The Problem of Bioassay and Comparative Potency of Diuretics

II. Carbonic Anhydrase Inhibitors as Oral Diuretics

By Ralph V. Ford, M.D., Charles L. Spurr, M.D., and John H. Moyer, M.D.

Oral diuretic agents are widely used with but little information concerning their relative diuretic and toxic effects. A method is reported here for the bioassay of oral diuretic agents so that their comparative potency may be established. Four carbonic anhydrase inhibitors are compared by this method.

As THE uses for diuretic agents have multiplied because of a greater understanding of the pathologic physiology of salt and water metabolism, a fertile field for the development of new agents that will increase the excretion of sodium and water has developed. Diuretics may be categorized according to their mechanisms of action.1 The most commonly used diuretic agents are, of course, the organomercurials and these are available for parenteral injection as well as oral use. The parenteral forms are used in markedly edematous states and the oral mercurials find wide usefulness for maintenance therapy. The mechanism of action of the mercurial compounds has been reported to be the inhibition of succinic dehydrogenase activity in Henle’s loop of the nephron.2 Second only in importance to the mercurial diuretics are the agents that promote the excretion of sodium and water by the inhibition of carbonic anhydrase activity in the distal convoluted tubule of the nephron.3 In general, these are sulfonamide derivatives that may be administered orally to assist the control of mild edema.

The purpose of the present study is to report the method for the bioassay of carbonic anhydrase inhibitors as diuretic agents with the result that the comparative potency of these agents may be established. The 4 drugs to be compared are all sulfonamide derivatives and include acetazolamide (Diamox),* 6-ethoxybenzothiazole-2-sulfonamide (U-4191),† Butamidine (SKF-4965)‡ and P-carboxybenzene sulfonamide (Dirmate)§ (fig. 1).

Materials and Methods

This bioassay was conducted on a metabolic ward, utilizing men with mild heart failure who had previously demonstrated evidence of edema but, who, at the time of the study, were relatively edema-free. Each of 4 drugs was given to 10 patients and frequently the same patients received more than one of the drugs. The patients drank 3000 ml of distilled water per 24 hours and ate a diet containing 50 mEq. of sodium per 24 hours. Twenty-four-hour urine specimens were analyzed for sodium, potassium, chloride, and volume. The patients were weighed each morning before breakfast and after voiding. After suitable control periods, the patient’s urinary excretion rate of sodium was approximately 90 to 95 per cent of the dietary sodium intake. The patient’s excretion rate for sodium continued at this constant level for at least 3 days. The patient was then given a single dose after breakfast of the drug to be tested on 2 consecutive days and the body weights and excretion rates of water and electrolytes were determined. After the drug was stopped the patient’s excretion rate of sodium usually decreased for 1 to 3 days, during which time the body stores of sodium were replenished and the patient once more began to excrete at a fairly constant rate, which was very close to his dietary sodium intake. Thus, it was usually 7 days between the administration of the different drugs to each patient (fig. 2) as well as between the different dose levels employed for each drug.

Since the range between threshold dose and the maximum effective dose for increasing water and

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* Furnished by Lederle Laboratories.
† Furnished by Upjohn Co.
‡ Furnished by Smith, Kline & French Laboratories.
§ Furnished by Sharp & Dohme, Division of Merck & Co.

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Fig. 1. Structural formulas for the test drugs emphasize their basic sulfonamide structure.

Fig. 2. The technic for studying these diuretic agents consists primarily of the observation of sodium excretion. The increases due to the various drugs are followed by 1- to 3-day periods of "compensation" during which sodium excretion is depressed.

electrolyte excretion with these drugs was so small, it was impossible to establish a good dose-response curve. However, in 3 of the 4 drugs tested, very small doses were administered in a log-dose incremental fashion until further increases in dosage resulted in no further increase in the excretion rate of sodium. This point was arbitrarily termed the apex of the curve. Figure 3 shows that with 1 of the experimental drugs studied (U-4191), there was a minor increase in the excretion rate for sodium at the small dose tested (33.75 mg.) followed by an increasing magnitude of response until the 125-mg. dose was reached, at which point the curve flattened out and became parallel to the base line. This same procedure was used in the establishment of the apex point for Diamox and SKF-4965, but not for Dирнate. After this apex was established, a dose of at least 2 times that dose at the apex was administered as the testing dose for the particular drug (fig. 3). The apex point for each of 3 drugs was roughly similar except that SKF-4965 was slightly more potent.

Although the curve as plotted shows a slightly greater increase at the 250-mg. dose point, this increase was shown to be not significant by statistical analysis.

Thus, in the cases of Diamox and U-4191, the dosage for each test was 250 mg. daily. Experimental preparation SKF-4965 was tested at 150 mg., since its apex dose was 75 mg. In the case of Dирнate, the apex was not established, but, following the recommendations of the pharmaceutical pharmacologist, a dose of 2000 mg. was administered as a single dose.

RESULTS

The values are recorded as the increases in sodium, potassium, chloride, water and the decrease in weight as an average of the 2 consecutive days during which the comparative test dose (twice the maximum effective dose for each drug) was administered.

In table 1, the changes following the administration of Diamox in excretion rates of electrolytes and water as well as the changes in weight are recorded. There was an average increase in the urinary excretion of sodium per 24 hours of 35 mEq. (p < .001), which is the average of 2 consecutive 24 hours during which the drug was administered at a dosage of 250 mg. each day. The excretion rate for potassium increased by 35 mEq. per 24 hours (p < .001), while the chloride excretion rate decreased by 9 mEq. per 24 hours (p < .01). The rate of water excretion increased by 0.44 L per 24 hours (p < .001) and the weight decreased by 0.3 Kg per 24 hours (p < .02). The increase in the excretion rate of sodium per 24 hours was 31 mEq. for Dирнate, 44 mEq. for U-4191, and 31 mEq. for SKF-4965. Changes in the excretion rates of potassium, chloride, and water as
The mechanism of the diuretic effect is the inhibition of the carbonic anhydrase enzyme system at the level of the distal tubule in the kidney. This enzyme system is responsible for the exchange of intracellular hydrogen ions with the intratubular sodium, which results in acidification of the urine. Since sulfanilamide and certain of its derivatives are capable of inhibiting this enzyme system, there is a greater loss of sodium by way of the urine following their administration. It thus seemed reasonable to search for relatively nontoxic inhibitors of carbonic anhydrase. This search has yielded at least 4 compounds whose activity in this regard is adequate, yet they are not too toxic for oral administration (fig. 1).

Among the drugs tested, Diamox is the only one that is clinically available at this time. Dinnate is not now and probably will not become commercially available. U-4191, and Butamide have not been marketed for clinical use as yet, but probably will be at some future date.

The indications for the use of these drugs include maintenance therapy between peritoneal mercurial injections for the treatment of severe congestive heart failure, the treatment of mild heart failure, premenstrual edema, the nephrotic syndrome (in the absence of acidosis), hepatic and nutritional edema, and occasionally in the control of the edema associated with steroid therapy. In the latter entity, so-called "steroid edema," the use of carbonic anhydrase inhibitors is somewhat limited because they produce a kaluresis, which may upset normal acid-base balance, since the primary therapy, namely steroids, also exert this same pharmacologic effect.

This wide usage for oral diuretics in general, and for carbonic anhydrase inhibitors specifically, necessitates a method whereby these drugs can be compared in terms of diuretic potency and the incidence and severity of side reactions. In previous studies,4, 5 the technics for bioassay of mercurial diuretics have been described. It has been shown that the response to a diuretic agent is dependent upon the dietary sodium intake as well as the dose of the diuretic agent. These observations thus have established the necessity of maintaining a constant dietary intake of sodium when doing comparative diuretic bioassays. Since these drugs are relatively potent (as compared to carbonic anhydrase inhibitors), the changes in the excretion of electrolytes, water, and weight can be measured accurately and a dosage-response curve can be determined. By comparing dosage-response curves the potency estimation of the various diuretic agents can be determined.

In contrast to the mercurials, the potency of carbonic anhydrase inhibitors as natriuretic agents is comparatively small. Further, the

### Table 1.—Summary of the Average Effect on Body Weight and the Excretion Rates of Water and Electrolytes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Increase in sodium excretion (mEq./24 hr.)</th>
<th>Increase in potassium excretion (mEq./24 hr.)</th>
<th>Change in chloride excretion (mEq./24 hr.)</th>
<th>Increase in water excretion (L./24 hr.)</th>
<th>Weight change (Kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamox</td>
<td>35</td>
<td>35</td>
<td>-9</td>
<td>0.44</td>
<td>-0.3</td>
</tr>
<tr>
<td>Dinnate</td>
<td>31</td>
<td>28</td>
<td>-6</td>
<td>0.24</td>
<td>-0.2</td>
</tr>
<tr>
<td>U-4191</td>
<td>44</td>
<td>37</td>
<td>-6</td>
<td>0.43</td>
<td>-0.6</td>
</tr>
<tr>
<td>SKF-4965</td>
<td>31</td>
<td>43</td>
<td>-11</td>
<td>0.20</td>
<td>-0.2</td>
</tr>
</tbody>
</table>
dose ranges of the carbonic anhydrase inhibitors that produce significant and increasing responses in the excretion of sodium and water are so narrow that the establishment of typical dose-response curves is practically impossible. Thus, it is necessary to determine the point at which a further increase in the dose of each of these drugs does not result in an additional increase in the excretion rate of water or electrolytes and a decrease in weight. Then, by using a dose greater than this apex (we have arbitrarily selected a dose 2 times that of the apex dose) and administering this dose a sufficient number of times to be statistically significant, one is able to compare the relative potency of the carbonic anhydrase inhibitors. This approach established the maximum response that can be obtained with the 4 compounds under study irrespective of the dose requirement. However, with one exception (Dinveste), the dosages of each drug that produced maximum diuresis and natriuresis were roughly similar. Dinveste was given at a much larger dose, but, since it is no longer available, it has been impossible for us to study the problem of determining the apex point of this drug. However, comparison of these drugs through an analysis of variance reveals no significant difference among them in their ability to influence the excretion rates of sodium and other electrolytes as well as water and weight change at the dosages administered. No adverse side effects were noted with these dosages.

It is of interest to compare an orally administered mercurial diuretic chloromerodrin (Neohydrin) to Diamox during continued administration of each compound, since continued daily diuretic therapy may be required for adequate treatment of patients suffering from severe sodium and water retention as in severe heart failure. In figure 4 it is seen that the changes resulting from the administration of oral Neohydrin increased with continued administration of the drug. However, the changes following the administration of Diamox illustrate that although on the first day the changes were roughly equal to that of Neohydrin in clinically tolerable doses there was a decreasing response to the continued administration of Diamox for 5 successive days. This difference has been explained as being due to the development of compensatory mechanisms against the systemic acidosis that results from the administration of Diamox continuously. The full explanation is not available but an important factor is the decreased filtered load of bicarbonate presented to the tubules. Under controlled metabolic conditions the administration of 30 mg. (3 tablets) of Neohydrin orally produces an acute (2-day period of daily administration) increase in sodium excretion that is equal to that following the administration of 250 mg. of Diamox.

**Summary**

Utilization of controlled metabolic conditions, which furnish the patient with constant dietary and water intake, and measurement of electrolyte and water excretion, as well as weight change, permit determination of the comparative diuretic potency of orally administered carbonic anhydrase inhibitors.

No significant difference in maximum di-
uretic effect was found among 4 carbonic anhydrase inhibitors, Diamox, Dirnate, SKF-4965, and U-4191.

The acute response to the 4 carbonic anhydrase inhibitors was approximately equal to 3 tablets (30 mg) of oral Neohydrin given as a single dose; but continued administration of Neohydrin on consecutive days resulted in an increase in the excretion rate of sodium, while the continued administration of Diamox as well as the other carbonic anhydrase inhibitors was accompanied by a declining rate of sodium excretion.

**SUMMARIO IN INTERLINGUA**

Sub conditiones metabolic regulare a provider le patiente de constante quantitates de natrium e aqua dietari, le mesuration del excretion de electrolytos e aqua insimul con determinationes de alterationes de peso permette le evaluation comparative del potentia diuretic de oralmente administrate inhibitores de anhydrase carbonic.

Nulle significative differentias del potentia diuretic eseva trovate inter le 4 inhibitores de anhydrase carbonic: Diamox (acetazoleamido), Dirnato, SKF-4965, e U-4919.

Le responsa acute al 4 inhibitores de anhydrase carbonic eseva approximativamente equal a 3 tabletas (30 mg) de chlormerodrina oral (Neohydrina) administrare in un dose unic. Sed continue administrationes de Neohydrina con intervallos de un die resultava in un augmento del excretion de natrium, durante que le administration continue de Diamox e del altere inhibitores de anhydrase carbonic eseva accompaniante de un reduction del excretion de natrium.

**REFERENCES**


The application of the principles of science to the diagnosis and treatment of disease is only one limited aspect of medical practice. The practice of medicine in its broadest sense includes the whole relationship of the physician with his patient. It is an art, based to an increasing extent on the medical sciences, but comprising much that still remains outside the realm of any science. The art of medicine and the science of medicine are not antagonistic but supplementary to each other. There is no more contradiction between the science of medicine and the art of medicine than between the science of aeronautics and the art of flying.—Francis W. Peabody, 1881–1927.
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