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The Diuretic action of carbonic anhydrase inhibitor (Diamos) on cirrhosis of the liver : a case study of three patients with cirrhosis of the liver and associated ascites

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THE DIURETIC ACTION
OF
CARBONIC ANHYDRASE INHIBITOR (DIAMOX)
ON CIRRHOSIS OF THE LIVER
A Case Study of Three Patients with Cirrhosis of the Liver and Associated Ascites

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- INTRODUCTION -

Due to the recent successful diuretic action of Diamox in patients with cardiac decompensation (2, 13, 14), an attempt was made in this study to evaluate its diuretic effectiveness in patients with cirrhosis of the liver and associated ascites.

Diamox, 2 acetylamino-1, 3, 4 thiadiazole-5-sulfanamide (6063) was developed in the search for a powerful, non-toxic inhibitor of carbonic anhydrase related to sulfanilamide.

The effect of sulfanilamide on acid-base balance, urinary and blood constituents was observed (1, 15, 23, 37, 38) approximately three years prior to the determination of its actual mode of action as a carbonic anhydrase inhibitor. These studies are worthy of mention, not only for historical purposes, but also for correlation and anticipation of the action of Diamox on the human and animal subject.

HISTORY AND DEVELOPMENT OF CARBONIC ANHYDRASE INHIBITOR AS A THERAPEUTIC AGENT

It was noted by Southworth in 1937 (37) that of fifty cases treated with para-amino-benzene-sulfonamide (Prontylin) in doses of 0.04 and 0.12 gm/kil/day, two cases showed clinical acidosis.

As a result of this a study was performed on fifteen patients using Protylin. CO_2 combining power determinations were made prior to giving the drug and at least one subsequent determination during or shortly after its administration. Each case showed a definite decrease in the CO_2 combining power; this decrease from 1.9 to 27.3 vol % with the mean being 14.1 vol %. In spite of these findings there was no clinical evidence of acidosis. The degree of fall in CO_2 was found to show a moderate correlation with the dose of Prontylin given in gms/kil in the previous 24 hours. In another study of 97 selected cases treated with sulfanilamide (blood concentrations were 10 mgm percent or more) hyperventilation and cyanosis were noted, together with an associated reduction in the CO_2 combining power

(10-15 vol %) and the pH of the urine was quite regularly above 7. (1)

Marshall et al (24) observed that after the administration of 0.1 gm/kil of sulfanilamide to dogs a decrease in the pH of the blood, a decrease in CO_2 content of true plasma and an increase in the pH of the urine was produced. They concluded from these experiments that a definite acidosis due to an alkali deficit was produced by sulfanilamide.

A study of humans by Hartmann et al (15) completely contradicted the findings of Marshall (24) concerning a fall in blood pH after the use of sulfanilamide. As was noted previously that the administration of moderately large doses of sulfanilamide commonly caused hyperventilation which simulated the dyspnea of acidosis (1, 24, 37), suspicion concerning the significance of such overbreathing was aroused; for it was observed that such hyperventilation seemed out of proportion to the rather moderate average reduction of the blood CO_2 content (15-20 vol %). (15) It was demonstrated that, coincident with the fall of the carbon dioxide content of serum, the pH of the urine was usually above 7. This observation led to the conclusion that the change in the acid base equilibrium was actually a carbon dioxide deficit type of alkalosis, secondary to primary hyperventilation and not acidosis.

Two normal adults and two children were studied (15) and changes in pH, bicarbonate content of urine, pH and carbon dioxide content of blood serum, as well as blood sulfanilamide and methemaglobin concentrations were measured after the administration of sulfanilamide. An alkaline urine with a simultaneous reduction of the CO_2 content of blood serum and a slight rise in serum pH was observed in every case. The production of this triad by hyperventilation was described by Collys in 1920. (8) The accepted explanation of these changes is that the hyperventilation lowers the CO_2 tension of the alveolar air and consequently that of plasma. The immediate result of the decreased plasma CO_2 tension is an increase in the ratio of $\frac{BHCO_3}{H_2CO_3}$ with an increase in the plasma pH.

One manifest compensatory response to this alteration is the increased excretion of bicarbonate by the kidney in an effort to reestablish the normal ratio between bicarbonate and carbonic acid, upon which the pH of the plasma depends. The reduction of the base bicarbonate in the serum should not be attributed wholly to the excretion of bicarbonate by the kidney. At an elevated pH of the blood serum, other blood buffers, particularly phosphates and proteinates, claim more base which is yielded by bicarbonate. In addition,

chloride shift from the red blood cell to the plasma would further tend to reduce bicarbonate, as would also the slight and transient ketosis which seems to be frequently associated with the alkalosis of hyperventilation (28) and which was also noted following the sulfanilamide administration.

Although the increase in pH values of the blood serum was not marked, they became more significant when one noticed that if the decrease in CO_2 content of the serum were to be explained on the basis of acidosis one could have expected a definite fall in the pH value of the serum of about 0.10. (28) The only other explanation for a fall in serum CO_2 content with a simultaneous increase in urinary pH to values above 7 is, as Marshall (24) suggests, a failure of the tubules to reabsorb bicarbonate. As will be mentioned later in this paper, the hypothesis proposed by Marshall proved to be correct and the elaborate experiments to contradict this supposition combined the use of ammonium chloride and sulfanilamide with the result that a bicarbonate free urine was maintained. Later experiments have shown that these two drugs seem to be antagonists. (14)

The hyperpnea observed during sulfanilamide therapy may be regarded as a secondary event compensatory to

plasma bicarbonate reduction caused by removal of fixed base in the urine. (3)

A study was undertaken to investigate the effect of sulfanilamide upon the urinary constituents. (38) The administration of this drug resulted in a marked increase both in twenty-four hour urine volume and in the renal excretion of sodium and potassium. The quantity of free ammonia in the urine was not altered significantly. A slight increase in pH of the twenty-four hour specimen was noted. There was no appreciable change in the excretion of chloride, inorganic phosphate or total nitrogen. In both the subjects that were adequately followed the sulfanilamide was almost completely eliminated forty-eight hours after the last dose, and in none of the three subjects did daily urine examinations reveal evidence of renal damage. Blood specimens were negative when examined spectroscopically for sulphemaglobin and methemaglobin, even though the subjects showed a slight blue discoloration.

Two normal dogs were placed on a constant metabolic regime and studies similar to those described in the human subjects were made. However, the changes were much smaller than those observed in the human subjects and the plasma CO_2 combining power was not altered. (38) This is also significant in as much as toxicology of a

drug is often judged by its effect on animals, as was this drug, and no ill effects are noted. The fact that these drugs do not affect the animal in as low a concentration as they do the human subject is not often considered. This applies to the sulfanilamide action upon dogs. This fallacy might also hold true in the current literature quoted by the producers of Diamox.

In 1940 Mann and Keilin (21) noted that the enzyme carbonic anhydrase, which catalyzes the reversible reaction $H_2CO_3 \rightleftarrows CO_2 + H_2O$, is a zinc protein compound and that the activity of this enzyme is very strongly and reversibly inhibited by potassium cyanide, hydrogen sulfide and sodium ozide. These inhibitors are not specific for carbonic anhydrase as they are shared also by other metallo-protein enzymes such as catalase, peroxidase, cytochrome oxidase and phenol oxidase.

Repeated references in the literature to the fact that the administration of some sulfonimide compounds is followed by a fall in the CO_2 combining power suggested the possibility that some of these compounds may have an inhibitory effect on carbonic anhydrase. This supposition was confirmed experimentally by testing the effect of sulfanilamide on the catalytic activity of carbonic anhydrase in blood, in gastric mucosa and in pure enzyme preparation. The results of these experiments show that

the sulfanilamide acts as a powerful inhibitor of carbonic anhydrase, exhibiting marked effects even in concentrations as low as 2×10^{-6} mol. (21)

The sulfonamide group appeared to be directly concerned with the inhibitory effect of these drugs since the replacement of both hydrogen atoms, or even one in the amido group, causes a complete loss of the inhibitory property. The enzyme seems to be an exceptionally sensitive biological test for detecting a few micrograms of an unsubstituted sulfanimide compound. It was also noted that the amino group in sulfanilamide which is responsible for its therapeutic properties (23) is not responsible for its inhibition of the carbonic anhydrase. (21)

It was shown one year later by Davenport and Wilhelm (11) that carbonic anhydrase is present in significant concentrations in the cortex of cat, dog and rat kidneys. Hober's experiments revealed that after the addition of sulfanilamide and of sulfonamides having an unsubstituted SO_2NH_2 group to the Ringer-phenol red perfusion fluid of an isolated frog kidney, the reaction of the secretion turns from acid to alkali, as indicated (16). He assumed that the change of reaction was due to the inhibitory action of sulfonimides upon carbonic anhydrase and that this enzyme is involved as a catalyzer in the reabsorption of bicarbonate from the kidney.

The action by which carbonic anhydrase affects the renal cellular mechanism in the acidification of urine was described by Pitts and Alexander (31) and will be discussed in another section of this paper.

The first therapeutic application of carbonic anhydrase inhibition was described by Schwartz (35) in the treatment of congestive heart failure. It was his contention that if a specific method were available for producing selective inhibitors of the renal tubular reabsorption of the sodium ion from the glomerular filtrate, it might prove useful in the study and treatment of edema.

This work was based upon the findings of Pitts and Alexander (31) in which they demonstrated that the renal tubules must make an active addition of acid to the glomerular filtrate to account for the acidity of the urine. The only source of acid large enough to account for the acidification of the urine was considered by them to be hydrogen ions derived from carbonic acid formed in the renal tubule cells. The loss of hydrogen ions by the tubular excretion would require the absorption of base from the glomerular filtrate to maintain ionic equilibrium. After the administration of sulfanilamide to dogs they found that the titratable acidity of urine fell and its pH increased. The following conclusion was postulated:

"The reduction in tubular excretion of hydrogen ions had resulted from the inhibition of carbonic acid formation."

It appeared to Schwartz (35) that with sulfanilamide administration and decreased acid excretion, the disturbance in acid-base balance should result in failure to reabsorb fixed base from the glomerular filtrate. This loss of fixed base if maintained might be expected to lead to diuresis and a loss of edema. His study of three patients to whom sulfanilamide in doses of four to six grams per day (serum level approximately 12 mgm/100cc) were administered substantiates this hypothesis. All patients showed an increase in excretion of sodium and potassium within the first twenty-four hours of sulfanilamide administration. The two patients in whom it was possible to continue the drug for a seven day period maintained a high daily output of sodium in the urine which was four to five times the control value and exhibited a weight loss that correlated with the increase in sodium output. The urine pH increased in all cases. At the same time there was a sharp fall in CO_2 combining power in serum accompanied by an increase in chloride. These changes can best be explained by an inhibition of carbonic anhydrase in renal parenchyma with retention of hydrogen ions and concomitant diminution

of sodium reabsorption. It was noted that the withdrawal of sulfanilamide resulted in a fall in sodium excretion to lower levels than those observed in the pre-treatment period ("rebound phenomenon") of the two patients observed for a prolonged period.

From a study in which a group of patients receiving two to three grams of sulfanilamide daily Roughton and his workers (34) found that during rest and moderate exercise (five or six times resting metabolism) the rate of CO_2 elimination was not impaired.

CHEMISTRY

Because sulfanilamide is too toxic for prolonged use since it may cause collapse, fever, tachycardia, leucopenia and agranulocytosis (5), it occurred to Roblin and Clapp (33) that heterocyclic sulfonamides might possess a high degree of inhibitory action on the enzyme carbonic anhydrase and provide a non-toxic drug. This idea was based upon the assumption that competition between carbon dioxide or bicarbonate ion and the sulfonamide group might account for the known inhibitory action of sulfanilamide and other unsubstituted sulfonamides on this enzyme. (21) A direct relationship had been established previously between acid dissociation constants of sulfanilamide derivatives and their competitive antagonism of p-amino-benzoic acid. Consequently, although no evidence for a competitive effect is known in the case of the carbonic anhydrase (10), it was anticipated that heterocyclic sulfonamides would be more highly acidic and might therefore exert a more powerful inhibitory action.

They reported upon the preparation of heterocyclic sulfonamides unsubstituted on the sulfonamide nitrogen, since the unsubstituted derivatives are the only type which have been reported to produce a high degree of enzymatic inhibition.

These compounds were synthesized from thio-heterocycles by low temperature oxidative chlorination followed by amidation of sulfonyl chlorides. This method was generally applicable to the preparation of heterocyclic sulfonamides which were not readily obtainable by other procedures.

In studies by Miller (26) using the colorimetric method of Philpot and Philpot (29), compounds were studied in comparison with sulfanilamide which was arbitrarily assigned the value of 1. Consequently, the larger the number the greater the anti-enzymatic activity. The inhibition of carbonic anhydrase by a series of heterocyclic and aromatic sulfonamides has been investigated. Some of the heterocyclic unsubstituted sulfonamides have been found to be 100-2000 times as active as sulfanilamide.

Diamox, 2 acetylamino--1, 3, 4 thiadiazole--5 sulfonamide, has an unsubstituted sulfonamide group, a heterocyclic ring and an acetylated amino group. The compound is a weak acid slightly soluble in water. The amino group, after removal of the acetyl radical by hydrolysis, does not react as a typical arylamine since it does not give a positive Bratton-Marshall reaction. It is 50 to 400 times as potent a carbonic anhydrase inhibitor as sulfanilamide. (26)

Pharmacology

Diamax has been shown to be well absorbed from the gastrointestinal tract of all species studies including man. It possesses a very low systemic toxicity and is excreted largely unchanged by the kidney in 24 hours. The clearance of Diamax is approximately two-thirds that of the simultaneously determined exogenous creatinine in the dog. (22) Studies with this carbonic anhydrase inhibiter in vivo and in vitro show that the interaction between the enzymes and the inhibitor is reversible.

After a single dose in dog, rat, and man, there is a copious diuresis of Na^+, K^+, HCO_3^- and H_2O. This effect in the dog is largely independent of dose in the range 10-1000 mg/kg. The urinary constituents return to normal after several days of administration. This restoration occurs even in treatment in which a high blood concentration of Diamax is maintained, however, metabolic acidosis which may appear by the second day of treatment continues as long as the dosage is maintained. The degree of acidosis, in general, depends on the calculated dosage in the range 1-100mgm/kg/day. Dogs have been kept for 5 months on a 100mg/kg/day dosage, with a blood ph of 7.0-7.2 and plasma bicarbonate of 10-15meq/l. (22)

After the administration of a single dose, or three doses at eight hour intervals, in one day, cessation of treatment is accompanied on the following day by a sharp rise in urinary ammonia, chloride, and titratable acidity; urinary excretion of bicarbonate, sodium, and potassium, are virtually wiped out in the recovery period. Coincidentally, the systemic acidosis rapidly reverts to normal.

Following 5 months of daily treatment and maintainance of acidosis, withdrawal of the drug is reflected in urine only by increased acidity and abolition of bicarbonate. Urinary chloride may rise and within a few days normal acid-base balance in the blood is restored. If the dog is then given Diamax, response is no different from that of the first day.

During the period of daily treatment (10 mg/kg-minimal acidoses) and after the initial diuresis, the stable urinary pattern could be altered maximally by a single larger dose of 200 mg/kg.

Janowitz and his colleagues (18, 19) studied the effects of carbonic anhydrase inhibition in relation to gastric secretion. Dogs with vagally denervated (Heidenhain) pouches were used in these experiments, and acid secretion was stimulated by the subcutaneous injection of histamine. When Diamax was injected intra-

venously into these animals in doses of 20-60 mg/kg body weight, it was found that from 80-97 per cent inhibition of HCL output (M-89 per cent) occurred after a latent period of 20-80 minutes. This inhibition persisted for some 3--6 hrs., but, apparently had recovered by 24 hrs. The following were noted: the inhibition which is manifested by decreased volume flow and acid concentration was never complete; the latent period was related inversely to the rate of secretion at the time of inhibition and, the degree of gastric carbonic anhydrase inhibition was well tolerated.

These findings are in keeping with the hypotheses that carbonic anhydrase activity during acid secretion catalyzes the rate at which carbon dioxide is hydrated to form carbonic acid. This serves to neutralize alkali within the parietal cell and furnishes bicarbonate ions for exchange with chloride ions of the blood. In the absence of this enzymatic activity, the rate of reaction is inadequate for the requirement of an actively secreting gastric mucose and consequently, the rate of formation of hydrochloric acid falls.

Hollander et al (17) report recent evidence to support the premise that carbonic anhydrase plays a major role in the intracellular conversion of CO_2 to H_2CO_3 and, therefore, in formation of HCO_3^- for secretion.

Following the intravenous injection in dogs of a sufficiently potent inhibitor of carbonic anhydrase (Diamax) the bicarbonate output in pancreatic juice stimulated by secretin was reduced as much as 97%. The small residue of about 3% may represent HCO_3^- either formed from CO_2 without the aid of the enzyme or derived as such from the blood.

It is stated in a Bulletin from Lederle based on work done by Maren which is not as yet in publication that the drug is not cumulative. It has no preference for any particular tissue except erythrocytes which it appears to saturate at a fairly low and fixed level for each species. However, there has been no symptomatic evidence of interference with respiratory exchange of carbon dioxide with the use of Diamax.

One may anticipate some symtomatic evidence in exhausting exercise based on the findings of Roughton (34) in which sulfanilamide was the carbonic anhydrase inhibitor used. The findings may be due to some effect of sulfanilamide other than its inhibitory mechanism of this enzyme; however, he found the following: that in exhausting exercise there is damming back of CO_2 which adds to the acidoses of lactate formation and results in prolonged dyspnea during recovery; that there is a psychological and generalized physical handicap in

subjects taking 2-3 grms daily and this is severe enough to make its prophylactic and therapeutic use unsuitable in patients expected to do exacting and strenuous work, especially when skill is required.

The Mechanism of Carbonic Anhydrase Inhibitor In The Kidney

The manner in which Diamox causes diuresis can only be explained adequately by first considering the current view on acid base balance and the ion exchange mechanism for urinary acidification. It is stated by Pitts (30, 31, 32) that the kidney participates in the regulation of body neutrality by stabilizing the plasma concentration of bicarbonate-bound base at a level of 25 to 27 mEq./L, and that the respiratory system also participates by stabalizing the plasma carbonic acid level at 1.25 to 1.35 m Eq./L. Concentrations of those two components together determine the reaction of the blood plasma and interstitial fluid which under normal conditions, is maintained, as is known, at the constant of a pH 7.4.

The problem of the renal stabilization associated with the concentration of bicarbonate is a dual one, involving both salvage of the filtered bicarbonate and restoration of base to the body which is utilized in neutralizing metabolic acids. In a quantative sense, salvage of the bicarbonate from the glomerular filtrate is the more significant; for, every day more than a pound of sodium salt is absorbed by the renal tubules. The efficiency of the absorptive mechanism of the kidney is such that under normal conditions less than 0.10 per cent of that filtered is wasted in the urine. Nevertheless,

following the ingestion of bicarbonate large quantities can be eliminated with only a slight increase in plasma level.

Another important function in acid base regulation is the tubular mechanisms which substitute hydrogen or ammonium ions for sodium ions in the tubular urine. By virtue of these substitutions, metabolic acids may be excreted in free titratable form or in combination with ammonia without sacrifice of the limited stores of body base. Both substituions are carried out by the distal segments of the renal tubules, and certain enzymes, namely, carbonic anhydrase, glutaminase and a group of amino acid oxidases have been assigned specifid functions.

It was demonstrated in animal experiments (31) that the quantity of acid excreted by the kidneys far exceeded the quantity of acid filtered through the glomeruli. Accordingly it has been demonstrated that a cellular mechanism must add acid to the glomerular filtrate as it passes through the renal tubules.

It is suggested that this addition of the acid is affected by a direct exchange of H^+ ions formed within the tubular cells for Na^+ ions which are present in tubular urine.

Acid base balance and ion exchange mechanism in urinary acidification

The diagram below is that of a cell from that portion of the distal tubule which is concerned with acidification of the urine, and will explain diagramatically these mechanisms of ion exchange.

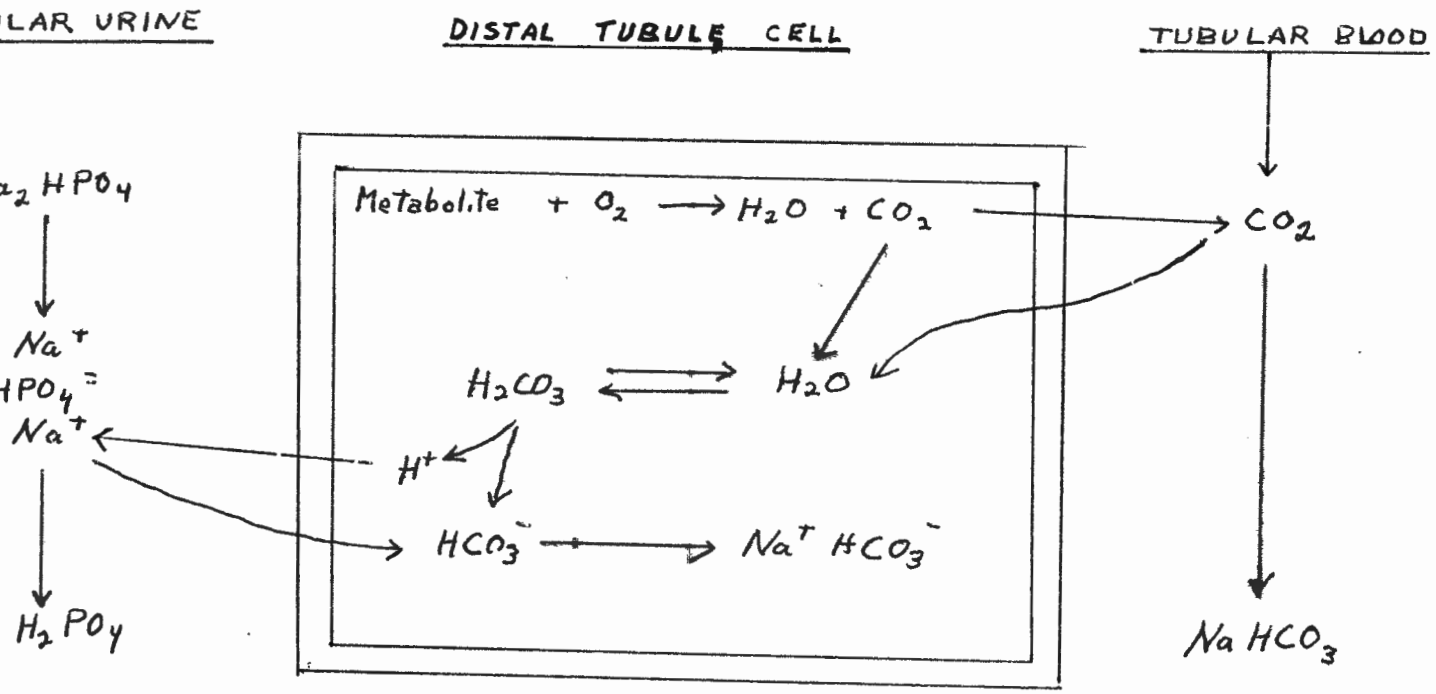

DIAGRAM from Pitts & Alexander

This cell is exposed on one side to the tubular blood and on the other side to the tubular urine. By virtue of its own metabolic activities, as well as its exposure to the renal capillary blood flow a continuous supply of carbon dioxide is made available to the cell. Because of its high concentration of carbonic anhydrase (11), the mediation of urine acidification by the conversions of the dissolved carbon dioxide gas and H20 to carbonic acid is accelerated into carbonic acid. ($CO_2 + H_2O \rightleftarrows H_2CO_3$). The enzyme is probably not essential for when it is inhibited by sulfanilamide, acid elimination continues but at a slower rate. Hydrogen ions which are dissociated from carbonic acid ($H_2CO_3 \rightleftarrows H^+ + HCO_3^-$) are exchanges across the luminal border of the cells for ions of fixed base in the tubular urine. The base, along with an equivalent quantity of bicarbonate is returned to the renal venous blood. The hydrogen ions, along with the anion residue are excreted in the urine as titratable acid.

Due to sulfanilamide which is a carbonic anhydrase inhibitor, the renal tubular replacement of sodium ions with hydrogen ions is effected and it therefore promotes the urinary loss of base. That is the base which would ordinarily be replaced by hydrogen ions and absorbed as bicarbonate is now excreted in the urine in

combination with buffer acids chiefly as disodium phosphate (Na_2HPO_4) and sodium bicarbonate ($NaHCO_3$).

1. Sodium

The Na ions present in tubular urine which enter the renal tubular cells in exchange for H^+ ions are absorbed into peritubular blood as sodium bicarbonate.

2. pK'

The pK' of urinary buffer influences the quantity of acid excreted; the lower the pK' the less acid excreted. Accordingly phosphate is a more effective urinary buffer than creatinine, and creatinine is more effective than p - amino-hippurate. (31)

3. Potassium

Inhibitions of carbonic anhydrase leads not only to loss of the capacity to acidify the urine but also produces a marked increase in potassium excretion. (4)

The amount of potassium usually excreted is approximately 10-15% of the amount made available for excretion by the process of glomerular filtrations. (4)

Extensive and conclusive evidence now indicates that the tubules do have the capacity to secrete as well as to reabsorb potassium, and, in fact, there are some reasons for suggesting that the secretory process contributes significantly to normal potassium excretion even though the total excreted is far below the amount filtered.

It has been known for some time that the administration of neutral potassium salts causes the urine pH to rise. Conversely, the excretion of potassium is increased by alkalosis. (27) In a few instances in which studies have been made, it has been found that alkalotic, potassium depleted individuals excreted a urine which was inappropriately acid and became alkaline only when potassium salts were administered. (20) These observations remained unexplained until the relationships were made even more apparent by the effects of certain carbonic anhydrase inhibitors.

Upon injection of Diamox, intravenously, in acidotic dogs there was noted a marked increase in excretion of potassium, While smaller than the increment of Na on an absolute basis, the increase in potassium excretion was much larger relative to the amounts of each cation filtered. In a number of such experiments, the increment in potassium excretion was larger than the amount filtered. An increase in magnitude was possible only if there was some augmentation in secretion of potassium. That actually all or most of this increment is attributable to secretion can be deduced from the effect of mercurial diuretics. On the augmented excretion produced by Diamox when mercurials are used there is essentially no effect of Diamox on potassium

excretion. Finally, when the mercurial effect is removed, as indicated, by the return of chloride excretion to control levels, potassium excretion goes up to the level usually observed after Diamox. The drugs were then administered in the reverse order. Potassium excretion was restored to control levels when the mercurial effect was superimposed on that of Diamox and rose again after BAL.

Due to these observations, along with those already referred to relating urine acidification and potassium excretion, it seems most logically to explain this by the hypothesis "that in secretion of potassium and hydrogen ions, both species enter into reversible combination with some common component so that the presence of one tends to exclude the other. Then other things being equal, it would be expected that an increase in potassium would reduce hydrogen ion secretion, a decrease in potassium would favor acid secretion, a decrease in hydrogen ion concentration would favor potassium excretion etc." (4)

Considering the relationship between plasma bicarbonate and the excretion of bicarbonate and titratable acid has led to the conclusion that in acidosis little, if any, bicarbonate reaches the distal hydrogen ion secreting mechanism and at the peak of bicarbonate

reabsorption the distal mechanism is responsible for only about 20% of the bicarbonate reabsorbed. (30)

It is worthy of note that the competition between hydrogen and potassium ion for secretion is not on a one to one basis, at least insofar as can be detected. The increment of hydrogen ion excreted which results from inhibition of carbonic anhydrase is considerably greater than the increment in potassium excretion which ensues. This may indicate that the turn-over rate for hydrogen ions is greater than that for potassium or that the reactions which limit the rates of secretions of the two ions are not those involved in the competition. The inequality in turn-over affords a possible explanation for the diuretic actions of potassium salts, that is, their capacity to cause a loss of sodium. Following the administration of potassium salts the ion exchange mechanism in the distal tubule is diverted in part to potassium exchange. This, in itself, involves no increase in sodium excretion since potassium is exchanged for sodium instead of hydrogen for sodium. However, the suppression of hydrogen ion secretion which accompanies this shift is greater than the increase in potassium excretion. The difference between the increment in potassium secretion and the decrement in hydrogen exchange would represent net loss of Na.

Clinical Effects of Diamox on Electrolytes And Urinary Output

The only literature in print to date, as far as the clinical effects of Diamox, has been in regard to its effect upon cardiac decompensation. (2, 13, 14) These findings will be summarized so that a comparison can be made with the findings presented in this paper concerning cirrhosis with ascites.

Water Excretion

In all patients in which Diamox was effective there was an increase in the twenty-four hour urine volume after oral administration. A maximum urine flow of 8 cc. per minute occurred ninety minutes after the oral dose whereas, a maximum urine flow of 10 cc. per minute was attained thirty minutes after the intervenous injection of Diamox in one patient.

Urinary Electrolytes

1. Sodium

The rate of sodium excretion increased in all subjects regardless of the diuretic response to Diamox. Increments in sodium excretion after a single course of Diamox varied from 37 to 188 meq. for a 24 hour period in those patients in which a satisfactory diuresis was obtained. The mean increase was 90 meq. In the group of patients in whom the resultant diuresis was insufficient to control the cardiac decompensation, the

increments of sodium varied from 1.0 to 90 meq. with a mean of 31 meq. over a 24 hour period.

Sodium excretion in the same patient mentioned in relation to diuresis showed an increase from 6 micro eq. to 120 micro eq. after Diamox was administered intravenously and a period of 90 minutes had elapsed. A rise from 1.6 micro eg. to 59.6 micro eg. per minute was noted after orally administered Diamox in the same patient.

2. Potassium

The excretion of potassium increased in all patients except one. No relationship was noted between changes in potassium excretion and the clinical response to the drug. In the responsive group, that being patients in whom diuresis was considered satisfactory, the range of increments of potassium excretion was 10 to 117 meq with a mean of 43 meq. for a 24 hour period. The unresponsive group showed a range from 10 to 118 meq. with a mean of 51 meq for 24 hours.

3. Chloride

The chloride response to Diamox is variable. In 16 patients a slight to moderate increase was noted, whereas in 8 patients the rate of excretion fell. The 24 hour changes varied from a decrease of 52 to an increase of 72 meq.

Plasma Electrolytes

There was no significant changes noted in the Na^+, K^+ or Cl^- levels of the plasma. The carbon dioxide content of plasma fell in most cases after the drug and in some this was accompanied by a slight fall in blood ph.

Dosage

It now appears that the optimal dose of Diamox for the treatment of edema of cardiac origin is 5 mgm/Kg once daily or once in 2 days. Small doses of 250-500 mgn daily are as effective as larger doses and are less likely to be accompanied by paracentesis.

It was also noted that Diamox alone or in combination with mercurial injections had little diuretic effect in the presence of anasarca and renal decompensation. However after the removal of ascitic fluid, the effectiveness of Diamox appeared to be increased.

Toxic Effects

No serious toxic manifestations were noted with Diamox. The mild acidosis was not accompanied by any of the typical signs or symptoms. Drowsiness, lassitude, mild paresthesias and anorexia have been reported as complications of Diamox therapy. (2, 14) No skin manifestations were noted, nor were there any changes in blood counts or urine suggesting any renal or hematopoietic toxicity.

Effect of Diamox In Treatment of Cirrhosis of The Liver With Ascites

a. Introduction

It is stated in a bulletin by Lederle that the results of treating cirrhosis of the liver with Diamox is equivocal, for both promising results (6, 7), and no effect have been reported. (9) As yet neither of these reports has been published.

It was also suggested (12) that careful supervision of these patients should be maintained since in a few instances disorientation and irrational behavior were noted to occur in this type of patient.

The object of this present study was to investigate the possible usefulness of Diamox as an oral diuretic in patients with cirrhosis of the liver and associated ascites.

b. Material and Methods

Three patients were investigated, one female, age 54, two males age 48 and 56 respectively. All patients were hospitalized during the period of study. A complete physical examination and liver function studies were performed on all patients. A definite fluid wave and hepatomegally could be demonstrated in each patient. Several parescentesis were performed in prior months in order to control the accumulation of ascitic fluids;

cell blocks taken from these specimens did not reveal neoplastic cells.

A diet consisting of a high carbohydrate, high protein, high caloric (3600 Cal approx) moderate fat, low salt was provided for these patients.

Controlled fluid intakes were not recorded and patients were allowed to drink fluids ad lib. Strict bed rest was not inforced.

Two twenty-four hour control urine volume specimens were collected together with urine and serum Na^+ and K^+ determinations, and a CO_2 combining power determination before Diamox was administered. The dosage of Diamox in all cases was 250 mgm each day. Daily recordings of 24 hour urinary output, urine Na^+ and K^+ levels, serum Na^+ and K^+ levels, and patients weight were recorded.

The blood chemistry was performed on fasting samples taken at the same time each day before breakfast. Venous blood was used in all determinations, however, it was not taken from the same vein each time.

The patients were weighed at approximately the same time each day. The Beckman Model DU Spectophotometer with flame attachment was used in the Na^+ and K^+ determinations and the Van Slykre method was used for CO2 combining power determinations.

Urine determinations were derived from spontaneously voided specimens.

c. Effect on Water Excretion

Full evaluation of H_2O excretion cannot be made for controlled fluid intake was not maintained as has already been mentioned. However, the 24 hour fluid output increased in all patients after the oral administration of 250 mgm of Diamox. The range of increments (after averaging the first two 24 hour volumes which were used as a control) ranged from 695 cc. to 1185 cc; the mean increment being 967 cc. An increased output was in general maintained throughout the period in which Diamox was administered and there was a slight decrease in urinary volume with the discontinuance of the drug in two cases.

d. Effect of Electrolyte Excretion

The rate of sodium excretion increased in all subjects; increments varied from 42.65 meq./24 hours to 90.28 meq./24 hours for the first 24 hour period. The mean being 59.21 meq. This increase in Na^+ excretion, regardless of the loss of weight, was maintained at a higher level than the initial values which were used as a control.

Potassium

The rate of K^+ excretion was increased in all patients; increments for the first 24 hour period varied from 22.46 to 51.15 meq/24 hour, the mean value being

33.96. An increased K excretion was maintained as long as the patient received the drug.

e. Effect on Plasma Electrolytes

There was no significant change in the serum levels of Na^+ or K^+, however, there was a drop in the carbon dioxide combining power of the plasma in all cases. The CO_2 combining power returned to its approximate initial value soon after the drug was discontinued. The range in increment varied from 1.5 meq. to 5.6 meq.; mean value being 3.22 meq.

f. Weight Response

Only one patient showed evidence of a continued reduction in weight. This patient weighed 194½ initially and after 12 days weighed 185½. The other patients showed no significant reduction; one patient remained approximately the same weight. The other patient continued to accumulate edema fluid even after a paresentesic in which 7,700 cc. of fluid was obtained.

g. Toxicity

No serious toxicity was manifested by the use of Diamox. One patient had the subjective sensation of burning in his eyes, and an erythematous, macular rash appeared on his forehead and zygomatic area of his face. This disappeared in two days after the drug was discontinued. Another patient complained of vertigo which

lasted 4 days. The drug was not discontinued in this individual and the sensation of dizziness left without altering the dosage of Diamox.

h. Discussion

The evaluation of Diamox in the treatment of cirrhosis of the liver with associated ascites is difficult for several reasons: (one) controls are difficult to establish, (two) many factors may influence the effects being measured and (three) because of the limited number of cases studied in this series.

Criteria utilized in the evaluation of the effectiveness of Diamox as an oral diuretic were: urinary output, excretion of Na, and reduction in weight.

Regardless of uncontrolled fluid intake, it is of interest to note that there was an increase in 24 hour urine volume output during the administration of Diamox. The oral dosage of 250 mgm per day being utilized in all cases.

Careful determinations of the daily weights probably serve as a reliable index of changes in water balance from day to day. A significant weight loss was demonstrated in one case; and maintainence of a fairly constant weight was seen in another case in which previous parescentesis were utilized to control the ascites. In the third patient Diamox did not show any significant

effect in weight reduction or maintainence at a constant level.

Na^+ and K^+ excretion increased in all patients after the administration of Diamox. In the one case in which no clinical improvement was noted there was still an increase in the Na^+ and K^+ excretion and urinary volume.

For a more detailed study it would be advisable to obtain a prolonged control period in which input, output, and exact Na intake were calculated. However, as has been suggested in other studies, other factors are introduced that may interfere with the proper evaluation of the effect of Diamox if such a period of control was maintained. Bed rest and restrictive intake of sodium may in themselves be capable of a favorable therapeutic response associated with an increased urinary volume excretion and an increased sodium excretion. These effects might erroneously be attributed to Diamox, whereas a control period of two days with a definite increase in urine volume and Na^+ excretion associated with significant weight loss and clinical improvement appearing in a 72 hour period after Diamox administration would more likely be due to the drug than a change in therapeutic management. It is also of interest that these patients were already on a low salt diet, therefore, it is the interpretation of this study that the increase in sodium

output was due to the effects of Diamox. Although Diamox produced a lowering of the CO_2 combining power of plasma there was no clinical evidence of acidosis.

There did not appear to be any serious toxic effects when the dosage of 250 mgm per day was administered. A skin rash developed but soon disappeared when the drug was discontinued; also, a sensation of vertigo and disequilibrium appeared to disappear without altering the dosage. Diamox was again administered after a few days to the patient who manifested a cutaneous eruption and there was no recurrence noted.

Although there was an increased Na^+ and K^+ excretion with the use of Diamox, the serum levels of these electrolytes were not altered significantly.

i. Summary

A Carbonic anhydrase inhibitor, 2, acetylamine 1, 3, 4, thiadiazole - 5 - sulfanilamide, (Diamox) was employed as an oral diuretic in three patients with cirrhosis of the liver and associated ascites.

Diamox produced a diuresis, weight loss and clinical improvement in patient (#1) a diuresis, maintenance of weight and clinical improvement in patient (#2) and an initial diuresis without any associated improvement in patient (#3).

Toxic side reactions were unimportant but did necessitate the discontinuance of the drug in one case for a few days.

j. Conclusion

The consistent correlation between weight loss, increased urinary output, increased sodium excretion and clinical improvement leads one to the following interpretation: Diamox may be of value in treating certain cases of cirrhosis of the liver. The criteria upon which one can state whether or not a patient will be amenable to this type of diuretic therapy cannot be formulated from this study.

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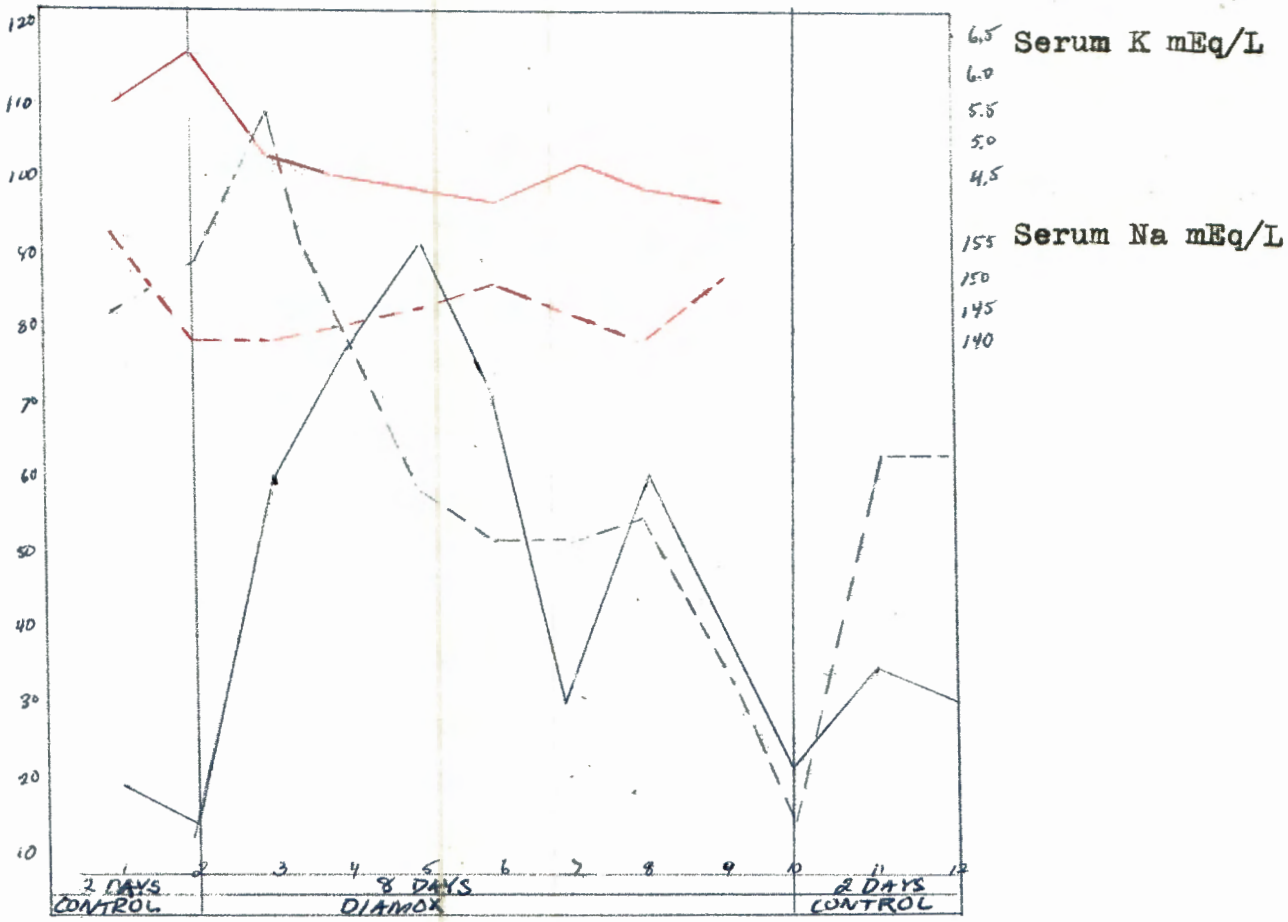
CASE L. H.



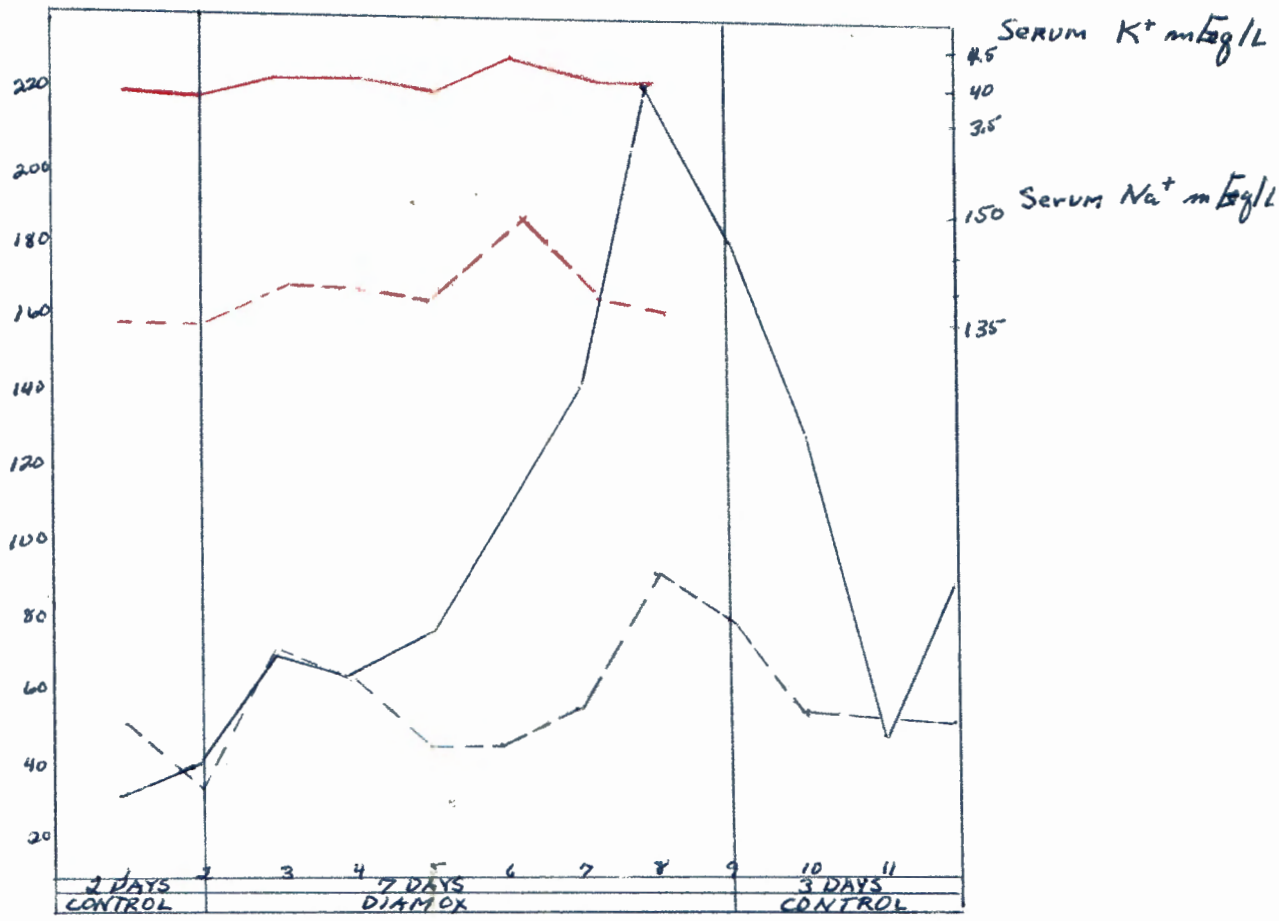
URINE SODIUM ——— mEq/24 hr.

URINE POTASSIUM — — — mEq/24 hr.

SERUM SODIUM — — — — mEq/L

SERUM POTASSIUM ——— mEq/L

CASE P. O.



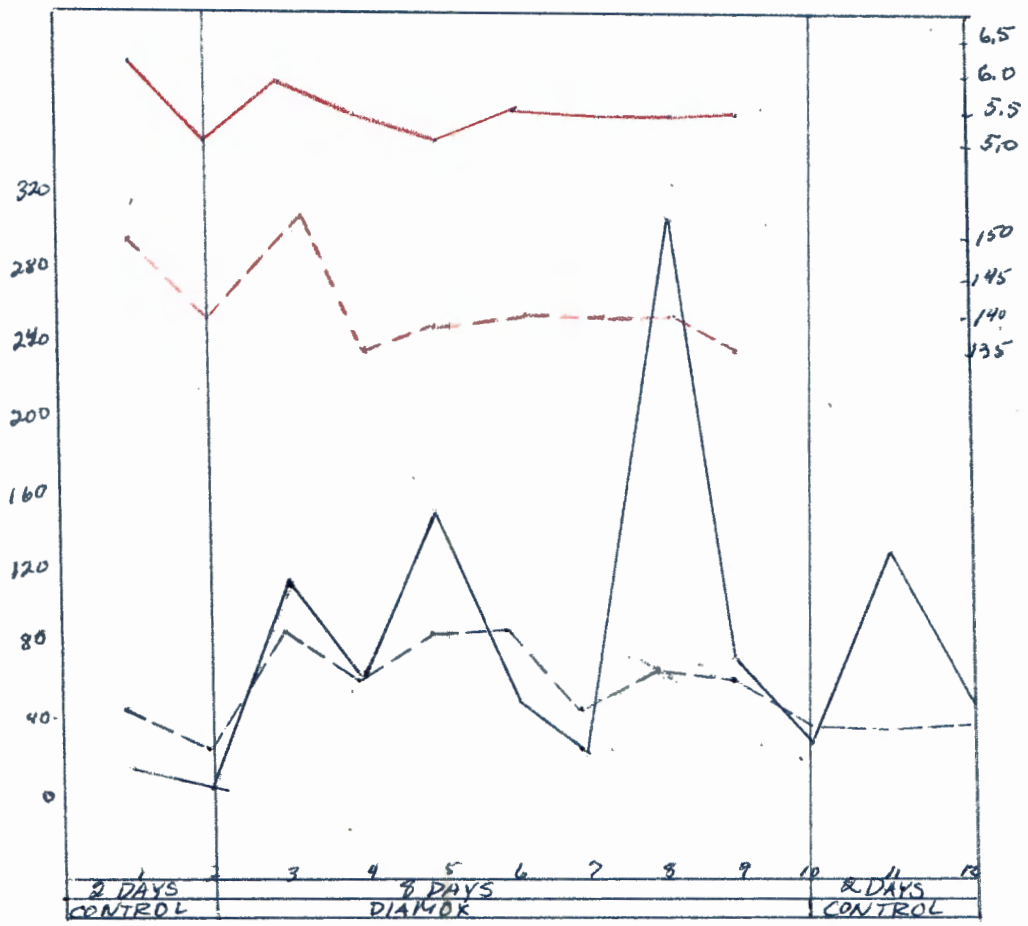
URINE SODIUM ——— mEq/24 hr.

URINE POTASSIUM — — -mEq/24 hr.

SERUM SODIUM — — — mEq/L

SERUM POTASSIUM ——— mEq/L

CASE W. D.



URINE SODIUM ——— mEq/24 hr.

URINE POTASSIUM – – – – mEq/24 hr.

SERUM SODIUM — — — — mEq/L

SERUM POTASSIUM ——— mEq/L

Ostergard, Peter

Date	13	14	15	16	17	18
Weight	194½	194½	194½	192	193	191
Urine						
Output	1350	1900	2810	2330	2120	1580
Na mEq/L	26	8.5	25	28	37	70
Na mEq/24 hr.	35	16.2	70.3	65.2	78.44	110
K mEq/L	39	19.3	25.6	28	21.9	29.8
K mEq/24 hr.	52.7	36.67	72.9	65.2	46.43	47.08
Serum						
Na mEq/L	135	135	140	140	138	150
K	3.9	3.8	4.1	4.1	3.9	4.4
Plasma						
CO_2 comb power		22.06 meq. 48.5 vol.%				

Ostergard, Peter

Date	19	20	21	22	23	24	26
Weight		190 3/4	188½	186½	185½		
Urine							
Output	2300	2950	2600	2025	1860	2300	
Na mEq/L	62	75	72	65	30	48	
mEq/24 hr.	142.6	221.25	187.2	131.6	55.8	100	
K mEq/L	25	31.25	30.9	27.9	27.9	22	
mEq/24 hr.	57.5	92.2	80.34	56.5	51.9	50.6	
Serum							
Na mEq/L	139	137	137				140
K mEq/L	4.1	4.1	4.1				4.6
Plasma							
		46.6 vol.%		42.8%			48.5%
CO_2 comb.		21.2 mEq		19.5meq.			22.1

12/19 - Pt. complains of burning sensations in his eyes; evidence of an erythematous, macular rash on face, forehead, cheeks.
12/20 - Symptoms
12/21 - Rash more evident; stop Diamox
12/24 - Rash completely cleared.

Halberg, Leona

Date	13	14	15	16	17	18
Weight in lbs.	179	182½	182½	180½	-	179½
Urine						
Output	660	710	1380	1160	950	900
Na mEq/L	30 mEq/L	19.	43	65	95	78
Na mEq/24 hr.	19.8	13.49	59.3	75.4	90.25	70.2
K mEq/L	123.75	123.7	77.7	67.5	62	56
K mEq/24 hr.	81.68	87.8	107.2	78.3	58.9	50.4
Serum						
Na	155	140	140	142	145	150
K	5.6	6.4	4.7	4.4	4.2	4.0
Plasma						
CO_2		59.8 vol.%				
Coml. power		27.2 meq.				

Comments
12/16 - pt. feels dizzy
12/17 - vertigo
12/18 - "Feels like I'm going to fall on my face when I walk."
12/19 - slightly dizzy today - "Not bad."
12/20 - no vertigo

Halberg, Leona

Date	19	20	21	22	23	24	26
Weight in lbs.	-	182 P.P.	179	179½	179		
Urine							
Output	1000	660	460	640	890	900	
Na mEq/L	30.0	95	82	33	40	33	
Na mEq/24 hr.	30.0	62.7	37.7	21.1	33.6	29.7	
K mEq/L	51.	83.75	72.5	24	70	68	
K mEq/24 hr.	51.0	55.24	33.35	15.4	62.3	61.2	
Serum							
Na	145	140	150				138
K	4.7	4.3	4.1				4.4
Plasma							
CO_2 comb.		47.5 vol.%		50.4			25.7 m
Power		21.6 mEq		23 meq.			57.0 v

Dougherty, William

Date	13	14	15	16	17	18
Weight in lbs.	165	170¼	171	170¼	171	157
Urine						
Output	833	785-	1830	1600	2300	1760
Na mEq/L	17.5	7.7	55	40	62	33
Na mEq/24 hr.	14.6	6.04	100.6	64	142.6	58
K mEq/L	50.0	35.4	47	38.6	35	49
K mEq/24 hr.	41.7	27.8	85.9	61.76	80.5	85
Serum						
Na	150	140	154	135	138	140
K	6.2	4.9	5.9	5.5	5.0	5.56
Plasma		24 meq.				
CO_2 coml power		53.2 vol %				

Paracentesis
12/17 - 7,700 cc of clear xanthocloromic fluid
Wt 174¼# Wt after 156#
Sp ls 1.009
Total protein 1.75 gm%
Smear - few pusules, r b c, acid f - t neg.

Diamox started 12/15

Dougherty, William

Date	19	20	21	22	23	24	26
Weight in lbs.	157	158	160	162½	162½		
Urine							
Output	2200	2130	2080	1380	1700	1700	
Na mEq/L	10.4	145.0	38	27	75	40	
mEq/24 hr.	22.88	308.85	79.04	37.3	134	68	
K mEq/L	27	36.25	35	40.1	28.75	34.3	
mEq/24 hr.	59.4	77.23	72.8	56	50.46	58	
Serum							
Na mEq/L	140	140	135				140
K mEq/L	5.4	5.3	5.4				5.4
Plasma		51.8		49.4%			
CO_2 comb.		23.3 mEq.		22.5			29.3 meq. 64.5 vol.%

Diamox disc. on 12/22

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