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DIAMOX®

ITS DEVELOPMENT, EFFECT ON BODY FUNCTIONS, AND PLACE IN CURRENT THERAPY

Robert C. Beckman

Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1955

Omaha, Nebraska

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April 2, 1955

To the Thesis Committee:

Mr. Beckman's investigation was pursued with vigor. At my suggestion, he took these pills himself to toxic levels and was able to write his paper with conviction.

Very truly yours,

Robert L. Grissom, M.D.,
Assistant Chairman,
Department of Internal Medicine.

RLG:jo

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INTRODUCTION

For slightly more than one year, a drug with many therapeutic possibilities has been available to the physician. Its pharmacological action is quite different from the commonly prescribed drugs and because of its relatively recent development, its properties may be obscure and its various effects on the organs of the body are very apt to be unfamiliar to the practioner as well as the medical student. It was this unfamiliarity with the drug which stimulated the writer to search the literature and produce the report which follows.

HISTORY OF CARBONIC ANHYDRASE INHIBITORS

Diamox® was discovered by Roblin and his colleagues in the laboratories of the Chemotherapy Division of the American Cyanamid Company at Stamford, Connecticut. Its development climaxed some ten years of research to produce a compound related to sulfanilamide which possessed its power to inhibit the enzyme carbonic anhydrase but was of low toxicity and free of bacteriostatic properties.

The search for an inhibitor of carbonic anhydrase began almost immediately after the isolation and description of this zinc protein enzyme in 1932 by Meldrum (1) and also by Brinkman and Margaria (2). Davenport (3,4,5) did much of the early research trying to determine the location and function of carbonic anhydrase in the various tissues of the body. He used thiocyanate and cyanide in his experiments as chemical inhibitors of the enzyme and perhaps he deserves the credit as the originator of the idea of a carbonic anhydrase inhibitor. Just prior to much of Davenport's work, Southworth (6) at Johns Hopkins Hospital published a most significant report of two cases of clinical acidosis which had developed after the administration of Para-amino benzene sulfonamide (Prontylin). Apparently this was the first report of certain sulfonamide compounds having an effect on the electrolyte metabolism of the body. He became interested in this effect of the then new sulfa drugs and studied fifteen cases in which the patients had received Prontylin and demonstrated in each case a consistent drop in the C O_2 combining power of the patient's plasma. Bosman and Perley (7) noticed that sulfanilamide produced a constant alkalinization of the urine in patients receiving the drug. Marshall (8) and his co-workers

reproduced in dogs these electrolyte changes by giving them sulfonamide. How sulfonamide was causing these changes was not understood until Mann and Keilin's (9) significant observation in 1940 that unsubstituted sulfonamide compounds were specific inhibitors of carbonic anhydrase. It was in 1942 that Mober (10) suggested the alkalinization of urine produced by sulfanilamide was due to the inhibition of carbonic anhydrase in the renal tubules. Davenport (11) had already proven that sulfanilamide was capable of inhibiting the secretion of acid by the gastric mucosa of dogs. Wood and Favour (12) also had shown that sulfanilamide could affect the carbonic anhydrase activity of the erythrocyte. Beckman (13) showed that sulfanilamide produced a sodium and potassium ion loss in the urine with a corresponding decrease in serum sodium, potassium, and C O_2 combining power.

Many investigators became interested in the role of carbonic anhydrase in the tissues and the alteration in the function of the tissues produced by inhibiting the enzyme with sulfanilamide. Such investigation is still in progress and many aspects of the problem unsolved. However, much benefit has accrued to clinical medicine. The first clinical

application was tried by Schwartz (14) in 1949 when he was interested in determining the effect of sulfanilamide on salt and water excretion in patients with congestive heart failure. All patients studied showed an increase in sodium and potassium excretion within the first twenty four hours after sulfanilamide administration and exhibited a significant weight loss. Because of the toxicity of sulfanilamide, the experiments had to be discontinued. However, the investigators were impressed with the effects produced in these cardiac patients and research to develop new compounds related to sulfanilamide which were capable of inhibiting carbonic anhydrase but had less toxicity was started.

The climax for the search occurred in 1950 with the development of the new series of heterocyclic sulfonamides by Dr. Roblin (15). These heterocyclic compounds proved to be very potent inhibitors of carbonic anhydrase and have very low toxic properties. One member of this new series of chemicals was 2-acetylamino 1,3,4 thiadiazole 5 sulfonamide, acetazoleamide, whose commercial name is Diamox®.

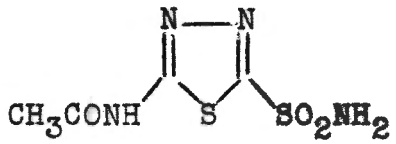
CHEMISTRY OF DIAMOX®

The heterocyclic sulfonamides were synthesized from thioheterocycles by low temperature oxidative chlorination followed by amidation of the sulfonyl chlorides.

Roblin's (15) interesting description which follows aptly explains the reasoning which led to the synthesis and investigation of the heterocyclic compounds for carbonic anhydrase inhibition activity. "In connection with a study of carbonic anhydrase inhibitors, it occurred to us that heterocyclic sulfonamides might possess a high degree of inhibitory action. This idea was based on the assumption that a competition between CO_2 or bicarbonate ion and the sulfonamide group might account for the known inhibitory action of sulfanilamide and other unsubstituted sulfonamides on this enzyme. A direct relationship had been established previously between the 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 carbonic anhydrase, it was anticipated that heterocyclic sulfonamides would be more highly acidic

and might therefore exert a more powerful inhibitory action."

The structure of Diamox® is:



The compound is a weak acid, slightly soluble in water. A soluble sodium salt may be prepared. The amino group after removal of the acetyl radical by hydrolysis does not react as a typical arylamine; it does not give a positive Bratton-Marshall reaction.

PHARMACOLOGY

The pharmacological action of Diamox® may be simply stated to be that of a specific inhibitor of the enzyme carbonic anhydrase. However, the situation becomes extremely complex in trying to understand the role of carbonic anhydrase in the overall physiology of the body. The enzyme has been found in erythrocytes, gastric mucosa, kidney, pancreas, brain, lens, ciliary body, testes, spleen, mucosa of duodenum,

ileum, and jejunum (16). The enzyme is definitely absent from normal plasma, urine, milk, sclera, gall bladder, skin, lungs, and thymus. Only trace amounts of carbonic anhydrase have been isolated from striated muscle, heart muscle, adrenal, spinal cord, and retina.

The Effect of Carbonic Anhydrase Inhibitors on:

Respiration: Without carbonic anhydrase the release of the amount of carbon dioxide liberated from the blood during one passage through the lungs would go to 90% of equilibrium in about one hundred seconds. Since erythrocytes spend less than one second in the lung capillaries, the need for enzymatic catalysis is readily seen. There is at least one thousand times as much enzyme present in the red blood cells as is needed to furnish the required catalysis for the dehydration of carbonic acid in the lungs. This explains the failure of carbonic anhydrase inhibitors to cause marked respiratory changes. Becker, Hodler and Fishman (17) were unable to detect any indication of impaired carbon dioxide transport in man when 50 mg/Kg of Diamox® were given by mouth. Roughton (18) showed that only in exhausting exercise was there some definite handicap to carbon dioxide removal resulting

in the accumulation of carbon dioxide when therapeutic blood levels of sulfanilamide were administered.

Renal Physiology: The kidney is the site where the role of carbonic Anhydrase has been best worked out. Carbonic anhydrase mediates the process of urine acidification by accelerating the conversion of carbon dioxide and water to carbonic acid ($CO_2 + H_2O \rightleftharpoons H_2CO_3$) in the distal tubular cells (19, 20). The ionization of carbonic acid to form hydrogen ion and bicarbonate ion ($H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$) is thus indirectly dependent on carbonic anhydrase activity. The hydrogen ions so formed take the place of the sodium ions of the buffers of the glomerular filtrate, chiefly in di-sodium phosphate (Na_2HPO_4) and sodium bicarbonate ($NaHCO_3$). The excretion of sodium acid phosphate (NaH_2PO_4) in the urine conserves sodium which returns to the blood as does the carbonic acid which is formed. The formation of ammonium ion, which also conserves base by buffering non-volatile strong acids is also dependent on the supply of hydrogen ions (19, 20).

When carbonic anhydrase activity is inhibited by Diamox®, much less hydrogen ion is available for the acidification process. The sodium ion of the buffers, no longer being replaced by hydrogen ion,

is excreted. Filtered bicarbonate ion, no longer forming carbonic acid in the lumen, is also excreted. Thus the urine after Diamox® administration contains sodium bicarbonate. At the same time, lack of hydrogen ion decreases the formation and excretion of ammonium ion. The excretion of potassium ions when Diamox® is given is a result of the decrease of hydrogen ion production. Berliner (21,22) has shown that potassium ions compete with hydrogen ions in the tubules for excretion. Hence, decrease of hydrogen ion secretion leads to an increased potassium output. Maren (23) has worked out the cyclic nature of response and recovery following Diamox®. These studies show that the loss of base occurs only in the six to twelve hour period following Diamox® administration. When low doses are given once a day for diuretic effect, full carbonic anhydrase activity begins to return six hours after dosage. With resumption of enzyme activity, bicarbonate, sodium, and potassium ions are retained and the titratable acidity and secretion of ammonium ions increase above pretreatment levels.

Gastric Secretion: Davenport (4,5) in 1939 described the presence of carbonic anhydrase in very high

concentration in the parietal cells of the gastric mucosa of cats and rats, and in 1941 he (11) demonstrated that the rate of acid secretion by the gastric mucosa could be diminished by the administration of high doses of sulfanilamide. Davies (24) reported in 1951 the complete inhibition of hydrochloric acid secretion in isolated frog and toad gastric mucosa by using p-sulphonamide benzoate. Because of the very high concentration of carbonic anhydrase in the gastric mucosa, very large amounts of inhibitor are required to affect the secretion of hydrochloric acid. Davies (24) estimates that only about 0.3% or less of the enzyme activity extractable from the oxyntic cells is actually required to catalyze the uptake of carbon dioxide even during high rates of hydrochloric acid secretion. Rehn (25) working with dogs found that Diamox® given intravenously did affect the rate of acid secretion in dosages of 40 mg/Kg, but even with such high dosages as 200 mg/Kg the secretory rate was not completely inhibited in some experiments. McGowan (26) gave eight patients doses of 5 to 30 mg/Kg of body weight without significant suppression of gastric acidity. However with increased dosages of 65 to 70 mg/Kg of body weight he found a definite decrease in acid concentration.

Interesting enough, two of these patients had active duodenal ulcers and two had inactive duodenal ulcers. As will be discussed later, the dosages required to decrease the acid secretion are in the range wnere unpleasant side reactions are experienced; therefore, the application of Diamox® in treating peptic ulcer patients is not practical.

Pancreatic Secretion: Carbonic anhydrase has been found in the pancreas in significant concentration but the mechanism of pancreatic external secretion still is uncertain. Birnbaum and Hollander (27) have shown in dogs with duodenal fistulae that intravenous Diamox® caused a reduction in the volume response of pancreatic secretion to secretin.

Ophthalmic Physiology: Carbonic anhydrase has been demonstrated in the ciliary body of the rabbit eye. Kinsey (28) determined that bicarbonate is present in higher concentration in the aqueous humor than in plasma. This is very suggestive of a functional role of carbonic anhydrase in the secretion of aqueous. Grant and Trotter (29) showed that Diamox® given intravenously was capable of producing a drop of intraocular pressure in rabbits, but it had no effect when dropped on the cornea or injected subcutaneously.

The presence of a high concentration of carbonic anhydrase in normal bovine lenses was demonstrated by Bakker (30) in 1939. In senile cataractous lenses, the carbonic anhydrase activity was found to be greatly reduced. The reduction correlated fairly closely with the degree of the opacity of the lens. This observation was made in cattle and also humans. Whether age affected the carbonic anhydrase activity in non-cataractous lenses was studied by Reich and Healy (31). No significant variation was found in the mean values representing the lens enzyme activity of three different age groups of cattle. Their conclusion was that it was unlikely that any causal relationship exists between a low lenticular carbonic anhydrase activity and senile cataractous change. This naturally assumes great importance in humans treated with Diamox® over a long period of time.

The decrease in intra-ocular pressure was shown by Becker (32) to occur in nephrectomized rabbits, and also in dogs and man made unresponsive to the diuretic action of Diamox® by ammonium chloride acidosis or by repeated doses of Diamox®. The reduced intra-ocular pressure produced seems to be a direct effect on the inhibition of formation of the aqueous humor and not due to the action of Diamox® on some other organ as the kidney.

Diamox® produces only a slight decrease in intra-ocular pressure in normal eyes. Becker (33) observed in eight normal eyes the following drop in intra-ocular pressure after administration of 500 mg of Diamox®:

Before:	17	17	15	15	17	17	19	19	mm. of Hg.
After:	12	12	11	11	14	14	17	17	mm. of Hg.

Grant and Trotter (29) investigated the effect of Diamox® in normal eyes using 250 mg doses twice a day for one to five days. With such dosages, no change in intra-ocular tension was noted in eight normal eyes and only 1-2 mm. decrease in eight other normal eyes.

The decrease in intra-ocular pressure in cases of glaucoma is very dramatic and will be discussed later under therapeutic uses of Diamox®.

The Fetus: It is interesting to note that Davenport (16) found that the carbonic anhydrase concentration of erythrocytes of fetal goats was very low and this has also been observed in premature infants. The concentration of the enzyme rises abruptly as term approaches. The origin of the enzyme has not been determined.

In animal experiments, there is one report (34) of an unfavorable reaction in one litter of puppies

born to a dog which had been on high dosage of Diamox® (100 mg/Kg) for the full period of gestation. There were only two offspring. Both died in the first week after birth. At autopsy one had subdural and intraventricular hemorrhage and the other puppy had an area of necrosis at the apex of the myocardium and also pneumonia. Six months after being taken off of the drug, this mother was mated and delivered a litter of five normal puppies. Another dog had received 33 mg/Kg per day orally for the last third of gestation and five days post partum. At term a litter of five was born. All developed normally. Thirteen pregnant rats received Diamox® in their diet for the last week of term in dosages of 50-300mg/Kg per day. Each litter had a normal partuition. Other attempts to reproduce adverse effects in newborn of various animals have not been successful. In the one instance in which unfavorable results were obtained, the dosage was very high and would never be equalled in therapy of human patients. The drug is considered safe for administration to pregnant patients experiencing pre-eclamptic symptoms and signs.

Central Nervous System: Ashby (35,36,37,38,39) has shown that carbonic anhydrase is present in the

nervous system of various species in patterns which tend to be peculiar to the species. There is usually a progressive rostral increase in carbonic anhydrase content of the tissue. The enzyme has been found to be present in both white and gray matter. Since no function for carbonic anhydrase has yet been discovered in the nervous system, it is extremely difficult to evaluate what effect Diamox® may have on this system. However the toxic symptoms of drowsiness and paresthesias observed in patients receiving daily dosages of one gram or more may be due to the effect of inhibition of carbonic anhydrase activity in the central nervous system. Roughton (18) in his studies of some effects of sulfanilamide on man observed a significant impairment in three subjects in their response to the Jonnson Code test which is assumed to measure a cortical level of response. The Woodworth-Wells Form Naming Test and Pursuit Meter were not significantly affected. There has been no record in the current literature of repeating these tests using Diamox® instead of sulfanilamide, but similar results would be anticipated.

Other Tissues: Kirk (40) has reported the presence of carbonic anhydrase in the media of the human aorta.

Mawson and Fischer (41) found the enzyme in the prostate of the rat. In these tissues as in the nervous system, the investigation of carbonic anhydrase activity is really just beginning and whether Diamox® has an effect on such activity must await further research and observation of patients treated with the drug over prolonged periods of time.

Tissues After Long Term Administration: Dogs have been given oral doses of 100 mg/Kg for sixteen months with no clinical or pathological signs of damage nor evidence of gastrointestional irritation. Rats have received 300 mg/Kg per day in the diet for six months with no adverse effects.(34) In prolonged experiments of this type there is a metabolic acidosis characterized by low pH and CO_2 combining power of the blood. This acidosis has never proved deleterious.

Absorption and Excretion of Diamox®: Following a single oral dose in man, dog, or rat, the drug is rapidly absorbed and is excreted largely unchanged by the kidney in six to twelve hours. Its renal clearance in the dog is about two-thirds that of the glomerular filtration rate.

THERAPEUTICS

Diuretic Agent in Congestive Heart Failure: "It would be highly desirable to obtain a diuretic agent that could be given orally and yet act as effectively as parenterally administered mercurial diuretics, without producing significant toxic manifestation." Apparently Diamox® is not quite the perfect solution but definitely approximates this wish expressed by Dr. Friedberg (42). A group of investigators at Galveston, Texas who have been studying diuretic drugs for about twenty years state: "The ideal drug for augmentation of urinary output has long been sought. We have found that Diamox® seems to meet most of the important qualifications of an ideal drug or diuretic. Diamox® is usually well tolerated orally, effective over relatively short periods of time, physiologically active, non-irritating, non-toxic, and with no significant side effects."

The evaluation of Diamox® at the present time is difficult because it is still a relatively new drug and as with all new drugs there are enthusiastic supporters and some who are not so enthusiastic. In five different controlled series, various investigators obtained results which showed definitely that Diamox®

was beneficial in certain cases of congestive heart failure. Best results were obtained in patients with predominantly left sided decompensation of mild to moderate severity. In patients with severe right heart failure with large accumulations of fluid, paracentesis initially gave the best results and then later Diamox® was able to prevent the reaccumulation of fluid. Friedberg (44) noted in 18 out of 26 patients with congestive heart failure a clinical response that compared favorably with that obtained after parenterally administered diuretics. These patients were ambulant and treated on an outpatient basis. Only patients were studied who had been found to require mercurial injections at intervals of ten days or less for control of heart failure. He found that if Diamox® is given three or more times a day the drug loses its diuretic activity completely or partially. If the drug is given in 250 mg doses once a day, this refractoriness did not appear in spite of continued therapy.

Belsky (45) gave Diamox® orally to thirteen patients with congestive heart failure who formerly had been given routine mercurial injections along with digitalis and low salt diet to control edema formation. He found that in nine cases mercurial injections could be dispensed with entirely. He noted that Diamox® was not

effective in the presence of anasarca and renal decompensation.

The Galveston group (43) reported in 1954 their study of thirty four hospitalized and twenty three outclinic patients. The twenty three outclinic patients were taken off routin courses of mercurial injections and ammonium chloride weekly or twice or thrice weekly and put on 500 mg Diamox® daily. The response was considered excellent in eleven, good in six, fair in three and poor in three. Two patients who had shown good response to Diamox® in the first course of 500 mg dosage seemed refractory after two months and began to gain weight. Diamox® was found to produce profuse diuresis occasionally but usually moderate diuresis. Their blood and urine studies again showed no evidence of any renal irritation. They found that ammonium chloride counteracts Diamox® diuresis and should not be administered before or with Diamox®. Two who had needed 2 cc Thiomerin three times a week were maintained alone on 500 mg of Diamox® daily.

The average clinical response in the thirty four hospitalized patients was considered excellent in ten, good in four, fair in ten and poor in six.

The patients in the whole group had the following types of disease:

Arteriosclerotic Heart Disease	18
Hypertensive Heart Disease	15
Combined arteriosclerotic and Hypertensive Heart Disease	9
Rheumatic Heart Disease	5
Syphilitic Heart Disease	7
Congenital Heart Disease	1
Liver Disease	2

Diamox®'s most severe test was given by Schwartz (46) who studied twenty six hospitalized patients with severe chronic congestive heart failure. Fifteen of these had failed to lose weight with their last dose of mercurials prior to the study with Diamox® and most of these were considered to have become resistant to the mercurials. Thirteen of the patients in this group had azotemia with NPN over 50 mg %. Schwartz admitted that "one would not choose such a group to demonstrate the optimal effects of a new diuretic agent; it is in this group that such an agent is most urgently needed." He found that only in one of these severe cardiacs did Diamox® prove to be a clinically effective diuretic agent. Less favorable results were especially observed in those patients with azotemia. Half of the group failed to lose weight and the remainder with one exception lost only two to

six pounds over an average period of seven days. Those fifteen who had previously failed to respond to mercurial treatment were given another trial of mercurial therapy immediately after the end of the treatment with Diamox®. Five of these responded with diuresis (weight loss of three pounds or more) to the first dose of mercury. Schwartz's conclusion which seems rather unfair when reviewing his cases was that Diamox® could hardly be of value in the management of hospital patients with severe congestive heart failure.

Later Schwartz (47) studied seventeen hospital patients with severe congestive heart failure due to chronic cor pulmonale. Fifteen of these had emphysema and the other two had kyphoscoliosis as the etiological basis of their cor pulmonale. These patients were treated with Diamox® in dosages mainly between 1.0 to 1.5 grams in divided doses over a twenty four hour period. Large diuresis and significant clinical improvement was noticed in one-half of those treated. Nine of the subjects lost an average of 15.5 pounds over periods of from five to twelve days. Seven of the patients failed to lose weight. Two lost one or two pounds. In both the responsive and unresponsive groups a decline in the CO_2 content of plasma was obtained.

Cardillo, Mullin, Scheffer and Lyons (48) reported in August 1954 the results of their study of twenty five ambulatory cardiac patients as to the relative efficiency of Thiomerin and Diamox over an eight week period. The mean weight loss observed with Thiomerin was:

Dosage:	.5cc	1.0cc	1.5cc	2.0cc
Mean weight loss:	.25lb	.98lb	1.5lbs	1.9lbs

The mean weight loss with Diamox® was:

Dosage:	250mg	500mg	750mg	1000mg
Mean weight loss:	1.3lbs	2.3lbs	2.2lbs	2.4lbs

With Diamox® it may be seen that an increased response is not obtained by increasing the dosage above 500 mg; but with Thiomerin, there is a linear response.

EMPHYSEMA

The use of Diamox® in patients with congestive heart failure secondary to emphysema has already been mentioned. There has been one report (49) of using Diamox® experimentally in the therapy of emphysema

without cardiac complications. The mechanism by which patients with respiratory acidosis would be benefitted by superimposing a metabolic acidosis is very obscure, but apparently the emphysematous patient obtains subjective relief. Six patents with emphysema who were in respiratory acidosis were studied and good results were obtained in five. One patient had been comatose prior to therapy and was maintained in an alert state for two separate periods without the use of a respirator while receiving Diamox®.

The one patient who did not respond to Diamox® therapy satisfactorily had shown initial improvement in his mental status but later his plasma CO_2 content rose. He became drowsyand required therapy with the Drinker respirator.

TREATMENT OF GLAUCOMA

Diamox® has proved to be a very valuable drug to the ophthamologist in the treatment of glaucomatous states. One investigator reports the successful use of the drug in over three hundred patients with glaucoma. In acute glaucoma the lowering of the intra-ocular pressure permits more adequate gonioscopic, ophthalmoscopic, tomographic, visual

and perimidric evaluation of the eye. The intra-ocular pressure can be maintained at lower levels in such self limited diseases as glaucoma associated with iritis, hyphema and glaucomato-cyclitic crisis. As a preoperative medication, Diamox® makes possible an operation on a softer, less inflamed eye with less likelihood of complications. By repeated administration of Diamox®, eyes with congenital glaucoma can be whitened and corneas cleared permitting goniotomy under direct visualization.

Dr. Becker (32) reported a pronounced decrease in intra-ocular pressure with Diamox® in twenty five eyes of fifteen patients with various types of glaucoma previously uncontrolled by conventional therapy. He found that following a single dose of 500-1000 mg, the intra-ocular pressure began to fall in 60-90 minutes, reached a minimum in 3-5 hours and returned to initial levels in 8-12 hours. Grant and Trotter (29) advise administration of Diamox® at 12 hour intervals has controlled intra-ocular pressure in most cases, but less frequent use has not.

Becker (32) demonstrated that one can maintain constantly depressed intra-ocular pressure in glaucomatous eyes by the around the clock use of Diamox® for more than ten months. He advises that

the effect on the lens, cornea, and other ocular tissues of continuous suppression of aqueous inflow needs further investigation.

In the treatment of glaucoma, Diamox® is used in conjunction with other time proven measures such as miotics and not in place of them.

INTRACTABLE EPILEPSY

In 1941, Cohen and Cobb (50) studied the anti-convulsive action of sulfanilamide in patients with epilepsy. Of the ten patients with severe epilepsy investigated, three exhibited marked improvement. One had moderate benefit. In four patients, there was a slight anti-convulsive effect. Two cases showed no obvious effect.

Using the more potent, less toxic carbonic anhydrase inhibitor, Diamox®, Bergstrom (51) administered the drug to forty two patients with intractable epilepsy in dosages of 10-30 mg/Kg per day. Ninety to one hundred percent seizure control was obtained in four patients and fifty to ninety percent control occurred in four others. None was made worse. In the observations of these forty two patients, it was determined that a dosage of 8 mg/Kg

per day resulted in a seventy percent inhibition of whole blood carbonic anhydrase activity in 3-4 hours. Twice this dosage did not produce any further significant metabolic effects.

The mechanism of the beneficial results seen is conjectural. It may be due to the moderate acidosis which develops or to direct inhibition of carbonic anhydrase in the brain at a cellular level.

TOXICITY

The most common complaint of patients receiving Diamox, especially those receiving dosages of one gram or over per day, is the development of "pins and needles" paresthesias particularly in face, hands or feet. These side effects disappear within a day after the drug is discontinued. Less frequently drowsiness may be experienced.

One febrile reaction to Diamox has been reported (52). The patient was positive he had never received any form of sulfa before. The drug fever developed after the third dose of Diamox® had been given. Later the patient was given a fourth dose to establish the nature of the reaction.

No skin manifestations have been reported nor any changes in urine other than the electrolyte effects.

The mild acidosis which developes with continued therapy is well tolerated by the patient.

In January 1955, the first case of agranulocytosis following Diamox® therapy was reported.(53) The patient was a sixty six year old white female who had been on a digitalis, low salt diet and Diamox regime for three months because of congestive heart failure. She was admitted to the hospital complaining of vertigo, syncope, numbness of left hand, weakness and malaise that had been increasing during the preceding four weeks. Her white blood count on admission was 5000 cells/cumm with a differential count of Segs.1, Band 2, Lymphs. 59, Monos. 38. Her hemoglobin was 14.1 grams and her red blood count was 5.56 million/cumm. Bone marrow studies showed granulopoiesis was diminished slightly with an arrest of maturation at the metamyelocyte level. The dosage of Diamox® this patient had been taking daily was 250 mg. It is interesting to note that she showed early toxic signs of the drug four weeks before admission to the hospital when complaints of numbness, malaise and weakness appeared. There symptoms were not understood and the patient was allowed to continue the drug without caution.

Treatment consisted mainly of discontinuing the diuretic and the patient responded readily.

Dogs can survive single oral doses of 2000 mg/Kg of Diamox®. Only when the dog is given 1000mg/Kg of Diamox® for three successive days can severe loss of potassium, resulting in death, be induced. The drug is not cumulative, and has no preference for any tissue, except erythrocytes, which it appears to saturate at a fairly low and fixed level for each species (34).

SUMMARY

A review of the events which led to the development of a potent carbonic anhydrase inhibitor by the American Cyanamid Company in 1950 has been discussed.

It was observed that sulfanilamide produced a metabolic acidosis in patients receiving the drug over a period of days. The associated change in the electrolyte pattern, the fall in the CO_2 combining power of serum, and the increase in urinary sodium, potassium, and bicarbonate suggested that such drugs capable of inhibiting carbonic anhydrase might be useful as a diuretic in the treatment of patients in congestive heart failure. The early trials confirmed the potentialities of such drugs, and research was begun to find new compounds which were less toxic and had greater carbonic anhydrase inhibiting power. The heterocyclic compound 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide proved to be a chemical with the desired properties. Since the enzyme carbonic anhydrase is present in many of the tissues of the body, it might be expected that Diamox® could have an effect on many of the body's physiological processes. However except in very high dosages this drug has little effect on the normal individual.

It is in the patient with increased fluid content, whether such fluid be in the interstial fluid as occurs in the patient with congestive heart failure or in the anterior chamber of the eye as occurs in the patient with glaucoma, that Diamox® exerts its most noticeable action. Diamox® has been proven by various investigators to be of benefit in the treatment of mild and moderate cases of congestive heart failure and in the therapy of the patient with glaucoma. Certain cases of epilepsy which have been resistant to therapy with the more commonly used drugs may be benefited by using Diamox®. Other conditions in which Diamox® has been found to be effective in relieving the symptoms and signs of excessive water retention are pre-menstrual tension and pre-eclampsia. Preliminary reports suggest that Diamox® may be of benefit in the treatment of emphysematous patients.

Both gastric and pancreatic secretion can be suppressed by the administration of Diamox® but the dosages required are in the level within which toxic symptoms are frequently experienced. The more common toxic symptoms are parasthesias of the face and extremeties and drowsiness. The metabolic acidosis which is produced in patients receiving the drug

over a period of days is well tolerated. One case of drug fever and one case of agranulocytosis have been reported as occurring in patients treated with Diamox®.

Finally, and probably most important, Diamox® because it is a potent carbonic anhydrase innibitor is a valuable research tool which investigators can utilize in determining the function the carbonic anhydrase enzyme has in the various tissues of the body. Although it is known that carbonic anhydrase occurs in the central nervous system, the aorta, the ocular lens, and many other tissues, the role of the enzyme in maintaining the function of these tissues is not known. It is with experiments utilizing Diamox® that the answers to these questions concerning the role of carbonic anhydrase will be obtained.

CONCLUSIONS

1. Diamox® is a potent carbonic anhydrase inhibitor and the effect of the drug upon a tissue is dependent upon the role the carbonic anhydrase enzyme assumes in the physiology of such tissue.

2. Investigators have proven that Diamox® can alter the function of the kidney, gastric mucosa, pancreas, ciliary body, and the erythrocyte.

3. Diamox® has been found to be effective in the treatment of glaucoma, mild, moderate and certain severe cases of congestive heart failure, and certain resistant cases of epilepsy.

4. Diamox® is an excellent research tool for the investigation of the function of carbonic anhydrase in the various tissues of the body, and as new facts are learned, further use of Diamox® in clinical medicine is anticipated.

5. The toxicity of Diamox® is very low; paresthesias and drowsiness are the most common symptoms that develop. Electrolyte changes cause a mild acidosis which is well tolerated.

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