THE EFFECT OF INTRAVENOUSLY ADMINISTERED 6063, THE CARBONIC ANHYDRASE INHIBITOR, 2-ACETYLAMINO-1,3,4-THIADIAZOLE-5-SULFONAMIDE, ON FLUID AND ELECTROLYTES IN NORMAL SUBJECTS AND PATIENTS WITH CONGESTIVE HEART FAILURE

By CHARLES K. FRIEDBERG, MARK HALPERN,[1] and ROBERT TAYMOR

(From the First Medical Service, The Mount Sinai Hospital, New York, N. Y.)

(Submitted for publication May 26, 1952; accepted September 24, 1952)

Many of the clinical features of congestive heart failure are determined by the abnormal accumulation of water in the extracellular space, an event largely dependent upon the abnormal renal retention of sodium. Although there is a lack of knowledge of the exact mechanism responsible for this abnormality in renal excretion, it is well known that agents that enhance the excretion of sodium are valuable in the treatment of heart failure.

The normal kidney plays an important part in preventing the sodium depletion of body fluids and maintaining the normal range of pH of the blood. This is accomplished by the tubular reabsorption of almost all the sodium which is filtered through the glomerulus and the conversion of the alkaline glomerular filtrate into an acid urine (1). The enzyme, carbonic anhydrase, found to be present in the renal cortex as well as other tissues such as gastric mucosa and erythrocytes of mammals (2), is regarded as an important element in the mechanism for the acidification of the urine (3) and the reabsorption of sodium. The inhibition of carbonic anhydrase and the prevention of the formation of an acid urine may serve as a method for reducing the abnormal sodium retention which is a feature of congestive heart failure.

Following the observation that sulfanilamide induced acidosis (4) with an alkaline urine (5) and an increased excretion of sodium and potassium (6, 7) and the demonstration that sulfonamide compounds were specific carbonic anhydrase inhibitors (8), Höber suggested that the alkalinization of the urine was due to the inhibition of carbonic anhydrase in the renal tubules (9). Subsequently, Pitts and Alexander showed that sulfanilamide reduced the capacity of the kidney to eliminate acid under maximal stimulation by a phosphate buffer load (3).

[1] Fellow of the Dazian Foundation.

Schwartz administered sulfanilamide to three patients with congestive heart failure and observed an increase in sodium, potassium, and water excretion (10). However, this drug was regarded as possessing too low an order of carbonic anhydrase inhibitor activity and as being too toxic for continued use.

Recently a series of more active carbonic anhydrase inhibitors of the sulfonamide group has been developed (11). One of these, the heterocyclic sulfonamide, 6063 (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide), is a carbonic anhydrase inhibitor which is 50 to 400 times more effective than sulfanilamide and nontoxic in effective doses (12). Studies in dogs (13) and a preliminary report of studies in human beings (14) have indicated that this compound markedly increases sodium, potassium, and bicarbonate excretion. This is a report of the study of the urinary excretion of water and electrolytes and of the blood electrolyte pattern following the administration of 6063 [2] to hospitalized patients with and without congestive heart failure.

MATERIAL AND METHODS

The character of the clinical material is presented in Table I. Of the fifteen subjects, there were ten men and five women whose ages ranged from twenty-three to eighty-three years. Three of the subjects had no evidence of cardiovascular or renal disease and were used as controls. The remaining twelve were patients with congestive heart failure of varied etiology as listed in Table I. These patients had dyspnea on exertion, orthopnea, a prolonged circulation time and an increased venous pressure. All the cardiac patients had been treated in the previous few weeks and had little or no clinical evidence of edema at the time 6063 was administered. The one exception was patient J. K., who presented moderate edema of the lower extremities. Two of the control and four of the cardiac subjects were on

[2] Supplied through the courtesy of Dr. J. M. Ruegsegger of Lederle Laboratories.

TABLE I

Patient	Age	Sex	Diagnosis	Sodium intake	Urine flow		24-Hour weight loss	Excretion rate										Urine pH	
								Na μEq./min.		K μEq./min.		Cl μEq./min.		HCO_3 μEq./min.		PO_4 μEq./min.		NH_3 μEq./min.			
					Cont.	Max.		Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Max.	Cont.	Min.	Cont.	Max.
				gm.	*cc./min.*		*Kg.*														
S. E.	55	M	Control	5.0–7.0	3.3	4.3	1.9	75.8	307	51.6	150	105	164	—	—	45.1	67.9	6.3	0.9	(5.5)	(7.8)
J. C.	23	M	Control	5.0–7.0	4.3	7.6	0.5	92.0	545	44.8	246	158	328	—	—	54.8	108	22.8	2.5	6.21	7.62
R. B.	35	F	Control	0.5	2.7	6.3	0.5	17.4	178	18.7	128	47.6	170	2.1	146	36.0	82.0	10.5	7.3	6.00	7.40
M. G.	44	M	Beriberi	5.0–7.0	0.9	1.7	0.9	169	325	73.0	141	—	—	—	—	45.2	77.0	—	—	(5.0)	(7.7)
F. L.	57	M	A.S.H.D.	5.0–7.0	1.8	6.0	2.7	96.3	294	62.8	138	87.3	142	—	—	49.4	62.4	—	3.8	—	7.70
J. K.	42	M	Cor Pulmonal	5.0–7.0	6.7	20.5	1.1	139	920	82.9	214	117	201	66.3	812	65.5	149	17.6	4.9	6.63	7.53
I. Q.	65	M	A.S.H.D.	5.0–7.0	4.5	7.9	1.1	72.2	321	72.7	170	76.2	113	16.2	302	21.3	105	7.9	2.8	6.55	7.50
H. C.	83	M	A.S.H.D.	0.5	2.5	6.6	0.7	20.7	132	28.2	142	15.0	30.0	—	—	26.7	77.2	2.5	1.4	(6.5)	(7.8)
G. B.	56	M	R.H.D.	0.5	0.3	1.7	1.1	7.6	155	14.5	114	15.8	49.3	—	—	30.7	76.5	3.4	1.3	(5.0)	(7.8)
R. H.	39	M	R.H.D.	0.2	0.5	3.6	0.9	2.3	104	35.3	168	22.0	62.1	—	—	5.9	51.0	5.7	1.5	(5.0)	(7.8)
L. I.	37	M	R.H.D.	0.2	0.6	9.2	1.0	1.8	275	21.0	348	6.1	59.6	—	—	35.6	155	13.6	3.2	5.75	7.59
S. R.	47	F	H.C.V.D.	0.2	1.1	3.6	0.7	2.5	50.0	42.8	290	3.1	8.5	11.8	269	23.5	58.4	20.7	1.1	7.04	7.66
S. S.	50	F	R.H.D.	0.2	0.5	2.9	1.5	26.8	196	28.5	144	19.0	31.4	4.6	224	29.0	58.8	6.5	3.2	6.62	7.58
M. B.	28	F	R.H.D.	0.2	0.4	1.6	0.6	0.3	7.5	17.4	67.0	0.7	9.4	0.1	43.3	30.7	47.0	14.8	1.9	5.40	7.59
S. F.	58	F	A.S.H.D.	0.2	3.0	3.6	1.5	7.9	79.2	61.9	122	11.5	30.6	21.6	115	28.2	30.6	7.5	1.5	6.91	7.62

Sodium intake refers to average daily sodium of diet on days preceding the experiment.
Cont.—Average of the rates observed during the control periods.
Max.—Maximum rate observed after 6063. Min.—Minimum rate observed after 6063.
pH values in ( ) determined by indicators. All other pH values determined with glass electrode.
R.H.D.—Rheumatic Heart Disease.
A.S.H.D.—Arteriosclerotic Heart Disease.
H.C.V.D.—Hypertensive Cardiovascular Disease.

a diet containing 5.0 to 7.0 grams of sodium per day prior to the experiment. The remaining subjects were on diets restricted to 0.2 to 0.5 grams of sodium per day for at least three days prior to the day of the experiment (Table I). These differences in dietary intake in the various control and cardiac subjects were not related to the character or severity of clinical manifestations but were arbitrarily determined for the purpose of this study. All subjects were fasting for at least twelve hours prior to the onset of the experiment and received no food during the observation period, which averaged six hours.

Hydration was instituted three to four hours prior to the start of the infusion of 6063 and maintained until the end of the experiment. Except for patients J. K. and S. F., all subjects received 250 cc. of water orally per hour. J. K. received 1,000 cc. of water per hour for four hours prior to the beginning of the control period and 500 cc. of water by mouth per hour until the observations were discontinued. S. F. received 250 cc. per hour for three hours before the control period and during the entire experiment plus an additional 250 cc. at the beginning of the control period and at the start of the infusion of 6063. The volume of fluid given intravenously was included in determining the fluid intake. After a series of control periods, 750 mg. of 6063 in 250 cc. of 5 per cent dextrose in water was administered intravenously over a period of twenty minutes. However, patient M. B. weighed only 42.3 Kg. and received 400 mg. of 6063.

The urine from all the women and three of the men was obtained through an indwelling catheter; the other male subjects voided directly into a container from the standing position. Massage over the suprapubic region was carried out at the end of each collection period to promote emptying of the bladder. In the seven patients in whom the carbon dioxide content of the urine was determined the specimen was passed directly into a vessel containing mineral oil and analyzed promptly. Blood specimens were obtained from a vein and heparin was used as an anticoagulant. The blood specimens for pH and carbon dioxide content were obtained by venipuncture without stasis and drawn into a greased syringe containing heparin and sodium fluoride to prevent glycolysis.

Plasma and urine sodium and potassium determinations were performed on a Janke flame photometer with lithium as an internal standard. Chlorides were determined by the method of Schales and Schales (15), creatinine by the method of Bonsnes and Taussky (16), and phosphorus by the method of Fiske and Subbarow (17). Titratable acidity was titrated with 0.1 N sodium hydroxide, phenol red being used as the indicator. Ammonia content of the urine was obtained by the aeration method of Van Slyke and Cullen (18) as modified by Summerson (19). The pH of the blood was determined at room temperature with a glass electrode and a Cambridge pH meter and converted to body temperature by subtracting 0.01 unit for each degree below 38° C. The carbon dioxide content of blood and urine was determined manometrically by the method of Van Slyke and Neill (20). The bicarbonate content was calculated by the Henderson-Hasselbalch equation with 6.10 as the pK for both blood and urine.

RESULTS

Effect on urinary flow and body weight (Table I)

The urinary flow increased in each case and there was a consistent loss of body weight varying from 0.5 to 2.7 Kg. within twenty-four hours after the administration of the drug. The patients used were almost all free of edema as a result of previous therapy. Since 6063 was being used primarily as a physiological tool to investigate renal behavior in heart failure and not to demonstrate its efficacy as a diuretic, it did not appear important to choose subjects with considerable edema. These facts must be considered in evaluating the relatively moderate increase in urinary flow and loss of weight.

Acid-base factors (Figure 1)

Following the administration of 6063 the urine invariably became alkaline in thirty minutes and remained so for the duration of the observation period of about six hours. The highest urinary pH appeared after one to two hours, generally reaching a slightly higher level than that of plasma and varying between 7.40 and 7.70. Titratable

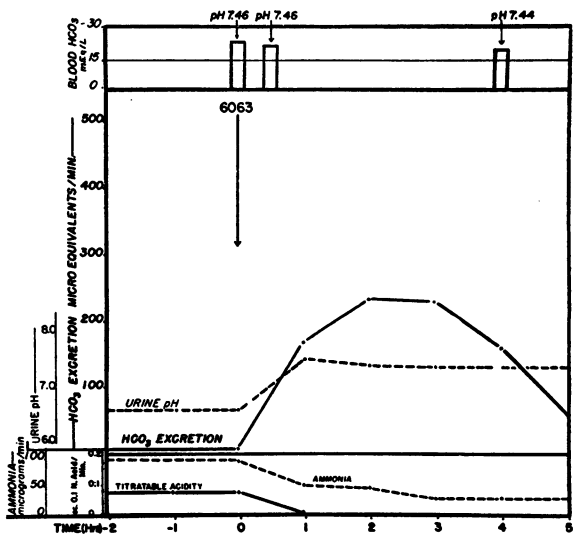

FIG. 1. THE EFFECT OF 6063 ON THE ACID-BASE REGULATORY ACTIVITY OF THE KIDNEY IN A PATIENT WITH CONGESTIVE HEART FAILURE AND ON A DIET RESTRICTED TO 200 MG. OF SODIUM DAILY

pH values at top of figure are those for blood.

acidity rapidly disappeared. The excretion of urinary ammonia was markedly diminished in all cases, but the ammonia continued to be present even when the urine was more alkaline than plasma.

Control specimens of urine were acid except in one patient, S. R., who was on a rice diet, and whose control urine had a pH of 7.04. In the control urines the bicarbonate excretion rate was low, ranging from 0.1 to 21.6 microequivalents per minute. In one patient, J. K., with cor pulmonale, the control bicarbonate excretion rate was 66.3 microequivalents per minute. In all cases there was a marked increase in bicarbonate excretion following the intravenous administration of 6063, the peaks ranging from 43 to 812 microequivalents per minute.

Blood bicarbonate and pH levels disclosed only a slight tendency to acidosis following a single intravenous administration of 6063. However, this evidence of acidosis due to 6063 was much more striking in other patients studied who were given 6063 orally for several days. In one such patient, R. B., who had received 600 mg. of 6063 orally per day for two days, the blood bicarbonate concentration fell to 18 milliequivalents per liter and was further reduced to 15 milliequivalents per liter after an intravenous injection of 6063. Despite the low blood bicarbonate concentration and a blood pH of 7.26 the urine was alkaline, containing a high concentration of bicarbonate and a low concentration of ammonia. The peak rate of bicarbonate excretion was 146 microequivalents per minute.

Effect on urinary electrolytes

The effect of intravenous 6063 on the individual major electrolytes in the urine is represented in Figure 2. These are the findings in S. S., a patient with congestive heart failure, who had been on a 500 mg. dietary intake of sodium. It is apparent that as the urinary flow increased there was marked increase in quantity as well as an increase in concentration of sodium, potassium, and bicarbonate, whereas chloride and phosphate excretion increased only slightly and less than the increase in flow.

The *sodium excretion* increased markedly in every instance, independently of the presence or absence of congestive heart failure. In the two control subjects receiving 5.0 to 7.0 grams of sodium daily, the sodium excretion increased to peak levels of between four and six times the control values. In absolute terms this represented an increase of 231 and 453 microequivalents of sodium per minute, respectively. In the normal subject receiving 500 mg. of sodium daily there was a tenfold increase in the rate of sodium excretion, but the absolute increase was only 161 microequivalents per minute. In the patients with congestive heart failure, the increment in sodium excretion appeared to be related to the sodium intake, being greater in those patients who were previously on a more liberal sodium diet. When the sodium was limited to 200 or 500 mg. daily, the absolute increase in sodium excretion varied from 7.2 to 273 microequivalents per minute. The differences between the increments of sodium excretion could not be correlated with any differences in the degree or type of heart failure or with any difference in responsiveness to therapy. There were insufficient data to determine whether there was a significant difference in the increments of sodium excretion between the control and cardiac groups of patients.

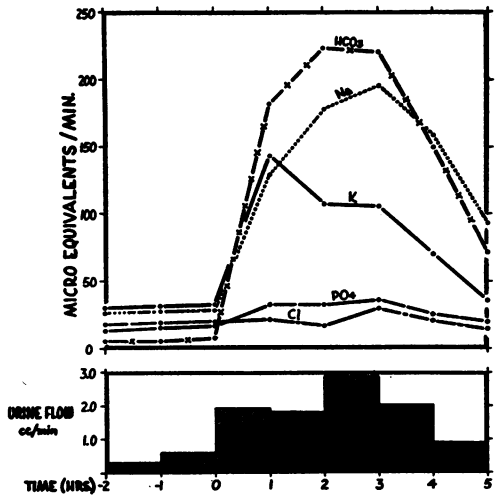

FIG. 2. THE EFFECT OF 6063 ON EXCRETION RATES OF WATER AND ELECTROLYTES

6063 was given at "0" time.

The *potassium excretion,* like that of sodium, increased in all cases after the intravenous administration of 6063. In general the degree of increase in potassium excretion did not vary as much as the increase in sodium excretion. The increment in potassium excretion bore no constant relationship to the increase in sodium excretion.

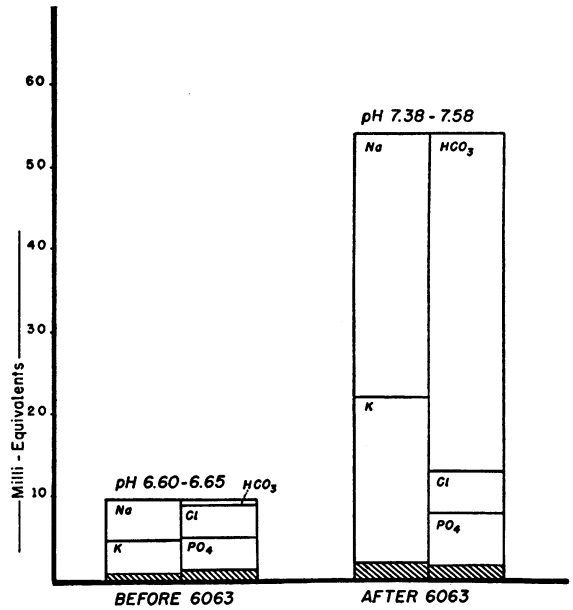

FIG. 3. THE TOTAL ELECTROLYTE CONTENT OF THE URINE FOR A THREE-HOUR PERIOD BEFORE AND A THREE-HOUR PERIOD AFTER THE ADMINISTRATION OF 6063

The cross-hatched areas are inserted as a diagrammatic representation of other unmeasured cations and anions but have no quantitative significance.

Chloride excretion was invariably augmented, but the absolute increase was quite small compared with that noted for sodium, potassium, and bicarbonate. Unlike the concentration of the latter electrolytes, that of urinary chloride was less following 6063 than preceding the administration of the drug. The absolute increase in chloride excretion appeared to be related to the increased urinary flow. The *excretion of phosphates* was increased consistently to the same or to a slightly higher degree than that of chloride.

The effect of 6063 upon the electrolyte spectrum of the urine and the change in total electrolyte excretion is represented schematically in Figure 3. The numbers denote the total excretion of electrolytes, in milliequivalents, for three hour periods before and after the intravenous administration of the drug. There are readily apparent absolute increases in potassium, sodium, and bicarbonate with relatively small increases in phosphate and chloride. In the case depicted, the single intravenous injection of 6063 increased the total excretion of base for a three-hour period by 45 milliequivalents.

Comparison of the effect of 6063 with that of a mercurial diuretic on urinary electrolytes

The difference between the pattern of urinary electrolytes after a mercurial diuretic and that after 6063 is indicated by the following representative observations on two patients, one of whom received an intravenous injection of 2 cc. of mercuhydrin and the other a single intravenous injection of 750 mg. of 6063. Both patients were essentially similar in respect to the type and severity of congestive heart failure and an almost identical diuresis and weight loss were observed in each. After the administration of 6063, the urinary flow rose from 0.6 to 9.2 cc. per minute, whereas after the mercurial the urinary flow increased from 0.2 to 11.4 cc. per minute. Both the onset and peak of action occurred earlier after 6063 than after the mercurial. Peak excretion rates of 275 microequivalents per minute for sodium and 59.6 microequivalents per minute for chloride were observed after the administration of 6063; maximum excretion rates after mercurial were 1,037 microequivalents per minute for sodium and 1,058 microequivalents per minute for chloride. Bicarbonate increased markedly after 6063, slightly after the mercurial.

Plasma electrolyte pattern

Reference has already been made to the observation that the relatively small effective single intravenous dose of 6063 administered in these experiments produced a slight and inconstant tendency towards acidosis as indicated by a reduction in the blood bicarbonate level and blood pH. No significant effect was noted on the plasma concentrations of sodium, potassium, chloride, phosphate, or creatinine.

Toxicity

No toxic effects were observed. The slight chemical acidosis that occurred in some subjects was not accompanied by any clinical symptoms.

DISCUSSION

The observations described above of the effect of the carbonic anhydrase inhibitor, 6063, on electrolyte excretion support the concept of Pitts and Alexander (3) that the enzyme, carbonic anhydrase, in the renal tubules plays an important

part in the acidification of the urine and the conservation of base. According to this theory, carbonic anhydrase accelerates the hydration of carbon dioxide to carbonic acid in the tubule cells and this acid, in turn, provides hydrogen ions which are exchanged for sodium ions in the tubular urine (Figure 4). The hydrogen ions combine with bicarbonate ions in the tubular lumen to form carbonic acid. This breaks down to provide carbon dioxide, which diffuses freely across the tubular membrane and into the tubular cell. The exchange of hydrogen for sodium ions converts the urinary alkaline phosphate to the acid form. The net result is the reabsorption of sodium and bicarbonate and the addition of hydrogen ions to the tubular urine. This process has generally been regarded as limited to the distal tubules (3), which are said to receive approximately the 15 per cent of glomerular filtrate not already reabsorbed in the proximal tubules (21). When, as in these experiments, the carbonic anhydrase was inhibited by 6063, both the sodium and the bicarbonate, which would have been reabsorbed if hydrogen ion were secreted, were now excreted and the urine remained alkaline.

It is of interest that the inhibition of carbonic anhydrase augments the excretion of sodium even from the kidney of a person in heart failure, despite the known tendency in such a kidney to increased reabsorption of sodium. The increment

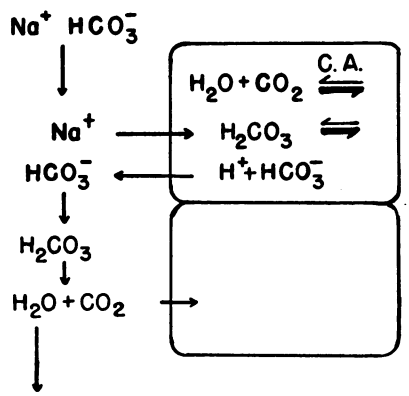

FIG. 4. MECHANISM OF HYDROGEN-SODIUM EXCHANGE AND OF BICARBONATE REABSORPTION

Carbonic anhydrase (C.A.) catalyses the hydration of CO_2 to carbonic acid which is a source of hydrogen ions. Sodium from the tubular urine is exchanged for this hydrogen ion. In the lumen this hydrogen ion combines with bicarbonate to form carbonic acid and subsequently H_2O and CO_2. The CO_2 diffuses back into the tubule cell.

in sodium excretion following 6063 is limited to that portion of filtered sodium which depends on the secretion of hydrogen for its reabsorption. There were insufficient data to indicate whether the inhibition of sodium reabsorption in subjects with heart failure was significantly more or less than in control subjects. Furthermore, this study provides no data to show whether the severity of edema present in a cardiac patient modifies the effect of 6063 on sodium excretion. The degree of increase in sodium excretion due to carbonic anhydrase inhibition appeared to be significantly influenced by the previous dietary intake of sodium. This relationship existed whether or not congestive heart failure was present. These observations suggest that variations in the dietary intake of sodium influence the quantity of sodium excretion related to urine acidification either by altering sodium concentration in the tubular cell or by some other mechanism. The effect of carbonic anhydrase inhibition on sodium excretion appears to be limited or modified by some such mechanism.

The interpretation of the increase in potassium excretion following 6063 is hampered by uncertainty as to the occurrence and quantity of tubular reabsorption or secretion of this ion in the distal tubule under normal circumstances. Studies of potassium excretion following the administration of 6063 to normal dogs (13) indicated that increased potassium excretion after this drug was due to increased tubular secretion of potassium. Berliner, Kennedy, and Orloff (13) suggested that there was competition between hydrogen and potassium for secretion by the tubular cells; inhibition of hydrogen secretion by the carbonic anhydrase inhibitor, 6063, permitted the increased secretion of potassium. The increased potassium secretion following 6063 in our human subjects is compatible with this concept of hydrogen-potassium competition.

It is apparent that the increased bicarbonate excretion accounted for almost all of the increase in anions excreted. Furthermore, it may be postulated that the inhibition of carbonic anhydrase and the consequent prevention of hydrogen ion secretion have their major effects upon the bicarbonate ion in the tubular urine, for unless it reacts with the hydrogen ion the bicarbonate is not reabsorbed. The sodium concentration in glomerular

filtrate greatly exceeds bicarbonate concentration, while in the urine excreted after 6063 the bicarbonate concentration was relatively higher. If the mechanism involved were simply inhibition of the exchange of hydrogen ion for sodium ion, one would expect the rise in excretion rates for sodium and bicarbonate to approximate each other. In this series of cases the rise in bicarbonate excretion exceeded that of sodium in five cases. This might be explained by a simultaneous inhibition of hydrogen-potassium exchange or, as has been suggested by Berliner and his co-workers (22), an exchange between sodium and potassium may take place under appropriate circumstances, with sodium being reabsorbed and potassium excreted.

We have no evidence to indicate whether the slight increases in chloride and phosphate excretion were direct consequences of the inhibition of carbonic anhydrase activity. It may be that the increase in total electrolyte excretion resulted in an increased rate of flow and this in turn caused a slight increase in the excretion of chloride (23). There are considerable experimental data to indicate that a simple osmotic diuresis will not increase phosphate excretion (23, 24). But studies on a group of patients similar to the subjects presented in this paper and under similar conditions of hydration showed a slight but consistent rise in phosphorus excretion during a urea diuresis (25). Since acidosis is associated with an increased phosphate excretion (26–28), it may be considered as a possible cause of the increment in phosphate excretion after 6063. However, the degree of acidosis following the latter drug was usually slight.

Although ordinarily acidosis greatly increases the ammonia excretion for any given urinary pH, ammonia production following 6063 was greatly diminished despite the presence of acidosis. As has been previously suggested by animal experiments by Ferguson (29) and by ammonium chloride experiments by Pitts (30), the results presented in this paper support the theory that the presence of hydrogen ions in the tubular urine is of considerably more importance in stimulating the excretion of ammonia by the kidney than acidosis of the blood. The extreme reduction in ammonia excretion following 6063 appears to be due to the inhibition of tubular secretion by hydrogen ion and consequent reduction in hydrogen ion concentration in the urine. However, ammonia excretion did not completely cease. This may indicate either that tubular secretion of hydrogen was incompletely inhibited or that the hydrogen ions necessary for ammonia excretion were made available by the buffer acids, carbonic acid or acid phosphate, in the alkaline tubular urine.

It is of interest to note that while both the mercurial and 6063 produced a sodium diuresis, after the former the urine usually contains a relative excess of chloride over sodium (31), whereas after the latter it contains a relative excess of bicarbonate and sodium over chloride. If the toxic effect of the mercurial on the tubule impairs tubular reabsorption of all electrolytes including sodium, chloride and bicarbonate, then most of the bicarbonate and some of the sodium may be reabsorbed subsequently by the acidifying mechanism. The chloride is not reabsorbed in the process of acidification of the urine and, therefore, is excreted in excess of sodium and bicarbonate. After 6063, only the electrolytes believed to be involved in the acidification are excreted in significantly larger amounts. There is only a slight indirect effect upon chloride excretion, a moderate increase in sodium excretion, and a maximal increase in bicarbonate excretion. Consequently there is an excess of bicarbonate excretion with acidosis after 6063 and an excess of chloride over sodium excretion with alkalosis after a mercurial.

SUMMARY AND CONCLUSIONS

1. The specific carbonic anhydrase inhibitor 6063 prevented acidification of the urine by the normal human kidney and the kidneys of patients with heart failure.

2. Bicarbonate, sodium, and potassium excretion were markedly increased whereas chloride and phosphate excretion were increased only slightly and ammonia excretion diminished.

3. The absolute increment in sodium excretion in these studies appeared to be related to the sodium intake in the days prior to the experiments and slightly if at all to the presence or absence of heart failure in these cases.

4. Moderate increases in the excretion of water and moderate weight loss in twenty-four hours occurred despite the fact that the cardiac patients had become free of edema previously or were refractory to mercurial diuretics.

5. There was slight acidosis but no significant change in the plasma concentration of sodium and potassium following a single intravenous injection of 6063. However, an intravenous injection of 6063 following two days of oral 6063 led to blood bicarbonate values in the range of 15 milliequivalents per liter. Despite this acidosis there was a marked excretion of sodium and bicarbonate, and the urine was alkaline.

6. The possible mechanism by which the carbonic anhydrase inhibitor, 6063, produced the observed electrolyte changes was discussed. It was postulated that bicarbonate reabsorption in the distal tubule depended upon hydrogen ion secretion, whereas reabsorption of the corresponding sodium ions could be accomplished by either hydrogen ion or potassium ion secretion.

REFERENCES

1. Henderson, L. J., A critical study of the process of acid excretion. J. Biol. Chem., 1911, **9**, 403.
2. Davenport, H. W., and Wilhelmi, A. E., Renal carbonic anhydrase. Proc. Soc. Exper. Biol. & Med., 1941, **48**, 53.
3. Pitts, R. F., and Alexander, R. S., The nature of the renal tubular mechanism for acidifying the urine. Am. J. Physiol., 1945, **144**, 239.
4. Southworth, H., Acidosis associated with administration of para-amino-benzene-sulfonamide (Prontylin). Proc. Soc. Exper. Biol. & Med., 1937, **36**, 58.
5. Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., The toxicity of sulfanilamide. J. A. M. A., 1938, **110**, 252.
6. Strauss, M. B., and Southworth, H., Urinary changes due to sulfanilamide administration. Bull. Johns Hopkins Hosp., 1938, **63**, 41.
7. Beckman, W. W., Rossmeisl, E. C., Pettengill, R. B., and Bauer, W., A study of the effects of sulfanilamide on acid-base metabolism . J. Clin. Invest., 1940, **19**, 635.
8. Mann, T., and Keilin, D., Sulphanilamide as a specific inhibitor of carbonic anhydrase. Nature, London, 1940, **146**, 164.
9. Höber, R., Effect of some sulfonamides on renal secretion. Proc. Soc. Exper. Biol. & Med., 1942, **49**, 87.
10. Schwartz, W. B., The effect of sulfanilamide on salt and water excretion in congestive heart failure. New England J. Med., 1949, **240**, 173.
11. Roblin, R. O. Jr., and Clapp, J. W., The preparation of heterocyclic sulfonamides. J. Am. Chem. Soc., 1950, **72**, 4890.
12. Miller, W. H., Dessert, A. M., and Roblin, R. O. Jr., Heterocyclic sulfonamides as carbonic anhydrase inhibitors. J. Am. Chem. Soc., 1950, **72**, 4893.
13. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J., Relationship between acidification of the urine and potassium metabolism, effect of carbonic anhydrase inhibition on potassium excretion. Am. J. Med., 1951, **11**, 274.
14. Friedberg, C. K., and Halpern, M., Effect of carbonic anhydrase inhibitor 6063 (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) on renal excretion of fluid and electrolytes in patients with congestive heart failure. Federation Proc., 1952, **11**, 49.
15. Schales, O., and Schales, S. S., A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem., 1941, **140**, 879.
16. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.
17. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
18. Van Slyke, D. D., and Cullen, J. E., The determination of urea by the urease method. J. Biol. Chem., 1916, **24**, 117.
19. Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry. Philadelphia, The Blakiston Company, 1947, p. 828.
20. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem., 1924, **61**, 523.
21. Pitts, R. F., Ayer, J. L., and Schiess, W. A., The renal regulation of acid-base balance in man. III. The reabsorption and excretion of bicarbonate. J. Clin. Invest., 1949, **28**, 35.
22. Berliner, R. W., Kennedy, T. J., Jr., and Hilton, J. G., Renal mechanisms for excretion of potassium. Am. J. Physiol., 1950, **162**, 348.
23. Mudge, G. H., Foulks, J., and Gilman, A., Effect of urea diuresis on renal excretion of electrolytes. Am. J. Physiol., 1949, **158**, 218.
24. Havard, R. E., and Reay, G. E., Note on the excretion of phosphate during water diuresis. Biochem. J., 1926, **20**, 99.
25. Friedberg, C. K., and Halpern, M., unpublished observations.
26. Harrison, H. E., and Harrison, H. C., The effect of acidosis upon the renal tubular reabsorption of phosphate. Am. J. Physiol., 1941, **134**, 781.
27. Ollayos, R. W., and Winkler, A. W., Urinary excretion and serum concentration of inorganic phosphate in man. J. Clin. Invest., 1943, **22**, 147.
28. Pitts, R. F., and Alexander, R. S., The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs. Am. J. Physiol., 1944, **142**, 648.
29. Ferguson, E. B., Jr., A study of the regulation of urinary ammonia excretion in the rat. J. Phys., 1951, **112**, 420.
30. Pitts, R. F., Renal excretion of acid. Federation Proc., 1948, **7**, 418.
31. Schwartz, W. B., and Wallace, W. M., Electrolyte equilibrium during mercurial diuresis. J. Clin. Invest., 1951, **30**, 1089.