule which approximates that for the entire nephron. Since the surface tubules of the dog kidney extend deeply into the renal medulla(9), the diffusion of urea into loop of Henle fluid would be facilitated by the progressive rise of urea concentration which is known to occur from the outer medulla to the tip of the papilla(10). Unfortunately, because the distal tubules are not accessible to renal micropuncture in the dog, it is not yet possible to quantitate urea addition to the loop of Henle fluid, or urea reabsorption by the distal tubule and collecting duct.

In summary, these renal micropuncture data suggest that urea addition to the loop of Henle fluid occurs in the dog as well as in the rat.

1. Lassiter, L. E., Gottschalk, C. W., Mylle, M., Am. J. Physiol., 1961, v200, 1139.
2. Ullrich, K. J., Schmidt-Nielsen, B., O'Dell, R., Pheling, G., Gottschalk, C. W., Lassiter, W. E., Mylle, M., ibid., 1963, v204, 527.
3. Bray, G. A., Prescott, A. S., J. Clin. Invest., 1961, v40, 1952.
4. Clapp, J. R., Watson, J. F., Berliner, R. W., Am. J. Physiol., 1963, v205, 273.
5. Fuhr, J., Kacqmarczyk, J., Kruttgen, C. D., Klin. Wochschr., 1955, v33, 729.
6. Chaney, A. L., Marbach, E. P., Clin. Chem., 1962, v8, 130.
7. Conway, E. J., Crosby, Lockwood & Son, London, 1957.
8. Jaenike, J. R., J. Clin. Invest., 1961, v40, 144.
9. Sperber, I., Zool. Bidray. Fran. Uppsala, 1944, v222, 325.
10. Robinson, R. R., Schmidt-Nielsen, B., J. Cell. & Comp. Physiol., 1963, v62, 147.

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Renal and Hepatic Effects on Potency of Testosterone Propionate in Chicken. (30579)

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The role of the liver in the inactivation of steroids is unquestionable. For instance, in man it is concerned with the inactivation of steroids(1). Estrogen in the rat is inactivated in the liver(2,3). In the chicken, testosterone is partially removed from the circulation by the liver and comb growth provides a sensitive indication of its hepatic inactivation(4). The renal portal system in the chicken kidney drains the blood from the hind limb and allows its direct access to the renal secretory system(5) offering a convenient way to study the role of the kidney in the processing of drugs when injected in the hind limb muscle. Similarly, the role of the liver can be conveniently determined by injecting the material into the gizzard muscle from which blood directly drains into the hepatic portal system. Injections into the pectoral muscle are drained to the general circulation. This unique circulatory system in the chicken provides for a comparison of potencies of active materials injected through these 3 routes, and may yield quantitative estimates of the effects of the liver and the kidney on the injected material.

Estrogenic stilbene derivatives in chickens have different potencies following peripheral, hepatic, and renal routes of administration (6). The subject of the present investigation was whether the testosterone has the same potency variability in chickens following all the above 3 mentioned routes.

Materials and method. Single comb white Leghorn cockerels, 21 days old, were maintained in wire floored cages. They were allowed Purina mash and water *ad lib.* Cockerels were arranged in groups of 10