Schultze and N. S. Mizuno for their interest in this work.

1. Bollman, J. L., Cain, J. C., Grindlay, J. H., *J. Lab. and Clin. Med.*, 1948, v33, 1949.
2. Glenn, W. W. L., Cresson, S. L., Bauer, F. X., Goldstein, F., Hoffman, O., Healey, J. E., Jr., *Surg. Gyn. and Obst.*, 1949, v89, 200.
3. Grindlay, J. H., Cain, J. C., Bollman, J. L., Mann, F. C., *Surgery*, 1950, v27, 152.
4. Mann, J. D., Higgins, G. M., *Blood*, 1950, v5, 177.
5. Rampone, A. J., *J. Appl. Physiol.*, 1959, v14, 150.
6. Shrewsbury, M. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 492.
7. Brown, C. S., Hardenbergh, E., *Surgery*, 1951, v29, 502.
8. Doemling, D. B., Steggerda, F. R., *J. Appl. Physiol.*, 1960, v15, 745.
9. Mizuno, N. S., personal communication.

Received December 18, 1962. P.S.E.B.M., 1963, v112.

## Alterations in Gastric Secretion of Rats Induced by a Potassium Deficient Diet.* (28189)

WILLIAM O. SMITH AND DONALD J. BAXTER (Introduced by G. S. Campbell)
(With the technical assistance of Howard Guiles)
*Medical Service, Oklahoma City V. A. Hospital and Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City*

The importance of potassium in maintenance of functional and structural integrity of the kidney in rats, dogs, and men, has been recently emphasized by numerous observers (1). Other organs of secretion have received less attention in this regard, although 2 previous studies have been carried out on the effect of potassium deficiency on gastric secretion in the rat(2) and in the dog(3). The present report confirms certain findings in these studies and extends the observations.

*Materials and methods.* Forty-eight female Holtzman rats with a starting weight of 160-200 g served as the experimental animals. One half of the rats were fed a potassium deficient diet of the following composition:

| | % |
|---|---|
| Corn starch | 64.2 |
| Casein | 30.0 |
| Butterfat | 3.5 |
| Calcium carbonate | 1.3 |
| Sodium chloride | 1.0 |
| Complete vitamin fortification (including trace elements) | |

This diet contains 0.23 meq of potassium per 100 g of diet by actual analysis. The other half of the animals were fed the identical diet but with 2.0 g of $KH_2PO_4$ added to each 100 g of diet. The rats were housed as triplets in metabolic cages, given demineralized drinking water, and each experimental triplet pair-fed with a control triplet.

Two weeks after the diet was begun, the rats were fasted for 48 hours and had pyloric ligation under ether anesthesia. Four hours later the rats were again anesthetized, blood was drawn from the inferior vena cava, and the gastric juice was harvested by opening the stomachs over a funnel and insuring a total collection. Sodium, potassium, magnesium, calcium, chloride, and phosphorus were measured on plasma and gastric juice, and free HCl and pepsin were additionally measured on the gastric juice. Sodium and potassium measurements were performed on a flame photometer with an internal lithium standard. Magnesium was determined by the technic of Simonsen, Westover and Wertman (4); calcium by an EDTA chelation method (5); chlorides by the Aminco-Cotlove chloride tritrator; phosphorus by the autoanalyzer; and pepsin by a modification of the method of Anson and Mirsky(6).

*Results* in the test animals were compared to those in the control animals in terms of both concentration and total 4-hour secretion of the various substances.

Table I lists values for some plasma electrolytes. The only statistically significant difference is in plasma potassium concentra-

* Supported in part by grant from Nat. Inst. Health, Bethesda, Md.

TABLE I. Plasma Electrolytes.

| | Control diet | K deficient diet | |
|---|---|---|---|
| Chloride | 85 ± 1 | 88 ± 1 | |
| Phosphorus | *9.2 ± .3 | 8.7 ± .2 | |
| Sodium | 144 ± 6 | 146 ± 9 | |
| Potassium | 5.4 ± .2 | 4.7 ± .2 | P<.05 |
| Magnesium | 1.62 ± .14 | 1.98 ± .13 | |
| Calcium | 6.1 ± .1 | 5.8 ± .3 | |

* mg %. All other values are mean ± S.E. in meq/l.

tion, where the test animals had a mean concentration of 4.7 ± 0.2 meq/l as compared with 5.4 ± 0.2 meq/l in the control group. Plasma magnesium concentration was slightly higher in the test animals but the statistical validity of the change is doubtful (p>.05).

The concentration of free HCl in the gastric juice of rats on the test diet was considerably lower than that of controls (72 ± 7 meq/l compared with 98 ± 4 meq/l (Table II). No significant changes were noted in the concentration of the other parameters studied.

In terms of total 4-hour excretion of the various substances, significant differences were observed (Table III). A marked decrease in free HCl, chloride, potassium and pepsin occurred in the deficient animals, as well as a lesser decrease in magnesium. The other parameters remained unchanged.

*Discussion.* Carone and Cooke(2) reported an increase in pH, a decrease in potassium and chloride concentrations, and an increase in sodium concentration in the gastric juice of rats made potassium deficient by diet and desoxycorticosterone acetate. They further showed that the figures returned toward normal after potassium had been restored to the diet. More recently DeMuro and associates (3) have reported a decreased concentration of hydrochloric acid in the gastric juice of dogs treated with desoxycorticosterone acetate. Normal values were restored after potassium replacement.

TABLE II. Gastric Juice—Concentration.

| | Control diet | K deficient diet | |
|---|---|---|---|
| Free HCl | 98 ± 4 | 72 ± 7 | P<.02 |
| Chloride | 157 ± 1 | 152 ± 3 | |
| Phosphorus | *4.7 ± .7 | 7.1 ± 1.2 | |
| Sodium | 28 ± 3 | 36 ± 3 | |
| Potassium | 17 ± 1 | 14 ± 1 | |
| Magnesium | 1.7 ± .2 | 2.6 ± .1 | |
| Calcium | 4.3 ± .4 | 5.7 ± .6 | |
| Pepsin | 13.0 ± .3 | 3.3 ± .4 | |

* mg%.
† mg tyrosine released/ml gastric juice.
All other values expressed as mean ± S.E. in meq/l.

The present study confirms the decrease in gastric juice hydrochloric acid concentration in rats given a potassium deficient diet alone. We were unable to demonstrate a change in chloride or potassium concentration. Although some increase in sodium concentration was observed, the change was not statistically significant (p>.05).

Previous studies(2,3) have not included the measurement of phosphorus, magnesium, calcium and pepsin in the gastric juice. Neither have they estimated total excretion of the various substances during a timed period. In terms of body economy total gastric secretion of a substance may be as important as, or more important than, the concentration of the substance alone. During a 4-hour period total secretion of HCl, chloride, potassium, magnesium and pepsin decreased significantly in the test group; secretion of phosphorus, sodium and calcium was not altered. The decrease in peptic activity suggests that the chief cells are affected in addition to the parietal cells.

TABLE III. Gastric Juice—Total Excretion.

| | Control diet | K deficient diet | |
|---|---|---|---|
| Free HCl | 254 ± 31 | 89 ± 22 | P<.05 |
| Chloride | 402 ± 40 | 210 ± 23 | P<.05 |
| Phosphorus | *117 ± 17 | 107 ± 22 | |
| Sodium | 69 ± 10 | 50 ± 4 | |
| Potassium | 42 ± 5 | 22 ± 2 | P<.01 |
| Magnesium | 4.4 ± .2 | 3.4 ± .3 | P<.02 |
| Calcium | 8.0 ± .7 | 5.8 ± .3 | |
| Pepsin | †72 ± 7 | 44 ± 7 | P<.05 |

* μg/100 g/4 hr.
† μg tyrosine released/100 g/4 hr.
All other values expressed as μeq/100 g/4 hr.

Failure to demonstrate an alteration in concentration or total secretion of sodium in our study is in contrast to the report of Carone and Cooke(2). This may be explained by the fact that these workers gave DOCA to most of their animals in addition to a potassium deficient diet, whereas the rats in our study were given a deficient diet alone.

Presently available knowledge of the role

of potassium in gastric secretory processes is too meager to allow an explanation of these alterations. Certainly, as discussed by Carone and Cooke(2), potassium is essential to the proper operation of energy transferring enzymatic reactions in carbohydrate metabolism and gastric secretory activity requires great energy expenditure.

These functional changes may be likened to those occurring in another secretory gland of high metabolic activity, the renal tubule, when potassium deficiency is induced.

*Summary.* The concentration of free HCl was found to be significantly decreased in the gastric juice of rats eating a potassium deficient diet. Total 4-hour secretion of free HCl, chloride, potassium, magnesium and pepsin was also significantly decreased but secretion rates of phosphorus, sodium and calcium were not altered.

1. Welt, L. G., Hollander, W., Jr., Blythe, W. B., *J. Chron. Dis.,* 1960, v11, 213.

2. Carone, F. A., Cooke, R. E., *Am. J. Physiol.,* 1953, v172, 684.

3. DeMuro, P., Rowinski, P., Calareau, I., Fraghi, A., *Acta Med. Scand.,* 1961, v170, 403.

4. Simonsen, D. G., Westover, L. M., Wertman, M., *J. Biol. Chem.,* 1947, v169, 39.

5. Zak, B., Hindman, W. M., Fisher, M., *Am. J. Clin. Path.,* 1956, v26, 1081.

6. Anson, M. L., Mirsky, A. R., *J. Gen. Physiol.,* 1932, v16, 59.

Received December 18, 1962. P.S.E.B.M., 1963, v112.

## Separation of Sulfated Urinary Acid Mucopolysaccharides Utilizing Cetyltrimethylammonium Bromide and Ethyl Alcohol.* (28190)

GEORGE H. STIDWORTHY, PEGGY JO ELLIS AND RAYMOND G. GOTTSCHALK

*Cancer Research Laboratory, Veterans Administration Center, Martinsburg, W. Va., and Department of Pathology, The George Washington University School of Medicine, Washington, D. C.*

Since Mörner in 1895 reported(1) the presence of sulfated acid mucopolysaccharides and mucopolysaccharide-protein complexes in human urine, limited information concerning these urinary components had been gained until the nineteen fifties when several different methods of preparation were reported. Fractions containing acid mucopolysaccharides have been isolated from urine by dialysis (2,3), heat treatment(4), ethanol precipitation(5), salting out(6), benzoic acid precipitation(4), or with a cationic detergent(7).

This paper deals with a method of quantitative separation and purification of acid mucopolysaccharides and mucoproteins from urine by precipitation with cetyltrimethylammonium bromide (CTAB) and ethyl alcohol. The procedure used mucopolysaccharide labelled *in vivo* with radioactive sulfate.

*Material and methods.* Twenty-four hour specimens of urine, collected from human subjects before and after a single intravenous injection with radioactive sulfate (about one mc of $Na_2S^{35}O_4$†), were preserved with thymol and stored at 4°C until analyzed. The procedure is shown in Fig. 1. An aliquot (usually one liter) from the daily collection was adjusted to pH 7 and allowed to stand in the cold at least 2 hours. The resultant precipitate was removed by centrifugation, dialyzed, assayed for uronic acid and radioactivity. The filtered urine was treated with CTAB (6 g/liter), shaken for one hour on a mechanical shaker and allowed to precipitate in the cold 4 to 6 days. After complete flocculation, the precipitate was removed by centrifugation (1000 × *g*). The supernatant was dialyzed, lyophilized and assayed for uronic acid and radioactivity. The precipitated complex was treated with sodium iodide (5% in ethanol) to release the cationic detergent. The freed quaternary ammonium ion is soluble in ethanol while the mucopoly-

* Aided in part by grant from Nat. Cancer Inst., USPHS.

† Abbott Laboratories.