SOME effects of water deprivation on renal function of cattle have been described. Most apparent, of course, is oliguresis. When cattle were deprived of water for 4 days, urine excretion was maintained for the first 24 hr. (Weeth, Sawhney and Lesperance, 1967). It then dropped 35% on the second day and was lowered by 70% on the fourth day. Cattle may adapt to infrequent drinking. When urine was collected from heifers on a once per 2 days watering regimen, the urine volume was 33% greater on the second day than on the first day after drinking (Weeth, Lesperance and Bohman, 1968). Urine specific gravity and osmotic pressure increased during water deprivation (Weeth and Lesperance, 1965); however, after 4 days deprivation the osmotic pressure was only 1289 mOsm/kg. Creatinine and osmolal clearances were reduced and plasma tonicity was increased (Weeth et al., 1967). The percent of filtered urea reabsorbed was increased, but the percent of filtered sodium reabsorbed was decreased by 4 days water deprivation.

The glomerular filtrate has been described as an ultrafiltrate of plasma with filtration occurring through a sieve-like glomerular wall having pores 75 to 100 Angstroms diameter (Pappenheimer, 1953). However, Chinard, Vosburgh and Enns (1955) concluded that their data on renal exchange rates were incompatible with filtration through glomerular pores. They proposed a diffusion concept, with the rate of transfer being determined by the diffusion coefficients of the exchanging molecules. Morphological studies of glomerular transport support the diffusion concept (Menefee and Mueller, 1967).

Whatever the mechanism of formation, the glomerular fluid normally contains some protein (Bott and Richards, 1941; Walker et al., 1941) and in proteinuria there can be structural changes in the glomerulus (Farquhar, Vernier and Good, 1957; Bencosme and Morrin, 1967). The present study was undertaken primarily to observe any changes which might occur in the urinary excretion of proteins by cattle during water deprivation. Other blood and urine changes associated with the dehydration are also reported.

Experimental Procedure

Two groups of six Hereford heifers each were subjected to 4 days water deprivation followed by 4 days ad libitum drinking in a single reversal experiment (Brandt, 1938). Initial body weight averaged 317 kilograms. Feed was mixed grass hay fed ad libitum. When serving as a control group, the heifers were managed the same except water was continuously available. Eight recovery days, during which no observations were made, were allowed between the reversal periods.

The experiment was conducted during summer, with heifers haltered in partially shaded pens. Average daily maximum and minimum temperatures were 33 and 3 C, respectively. Relative humidity averaged 40% and water loss from an evaporating pan averaged 7.4 mm per day. Descriptively, the climate was warm during the day, cool at night and dry.

Total daily urine was collected via indwelling, inflation catheters. A few crystals of thymol were added to the collection vessels. The collected urine was weighed twice daily, an aliquot taken, filtered with coarse paper and stored at --19 C. The sample was again filtered through Whatman No. 2 paper before each analytical procedure. On days 0, 2, 4, 6 and 8 of each period a sample of jugular blood was collected into heparinized tubes. In addition a sample was collected from dehydrated heifers on day 4 approximately 1½ hr. after their first drink.

For evidence of anhydremia, hematocrits were determined by capillary tube centrifugation. Plasma protein concentration was estimated from the refractive index (Weeth and Speth, 1968). Plasma proteins were separated by paper-strip electrophoresis in Durrum-type cells using veronal buffer (pH 8.6, ionic strength 0.075), and constant current of 5 ma per eight strip cell for 16 hr. (Block, Durrum and Zweig, 1955). The paper-adsorbed proteins were stained with bromphenol blue. Per-
percentage distribution of the proteins was then estimated with the Spinco Model RB Analytrol.

Total solids concentration of urine was estimated from the refractive index (Weeth, Witton and Speth, 1969). Urine protein concentration was estimated using the Lowry procedure as modified by Sailer and Gerstenfeld (1964), except that the technique of Rigas and Heller (1951) was used to precipitate proteins. The 5% trichloroacetic acid wash was used to separate mucoproteins. It was necessary to concentrate urine proteins before electrophoresis. This was done by vacuum filtration through a collodion sac. The sac was suspended in veronal buffer or physiological saline during filtration. Urine protein observations were made only on samples collected the days of the blood samplings.

Results and Discussion

The heifers lost an average of 53 kg or 16.9% of their initial body weight during the 4 days water deprivation. Animals while drinking ad libitum gained an average of 10 kg during the 8-day periods, so the urine collection routine was not too disturbing. The weight lost during dehydration had been regained after 4 days of rehydration. During the first 13 hr. of rehydration the heifers drank an average of 40 kg of water, which was equivalent to 12.9% of their initial body weight. During the first 24 hr. they drank an average of 64 kg of water. This was equivalent to 120.9% of the weight lost during dehydration. Similar rapid rehydration following 4 days water deprivation has been noted by Bianca, Findlay and McLean (1965) and Weeth et al. (1967).

Urine weight was reduced 31.1% during the first day of water deprivation. It continued to drop, but at a slower rate during the following days (table 1). It decreased only an insignificant 8.8% between the third and fourth days. Excretion on the fourth day was 0.8 kg/100 kg of body weight. Perhaps this was near maximum oliguresis for these

| TABLE 1. MEANS* FOR URINE WEIGHTS AND VARIOUS CONSTITUENTS OF URINE OF HEIFERS DEPRIVED OF DRINKING WATER 4 DAYS FOLLOWED BY 4 DAYS REHYDRATION |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Item                           | 0b              | 2               | 4               | 6               | 8               |
| Excreted, kg/day               |                 |                 |                 |                 |                 |
| Control                        | 9.2             | 9.0             | 9.9             | 9.5             | 9.5             |
| Deprived                       | 7.9             | 3.5**           | 2.1**           | 10.7            | 12.2*           |
| Total solids, g/100 g          |                 |                 |                 |                 |                 |
| Control                        | 6.18            | 6.13            | 6.39            | 6.42            | 6.57            |
| Deprived                       | 7.06            | 8.99**          | 8.63**          | 4.16**          | 4.57**          |
| Protein concentration, mg/100 ml|                 |                 |                 |                 |                 |
| Control                        | 78.8            | 65.8            | 59.4            | 55.7            | 53.4            |
| Deprived                       | 90.9            | 343.6**         | 230.1**         | 47.9            | 30.1            |
| Protein excretion, g/day       |                 |                 |                 |                 |                 |
| Control                        | 6.86            | 5.47            | 5.18            | 4.66            | 4.68            |
| Deprived                       | 6.68            | 11.56*          | 4.75            | 4.19            | 3.40            |
| Albumin concentration, mg/100 ml|                 |                 |                 |                 |                 |
| Control                        | 34.0            | 27.1            | 23.5            | 21.0            | 20.3            |
| Deprived                       | 41.5            | 175.0**         | 111.8**         | 16.1            | 10.6            |
| Globulin concentration, mg/100 ml|                 |                 |                 |                 |                 |
| Control                        | 34.8            | 27.4            | 22.6            | 21.5            | 21.9            |
| Deprived                       | 36.0            | 145.1**         | 93.9**          | 18.0            | 12.6            |
| Ua/Pl: Pae/a                    |                 |                 |                 |                 |                 |
| Control                        | 1.48            | 1.27            | 1.47            | 1.36            | 1.34            |
| Deprived                       | 1.58            | 1.83**          | 2.20**          | 1.59            | 1.41            |

* Twelve observations per mean.
† All heifers drinking ad libitum on day 0.
‡ Drinking ad libitum on all days.
§ Ratio of urine albumin/globulin to plasma albumin/globulin concentrations.
| Differs from control on same day at P<.05.
** Differs from control on same day at P<.01.
animals. The heifers were oliguric on the first day of rehydration. Urine excretion then increased and exceeded that of animals drinking \textit{ad libitum} by 28.6\% on the fourth day. Bianca \textit{et al.} (1965) noted a similar diuresis during rehydration. Urine total solids concentration was increased by deprivation, but daily excretion of total solids was reduced. This is consistent with the decreased osmolar clearance and plasma hypertonicity observed during dehydration (Weeth and Lesperance, 1965). During rehydration the concentration of total solids was reduced. Total solids excretion was reduced in the second day, but not in the fourth day urine.

Hematocrit values rose progressively during water deprivation (table 2). There was no change 1\frac{1}{2} hr. after the first rehydration drink. Hematocrits were still elevated on the second day of rehydration. Bianca \textit{et al.} (1965) reported a sharp rise in hematocrits on the first day of rehydration. Plasma protein concentration followed the same trend during dehydration, both indices reflecting anhydremia of about the same magnitude. The two did not change similarly during rehydration, because protein concentration was reduced at both days 2 and 4 of rehydration. Macfarlane \textit{et al.} (1961) considered plasma protein concentration a more sensitive index in dehydrated sheep.

Plasma albumin averaged 3.11±0.043 g/100 ml, or 40.8\% of total protein, in heifers drinking \textit{ad libitum}. The concentration of albumin did not increase during the hyperproteinemia of dehydration. Plasma albumin concentration was reduced in rehydrating heifers after both 2 and 4 days of \textit{ad libitum} drinking. The hyperproteinemia of dehydration was due to increased globulin concentration. Total globulin concentration was increased 17.0 and 28.8\% on the second and fourth days of deprivation, respectively. The hyperproteinemia resulted mainly from increased concentration of \(\gamma\)-globulin, which was significantly elevated on both the second and fourth days of dehydration. The \(\alpha\)-and \(\beta\)-globulins were increased on the fourth day only. There were differential changes in plasma protein profiles during rehydration. Albumin concentrations were reduced in the heifers while rehydrating, but globulin concentrations did not differ from those of heifers drinking \textit{ad libitum}. There were no changes in plasma protein profiles 1\frac{1}{2} hr. after the dehydrated heifers were allowed to drink.

These changes in protein profiles with water deprivation and rehydration do not agree with
those observed by Wehmeyer (1954). He deprived dairy cows of feed and water for 2 days and noted a 22.3% increase in plasma albumin with no change in globulin. Two hours after the fast, albumin concentrations had fallen to prefast levels, but globulins were elevated. Meyer, Weir and Smith (1955) observed increased albumin concentration in sheep shrunk 36 hours. Albumin concentration then decreased when the sheep received feed and water. Kagan (1943) noted, however, that the hyperproteinemia of dehydration in man was due to increased serum globulin, while albumin remained at the upper level of normal or was decreased. Senay and Christensen (1965) observed augmentation of both fractions in man during dehydration, but the increase in globulin was about double that of albumin. It does appear that the hyperproteinemia seen during dehydration is more than a simple removal of water from plasma.

The urine of heifers while drinking ad libitum contained 62.6 ± 4.87 mg of protein per 100 ml urine. The concentration range was great, from 15.8 to 152.5 mg/100 ml. An average of 18.5% of this protein was soluble in 5% trichloroacetic acid. In human urine 5% trichloroacetic acid soluble protein was assumed to be mucoprotein of renal tract origin (Saifer and Gerstenfeld, 1964). Water deprivation caused a marked proteinuria, with concentrations being elevated 422.2 and 287.4% on days 2 and 4, respectively (table 1). Terry et al. (1948) identified proteins of plasma protein concentration and proteinuria in dogs injected intraperitoneally with plasma. Proteinuria occurred when plasma protein concentration was above 9.7 g/100 ml. In the present study, there was a significant increase in urine protein concentration when plasma protein concentration averaged 8.53 g/100 ml. Total protein excretion was increased on day 2 of water deprivation, but not on day 4 because of the low urine volume and lessered proteinuria. There is no apparent reason for this. Macfarlane et al. (1961) did note with sheep that urine nitrogen excretion usually fell after the third day of water deprivation. There were no significant differences in urine protein concentrations or total excretion on the second and fourth days of rehydration.

The proteins of urine were not well separated by paper-strip electrophoresis. There was a distinct concentration corresponding to plasma albumin. There was a concentration corresponding to plasma α-globulin, but a band in the area of plasma β-globulin was usually poorly separated from adjacent α- and γ-globulin. Others (McGarry, Sehon and Rose, 1955; Rowe, 1957; Miyasato and Pollak, 1966) have encountered problems in concentrating and electrophorizing urine proteins. The ultrafiltration technique did not alter the protein profiles of twelve plasma samples which were compared straight and after ultrafiltration of a 1:50 veronal buffer dilution.

The albumin/globulin (A/G) ratio of urine collected from the heifers while drinking ad libitum was 0.95 ± 0.023. This was significantly higher than the plasma A/G ratio (0.70 ± 0.018). The ratio difference was due to a relatively lower percent of γ-globulin in the urine proteins. The urine protein profile appears to vary with species, and perhaps technique. The protein in dog urine is 60 to 75% albumin (Terry et al., 1948). Rat urine proteins are mostly α- and β-globulin with smaller amounts of γ-globulin and albumin (Sellers et al., 1952). In man urine has a lower A/G ratio than plasma (Rigas and Heller, 1951; McGarry et al., 1955) and the globulin present is mainly α-globulin. Cornelius et al. (1961) identified proteins of plasma and urinary tract origin in sheep urine. The plasma proteins in urine were principally α-globulins, with somewhat less albumin and small percentages of β- and γ-globulin. Packett and Coburn (1955), however, found one-half the protein in sheep urine was mucoprotein. They usually obtained only one band on electrophoresis of dialyzed urine, and serum proteins were rarely detected in urine.

The urine A/G ratio was increased by water deprivation (figure 1), because albumin concentration increased more than globulin concentration. At the same time the plasma A/G ratio was reduced. This is shown in table 1 by the increased urine A/G to plasma A/G ratios on days 2 and 4. The hyperproteinemia and proteinuria of dehydration appear to be differential. A similar differential proteinuria was noted by Terry et al. (1948) in dogs and by Rowe (1957) in man. The latter observed a lower serum albumin concentration in nephrotic serum, but in nephrotic urine the A/G ratio was increased. Sellers et al. (1952) produced proteinuria in rats by injection of renin. The predominant urine protein in these rats was albumin.

The principal filtration barrier between the glomerular endothelium and the visceral epithelium of Bowman's capsule appears to
be the basement membrane (Farquhar, Wissig and Palade, 1961; Bencosme and Morrin, 1967; Menefee and Mueller, 1967). Protein resorption does occur in the renal tubules (Schiller, Schayer and Hess, 1953; Oliver, MacDowell and Lee, 1954; Wilde, Legan and Southwick, 1968). Since the process resembles pinocytosis it probably is not dependent on molecular size.

Whatever the renal changes induced in the dehydrated heifers by the 4 days of water deprivation, the animals apparently recovered during rehydration. Urine protein profiles on the second and fourth days of rehydration did not differ from those of the heifers when drinking ad libitum. This conclusion is consonant with the morphological studies of Terry et al. (1948) and Baxter and Cotzias (1949) on animals recovered from proteinuria.

Summary

Total urine was collected from 12 Hereford heifers during 4 days of water deprivation followed by 4 days of ad libitum drinking in a single reversal experiment. A blood sample was collected on days 0, 2, 4, 6 and 8. The protein concentrations in plasma and urine were determined on these days, and protein profiles were established by paper electrophoresis.

There were differential protein changes in plasma and urine during dehydration and in plasma during rehydration. A hyperproteineemia of dehydration was due to increased globulin concentration. Albumin concentration remained unchanged. The γ-globulins were the principal contributors to the hyperglobulinemia. On the second and fourth days of rehydration, plasma protein concentrations were below those of animals drinking ad libitum because of reduced albumin concentration.

Average urine protein concentration of heifers while drinking ad libitum was 62.6 ± 4.87 mg/100 ml. The A/G ratio of urine (0.95 ± 0.023) was significantly higher than
that of plasma (0.70±0.018). Urine contained relatively less γ-globulin. Water deprivation caused proteinuria, although total urine protein excretion was increased only on the second day. Both urine albumin and globulin concentrations increased during deprivation. However, albumin concentration increased more than did globulin concentration. Urine protein profiles on the second and fourth days of rehydration did not differ from those of the heifers when drinking ad libitum. The heifers apparently had recovered from the proteinuria of dehydration.

Literature Cited

