content was greatly elevated. Hyperglycemia produced by glucose injection caused little change in the glycogen content of neuro- and adenohypophysis and also in the adrenal medulla, but significantly decreased that of the adrenal cortex. Fall in blood sugar levels (insulin injection) decreases the glycogen content of all the components of the hypophysis and adrenal glands. Perhaps the changes in glycogen content are related to hormonal synthesis and secretion. Although injection of ACTH causes a 27% decrease in the glycogen content of the normal rat adrenal cortex at 2 and 4 hours, no significant decrease was found in the diabetic animals at these time periods.

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Urea Excretion in the Potassium-Deficient Rat.* (31836)

A. L. FINN† AND L. G. WELT‡

Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina

The pathogenesis of the renal concentrating defect accompanying potassium deficiency in mammals(1,2) remains obscure. Although some evidence suggests that there may be a defect in the absorption of sodium from the ascending limb of the loop of Henle(3), it is clear that the countercurrent mechanism is operative in potassium depletion, since early distal tubular fluid is similarly hypotonic in potassium depleted and control animals(4,5). The impairment of urinary concentrating ability could also be due to a decrease in the permeability of the distal tubule and/or collecting duct system, from which, in the presence of antidiuretic hormone, water moves into the interstitium along its osmotic gradient.

Distal tubular permeability has been indirectly evaluated in potassium depletion and appears to be impaired in the dog(6) and in man(7,8), but direct micropuncture studies have effectively ruled out this possibility in the rat under ordinary solute loads(4,5). With regard to the role of the collecting duct, direct micropuncture data are unvailable in the rat but there appears to be no osmotic disequilibrium across the collecting duct of the potassium-depleted hamster(9).

Current concepts of renal physiology indicate that urea plays an important role in the concentrating mechanism(10,11,12,13). Most available evidence suggests that urea is

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† Work done during tenure of a Postdoctoral Fellowship from Nat. Heart Inst. Present address: Depts. of Physiology and Medicine, Yale University School of Med., New Haven, Conn.

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reabsorbed from the collecting duct, as from other parts of the nephron, as a consequence of the increase in its concentration resulting from the reabsorption of water. In addition to the passive reabsorption of urea from the tubule, there may well be a mechanism in the mammal for the transport of this substance from the lumen of the terminal portion of the nephron against its concentration gradient (10,14,15,16).

The purpose of this study was to evaluate, under conditions of hypertonic osmotic diuresis, the excretion of water and urea in potassium-depleted and control animals, in attempt to elucidate further the renal pathophysiology in potassium-deficient rats with particular reference to the concentrating defect.

Materials and methods. Female Sprague-Dawley rats, weighing 200-350 g, were fed 12 g daily of an electrolyte-free diet and gavaged with an electrolyte solution with or without potassium. The 10 potassium-depleted animals received the same solute load as did the 9 controls; the regimen continued for from 35 to 50 days, and 24 hours prior to a study, the animal concerned was deprived of water. Fifty milliunits of vasopressin tannate in oil (Pitressin, Parke-Davis & Co.) were administered at 8 a.m. and at 3 p.m. and the osmolality of the overnight urine collection was measured.

The animal was then anesthetized, with sodium pentoparbital the jugular vein cannulated and a catheter placed in the bladder. A priming dose of inulin and a sustaining infusion of inulin in isotonic saline were begun, the latter continuing until a positive fluid balance of approximately 10% of body weight was achieved. At this point, the infusion was changed to 25% mannitol or 5% saline and continued for from 30 to 50 minutes, during which time urine collections were made at ½- to 3½-minute intervals. Blood was collected at 5- to 10-minute intervals, and plasma concentrations of urea nitrogen, osmolality, and inulin were determined. Urea was measured with the Technicon Auto-Analyzer, osmolality with the Fiske Osmometer, and inulin by the method previously used in this laboratory(17). At termination of each experiment, the animal was killed and its muscle potassium determined as previously described(1).

In regard to the parameters evaluated here, there were no differences between animals infused with hypertonic mannitol and those given hypertonic saline, so that for the remainder of this paper, these two types of infusions will not be differentiated.

Results. Muscle potassium was 41.4 ± 2.7 (S.D.) mEq/100 g fat-free dry solids in the control and 30.4 ± 3.5 in the experimental group ($P<.01$). The maximum overnight urinary osmolality in the experimental group was 1925 ± 343 mOsm/kg while the controls achieved a value of 2567 ± 274 ($P<.01$).

Data regarding urea excretion are presented in Fig. 1-3. On each ordinate is plotted the fraction of the filtered urea which is excreted into the urine, C_{Urea}/C_{Inulin}.

Fig. 1 demonstrates that at high urine flows the clearance ratio was significantly greater in the potassium-depleted than in the control animals. The flow rates, along the abscissa, are grouped at intervals. These same data are plotted somewhat differently in Fig. 2, where the abscissa represents the U_{Inulin}/P_{Inulin} ratio. Here it may readily be seen that the clearance ratios were significantly greater in the potassium-depleted animals compared to the controls over the entire range of fractional water reabsorption, except at extremely low values, where the paucity of observations presumably accounts for the failure to note any difference between the two groups.

Similarly, Fig. 3 reveals that at virtually all osmolalities observed, the C_{Urea}/C_{Inulin} ratio was significantly higher in the experimental animals.

It should be noted that although the filtered loads of urea were identical in the two groups, the blood urea nitrogen was somewhat higher (18 mg% *vs* 14 mg%), and the GFR correspondingly lower, in the experimental groups.

The brisk osmotic diuresis which occurred in these animals is indicated by the low urine/plasma inulin ratios which were achieved, the values not infrequently being less than three. Despite this, we were at no time able

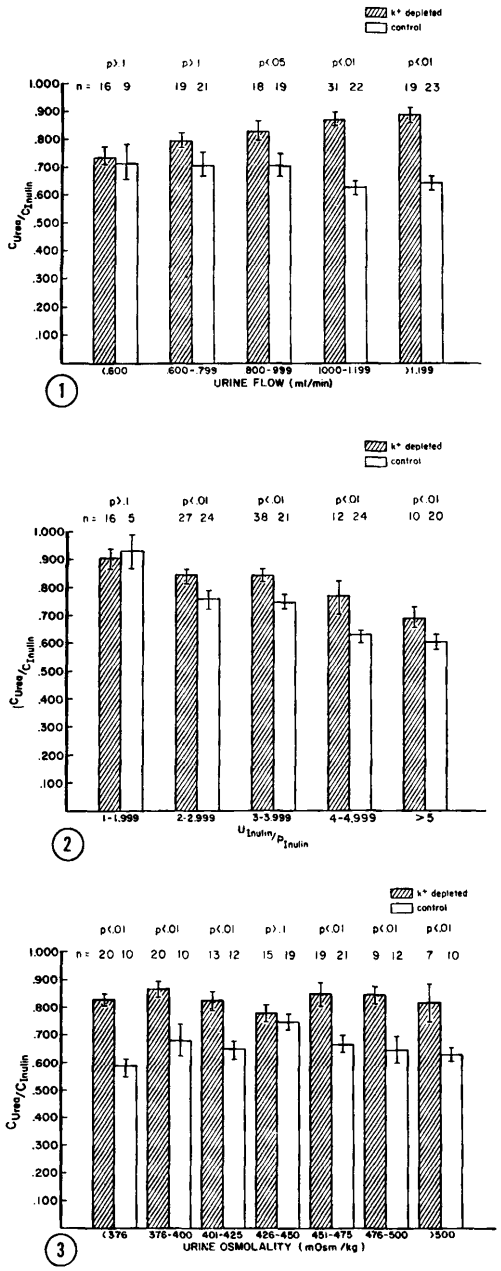

FIG. 1. Relationship between urea excretion and urine flow. Height of each bar represents percentage of the filtered load of urea that was excreted, or the clearance ratio C_{Urea}/C_{Inulin}. Lines associated with each bar denote standard error of mean. The p value over each pair of bars represents the probability, calculated on the basis of Student's t test, that such a difference between experimental and control groups might be due to chance. Figure depicts data obtained from 103 clearance periods in 10 experimental animals, and 94 clearance periods in 9 control animals. Urine flow was falling or stationary in approximately 60% of all collection periods in both experimental and control groups, and rising in the remaining 40%. However, the results were not different from those shown when the data were further separated on this basis.

FIG. 2. Relationship between urea reabsorption and water reabsorption. U_{Inulin}/P_{Inulin} ratio is utilized as a measure of water reabsorption. Other aspects as in Fig. 1.

FIG. 3. Relationship between urea excretion and urine osmolality. Representation is as in Fig. 1 and 2.

to demonstrate the formation of urine hypotonic to plasma in animals from either group.

Discussion. The potassium-depleted rats were, as expected, unable to concentrate their urine maximally and could not reabsorb water normally during osmotic diuresis. The failure to observe urine hypotonic to plasma despite the very low U/P inulin ratios supports the conclusion that the defect in the renal tubule is not solely a failure of osmotic equilibration between distal tubular fluid and its surroundings.§

Our data reveal that potassium-depleted animals excrete a consistently greater fraction of the filtered urea than normal controls, despite similar filtered loads, during acutely induced osmotic diuresis. There are several possible explanations for this.

First, there may be a decrease in permeability of the proximal convoluted tubule to urea in the potassium-depleted animals, such that a greater portion of filtered urea escapes back diffusion. However, such a defect would result in a urea solute diuresis which would limit the minimal as well as the maximal urinary osmolality. Most studies(5,18,19), however, have indicated that potassium-

§ It should be noted that there was a relative paucity of observations in the lowest osmolality ranges among the control animals, and a similar decrease in observations at the highest osmolalities among the experimental animals, despite similar urine flows and osmolal clearances. These data suggest that there may, in fact, be failure of distal tubular equilibration in the potassium-depleted group, but in the absence of observations, in even a single period, of urine hypotonic to plasma, conclusive evidence of such a disequilibrium is lacking. In addition, this distribution of observations would be consistent with a decrease in proximal tubular urea reabsorption in the experimental animals; however, for reasons to be discussed, this is an unlikely possibility.

depleted animals maintain normal or nearly normal ability to dilute the urine. In addition, there is no evidence that the minimal azotemia observed here would, of itself, affect urea clearance.

Second, there might be a diminution in membrane permeability to water resulting in failure of osmotic equilibration of fluid in the collecting duct with that in the loops of Henle and in the interstitial spaces. If this were so, less water and, consequently, less urea would leave the collecting duct system; hence, the osmolality of fluid in the collecting ducts would be lower than that of the loops of Henle at the same level. This would result in the elaboration, at any given total solute concentration, of urine containing a higher proportion of urea (and a lower proportion of other solutes) than if the osmolality of collecting duct and loop of Henle fluid were the same. (A decrease in active sodium transport, *per se*, would not affect osmotic equilibration and there would be no differences in urea excretion at similar urine osmolalities.)

In this regard, it is of interest that the increase in permeability to water following the addition of antidiuretic hormone to the isolated toad bladder is blunted in the absence of potassium(20,21). On the other hand, no osmotic disequilibrium was found in the kidney of the potassium-depleted hamster under varying osmotic loads in the presence of antidiuretic hormone(9).

Third, there could be a decrease in the permeability of the distal tubule to urea. It seems clear that the permeability of membranes to urea is less than to water(22,23) and that it is, therefore, conceivable that passive urea movement might be diminished without any change in water movement. Such a defect could account for all of the data reported here, and we cannot at present eliminate this hypothesis.

Finally, recent evidence(10,14-16) suggests that urea may be actively transported in the mammalian kidney. Our findings of increased urea excretion in an acutely induced osmotic diuresis in the potassium-depleted rat would be consistent with inhibition of such a transfer system.

Summary. Potassium-depleted rats excrete a consistently greater fraction of filtered urea than control animals at virtually all urine osmolalities and relative and absolute rates of water excretion. Furthermore, despite the reduction in Urine : Plasma inulin ratios to values below 2, no animals excreted urine that was hypotonic to plasma. The data suggest that in potassium depletion there is either a diminished permeability of the collecting tubule to water and/or urea, or inhibition of a transfer system whereby urea is reabsorbed from the collecting tubule against a concentration gradient.

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Hematopoietic Changes in Mice Following *Bordetella pertussis* Vaccine.* (31837)

GEORGE J. FRUHMAN (Introduced by W. Etkin)
Department of Anatomy, Albert Einstein College of Medicine, New York City

Injection of *B. pertussis* vaccine into mice causes marked hematologic changes which include hyperleukocytosis, depletion of small lymphocytes from lymphatic tissues, increased numbers of polymorphonuclear leukocytes in the splenic red pulp, and splenomegaly(1). Although both the spleen and the bone marrow were examined in the aforementioned study, no erythropoietic changes were noted in these organs. In light of the well-known ability of certain bacteria to stimulate splenic erythropoiesis in laboratory animals(2,3), this problem was reinvestigated with the use of morphologic and isotopic technics.

Methods. Adult, male CF#1 mice (Carworth, Inc., New City, N.Y.) weighing 25-30 g were housed individually and were fed a standard laboratory ration and water. Mice were injected intraperitoneally with 0.3 ml *B. pertussis* vaccine (Parke-Davis and Co., Detroit, Mich.) which contained approximately 18×10^9 killed bacteria in 0.9% NaCl solution with 0.01% thimerosal as a preservative. Control mice were injected intraperitoneally with 0.3 ml sterile, nonpyrogenic saline (McGaw Lab., Inc., Milledgeville, Ga.).

Touch preparations of fresh spleen and bone marrow were stained with a Wright-Giemsa mixture; histologic sections of spleen were stained with hematoxylin and eosin. Mice were injected subcutaneously with 0.1 μc Fe^{59} (E.R. Squibb and Sons, New Brunswick, N.J.) and its uptake measured in bone marrow and spleen(4). Quantitative studies of the local peritoneal fluid cellular response were made with the aid of an electronic cell counter(5). Circulating erythrocyte concentrations were determined with an electronic cell counter. Reticulocytes were stained with new-methylene blue.

Results. Seven days following injection of the vaccine, splenic weight was 320 $\pm$ 6 mg (M $\pm$ S.E.) as compared to 104 $\pm$ 3 mg in saline-injected control mice. Spleen sections revealed a marked decrease in white pulp and hyperemia in the red pulp. Cytologic examination of spleen prints disclosed the presence of numerous foci of developing nucleated erythroid and myeloid cells, their numbers far exceeding those seen in control animals (Fig. 1-3). In contrast to the increased splenic erythropoiesis, the fresh tibial and femoral bone marrow of vaccine-injected mice was grossly yellow as compared to the normal pink appearance seen in all the control animals, and the percentage of nucleated erythroid cells in bone marrow of vaccine-treated mice was only 6 $\pm$ 0.7% as compared to 19 $\pm$ 2.0% in control mice. Nine mice were examined in each group. Granulopoiesis appeared to be stimulated in the vaccine-injected mice as evidenced by the preponderance of myeloblasts and myelocytes, and the frequent appearance of mitosis in these cells.

Peritoneal fluid studies gave further indication of an intense leukocytic stimulation caused by the vaccine (Table I). Although 7 days had elapsed, neutrophils were still abundant. There were many enlarged macrophages containing phagocytic vacuoles; some were in mitosis. Cells resembling plasmocytes were seen and this cell type was never observed in control mice.

Ferrokinetic studies confirmed and extended the morphologic data dealing with

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