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Urine Concentration and Dilution: Effect on Red Cell Survival (39022)

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According to the counter-current theory of urine concentration, osmolality of plasma, interstitial fluid and tubular urine increases progressively from renal cortex to medulla (1–3). However, at any one level parallel to the long axis of the kidney, the osmolality of all three fluids is approximately the same (4, 5). This is attributed to trapping of sodium chloride and urea in the medulla (3, 6, 7). When the kidney is excreting concentrated urine, red cells passing through the renal medulla must, therefore, depending on the species, be subjected to osmotic pressures ranging from two to ten times greater than that of circulating plasma. On the other hand, the excretion of dilute urine as in hydration or diabetes insipidus, would expose red cells to an osmolality of only one-third to one-fourth normal of peripheral plasma. This suggested to us that such an osmolar stress might affect the lifespan of the red cell. Accordingly, we studied red cell survival in dogs subjected to prolonged dehydration followed by rehydration and in rats with congenital diabetes insipidus before and during treatment with pitressin. The results indicate that extremes of hydration or dehydration as reflected in the excretion of dilute or concentrated urine respectively, decreased chromium (Cr)^{51}-tagged red cell survival in the general circulation.

Methods and procedure. Four mongrel dogs weighing 23–26 lb were dehydrated for a period of five weeks by limiting their fluid intake to 250 ml/day. Solid food was not restricted. Body weight, urine output, serum and urine osmolality, blood urea nitrogen and hematocrit were determined repeatedly before and during the period of water restriction. Red cell survival measurements were made at the beginning of the third week according to a modification of the method of Stohlman and Schneiderman (8). Red blood cells (RBC) were tagged with ^{51}Cr by introducing 40 μCi of radioactive sodium chromate into 10 ml of fresh blood collected in anticoagulant acid–citrate–dextrose (ACD) solution. The blood was mixed and incubated for 20 min at room temperature, 25 mg of ascorbate was added and then injected intravenously into each dog. Twenty minutes later and then twice weekly, 30-ml samples of venous blood were collected from each dog. After centrifugation, the red cell sediment of 10 ml of blood was counted in a scintillation counter (Nuclear Chicago Model 33 13A). One hundred thousand counts were measured, corrected for physical decay, and the activity calculated as cpm/ml packed cells. After termination of water restriction, the dogs were given water *ad lib.* for 6 weeks after which ^{51}Cr RBC survival determinations were repeated.

Red cell survival of four rats with hereditary diabetes insipidus (Brattleboro strain)[2] weighing 170–219 g was measured by a modification of the method of McKee *et al.* (9). Under light ether anesthesia, venous blood (0.2 ml) was obtained from the tail vein of each rat using a 1 ml tuberculin syringe which contained a mixture of 3 μCi of ^{51}Cr and 0.2 ml of ACD solution. After incubation at room temperature for 20 min the blood mixture was injected into the tail vein through an indwelling polyethylene catheter which was removed after the mixed control sample was obtained for radioactivity measurement and the results were expressed as cpm ml of whole blood.

These studies were repeated on the same rats after treatment with 0.1 ml of pitressin tannate (4-mU) given subcutaneously daily or every other day for 3 weeks until the study

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was terminated. Urine output was measured daily and urine osmolality was determined by freezing point depression using the Fiske osmometer. Hematocrit was determined by a standard micromethod.

Results. ^{51}Cr RBC survival in the four dogs during water deprivation and the subsequent state of *ad lib.* hydration are shown in Figs. 1 and 2. In Fig. 1 remaining radioactivity expressed as a percentage of original ^{51}Cr value is plotted on a log scale on the ordinate with time in days on the abscissa. The half-life ($T_{½}$) is expressed as the time in days at which 50% of the original activity measured on the first day remains. The $T_{½}$ of dogs 1–4 during the water deprivation period was 8, 5, 2 and 1½ days respectively. After water was given *ad lib.* the $T_{½}$ of the same dogs increased to 23, 14, 13.5, and 23 days, respectively. The parameters used to follow the course of the water restriction period are shown in Fig. 2. The range of values for urine output, urine and serum osmolality, and hematocrit returned toward normal when water *ad lib.* was made available. Blood urea nitrogen stayed within normal range and did not change during the period of study.

The ^{51}Cr RBC survival of four rats with congenital diabetes insipidus before and during pitressin tannate administration are shown in Figs. 3 and 4. The $T_{1/2}$ of the ^{51}Cr-

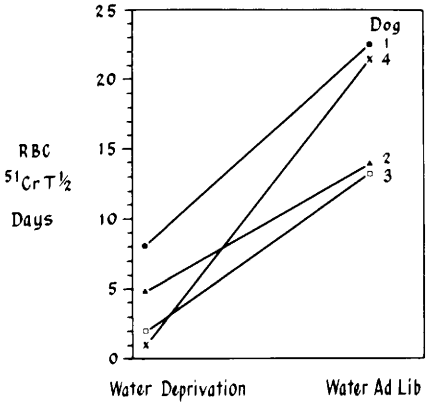

FIG. 1. Change in half-life of red cells of four dogs during water restriction and free water drinking. In each instance half-life decreased during water restriction (water limited to 250 ml/day plus dry food) when concentrated urine was excreted and increased to normal when water *ad lib.* was allowed. Paired *t* test analysis, $t = 5.2547$, $P < .02$.

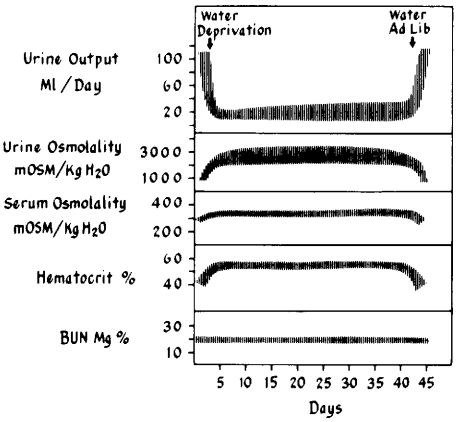

FIG. 2. Urine and blood data in four dogs during water restriction and *ad lib.* The nonuniform limits of the shaded graphs encompasses all the points of the four animals.

labeled RBC of untreated rats was 12, 15, 16, and 13 days, respectively. During pitressin administration, the $T_{1/2}$ increased to 20, 22, 19, and 21 days, respectively. These latter values fall within the normal range of ^{51}Cr RBC survival for a different rat strain reported by others (9). Pitressin tannate administration for 4 weeks produced a marked reduction in urine output and a corresponding increase in urine osmolality in each rat (Fig. 4). The mean daily urine output prior to pitressin ranged from 131 to 195 ml and 20 to 45 ml during pitressin; corresponding ranges for mean urine osmolalities were 150–163 mOsm/kg H_2O and 1532–2137 mOsm/kg H_2O, respectively.

Discussion. In 1951, Wirz, Hargitay and Kuhn (1) introduced a new concept to explain the mechanism of urine concentration. Noting that the loop of Henle resembled a hairpin loop, they postulated that the kidney concentrated urine by means of a counter-current multiplier system in which tubular fluid would progressively become more concentrated as it traveled from cortex to medulla, becoming maximally concentrated at the papillary tip. The anatomical arrangement of Henle's loop and vasa recta which parallel them are such that osmolality of tubular fluid, interstitial fluid, and plasma increases progressively from the outer cortex to medulla. Since equilibrium is complete or nearly so at any horizontal level, little or no

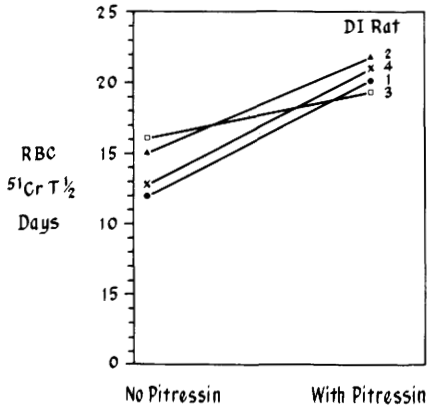

FIG. 3. Changes in half-life of red cells in four rats with diabetes insipidus when left untreated and after pitressin treatment. Life span was reduced in each rat without treatment and normalized with treatment. Paired t test analysis, $t = 5.4611$, $P < .02$.

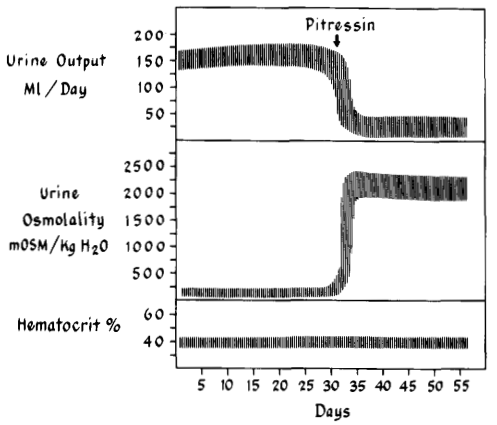

FIG. 4. Urine volume, concentration and red cell hematocrit in rats with diabetes inipidus before and after treatment with pitressin. Changes should be compared with changes in half-life shown in Fig. 3.

gradient exists between these three compartments in the normal kidney (4–7, 10). However, in hamsters with diabetes insipidus, micropuncture studies have revealed a significant difference in osmolality between vasa recta blood and loop of Henle fluid versus collecting duct urine. Osmolality in the former structures ranged approximately between 250–500 mOsm/kg H_2O while at the same horizontal level collecting duct urine ranged between 100–250 (11). Vasopressin renders the distal tubules and collecting ducts permeable to water, facilitating its reabsorption, and thereby permitting the excretion of concentrated urine which in the human can reach a maximum of 1400 mOsm/kg H_2O and in Jerboa 6450 mOsm/kg H_2O (12). In human diabetes insipidus, dilute urine with an osmalality as low as 70–80 mOsm/kg H_2O may be excreted. Therefore, red cells passing through the vasa recta must be subjected to these extremes of osmolality and react accordingly. Indeed, Ullrich *et al.* sampled blood from the papilla of water deprived hamsters and described crenation of red cells (13). In our studies, when medullary osmolality was increased by subjecting dogs to water restriction, survival time of ^{51}Cr RBC in the circulation was uniformly decreased to one-seventh of normal as compared to survival during the period of normal hydration in the same dogs. In untreated rats with diabetes insipidus ^{51}Cr RBC survival was reduced only 30% below the level of the treated state. While plasma osmolality was not measured, it can be assumed that in untreated diabetes insipidus even with free access to water there may be a moderate degree of hemoconcentration and hyperosmolality. Therefore, the 30% reduction in red cell lifespan could result from a combination of red cell exposure to slightly hyperosmotic circulatory plasma and to moderately hypo-osmotic plasma in the vasa recta. Treatment with pitressin would result in exposure of RBC to more concentrated medullary plasma which would tend to reduce red blood survival. However, this effect may be offset by restoring osmolality of circulating plasma to normal.

This study suggests that the kidney, which is recognized as a main source of erythropoeitin and hence an important organ regulating erythropoiesis, may also influence red cell destruction depending on the osmolality of the urine that is excreted. In the rat and dog studies reported here, red cell survival was decreased when the final concentration of urine was very dilute or very concentrated. Whether the rate of erythropoeitin release and urine concentration are related in the nature of a feedback mechanism is unknown.

Conclusion. Survival time of ^{51}Cr-labeled red cells was determined in dogs subjected to water restriction excreting concentrated

urine and again following a 6-week period of *ad lib.* water intake. During water restriction, RBC survival was reduced to one-seventh of normal. In rats with untreated diabetes insipidus, ^{51}Cr RBC survival was reduced 30% as compared to the pitressin-treated state. It appears that RBC survival may be influenced by osmotic stress associated with the state of urine concentration and dilution.

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