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ON THE METABOLISM OF METHYLATED DISULPHONAMIDES WITH A DIURETIC ACTION—A CONTRIBUTION TO THE MECHANISM OF ACTION OF CHLOROTHIAZIDE AND SIMILAR COMPOUNDS

BY

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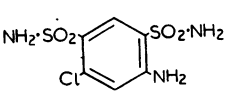
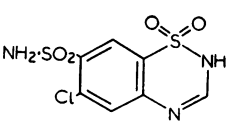
The metabolism of two substances possessing a diuretic action, 4-amino-6-chlorobenzene-1,3-disulphonmethylamide (III) and 6-chloro-3,4-dihydro-2-methyl-7-methylsulphamoyl-2*H*-1,2,4-benzo[*e*]thiadiazine-1,1-dioxide (IV), has been studied on rats and human subjects. It has been shown that these compounds are not demethylated in the $-SO_2.NHCH_3$ group. They therefore do not act through liberation of substances with unsubstituted sulphonamido-groups which might inhibit carbonic anhydrase, and their mechanism of diuretic action cannot be considered due to inhibition of this enzyme.

Chlorothiazide (II) (Novello & Sprague, 1957) is the prototype of a new class of diuretics which, like acetazolamide, the classic inhibitor of carbonic anhydrase, possess unsubstituted sulphonamido-groups which are responsible for the *in vitro* inhibition of carbonic anhydrase. However, there are substantial differences in the mechanism of action of these substances *in vivo*. They can be active *in vivo* without exerting an inhibiting action on carbonic anhydrase (Parenti & Capraro, 1959; Marro & Azzolini, 1960; Marro & Pesente, 1960).



I



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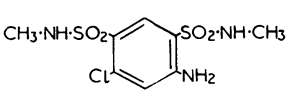
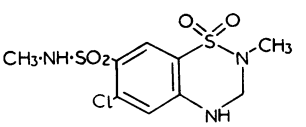


III



IV

We have made another contribution to the problem (Logemann, Giraldi & Parenti, 1958, 1959; Giraldi & Parenti, 1960) by the synthesis of methylated sulphonamides (III and IV) possessing a diuretic action. Unlike compounds I and II, compounds

III and IV are not *in vitro* inhibitors of carbonic anhydrase, since inhibition of the enzyme is specifically connected with the presence of an unsubstituted sulphonamido-group (Mann & Keilin, 1940). However, they produce a diuresis which is comparable with the corresponding non-methylated substances. Nevertheless, there are qualitative differences. The diuretic action of compound IV is less marked but more prolonged. This is of some importance in medical practice (Rulli, Concina & Bonanni, 1961), since this kind of action is advisable in patients with decompensated cardiac failure and oedema when the haemodynamic situation should not be disturbed too abruptly. The same concept may also hold true in the utilization of these substances as diuretics in other indications, for instance, in the treatment of hypertensive states and in slimming treatments.

Some authors (Beyer & Baer, 1960; Lund & Kobinger, 1960) postulate the *in vivo* splitting-off of the methyl group in our substances, in such a way that the diuretic action would be linked to the presence of unsubstituted sulphonamido-groups, in analogy to isopropylacetazolamide which is transformed into acetazolamide in dogs and rats (Maren, 1956). However, the corresponding methyl derivative was demethylated to only a slight extent. The isopropyl derivative corresponding to our compound III is inactive as a diuretic, while the methylated compounds III and IV are highly active. It is therefore impossible to suggest any parallelism between acetazolamide and its derivatives and the substances described by us.

We have re-examined the metabolism of the methylated compounds III and IV, although Giraldi (1959) in experiments on rats has already demonstrated that there is no splitting-off of the methyl group.

METHODS

A total of 2 g of compound III was administered orally to human subjects and 24-hr urine specimens were collected. Substance IV was administered orally to 20 rats (100 mg/rat), each weighing 90 to 120 g, as a 10% suspension in gum arabic, and the urine collected for 24 hr.

A total of 200 mg of compound IV was administered orally to human subjects and the urine collected over a 24-hr period. The products eliminated were determined by means of paper chromatography.

The urine of subjects receiving compound III was transferred to Whatman 3 MM paper and descending chromatography carried out using n-butanol–ammonia–water mixture (250:72:178). After drying, the chromatogram was developed by spraying with alcoholic hydrochloric acid and amyl nitrite, followed 2 min later by a 1% *N*-naphthylethylenediamine solution in n-butanol–water mixture (95:5).

The urine of the rats and human subjects receiving compound IV was hydrolysed with half quantities of 5 N sodium hydroxide in order to open the heterocyclic ring; this treatment leads to the appearance of a free amino-group (by this process compound IV is transformed into compound III). The solution was then neutralized with 5 N hydrochloric acid, applied to Whatman 3 MM paper, and subjected to chromatography as above.

The urine of human subjects treated with compound IV was hydrolysed as above, but it could not undergo direct chromatography because, on treatment with 5 N sodium hydroxide, decomposition products of pigments are formed. The urine was therefore extracted with ethyl acetate and the extract then underwent chromatography on Whatman 3 MM paper on a preparative scale. Portions of the paper from the positions corresponding to the diazotization stains were eluted with acetone and the acetone solution was then again chromatographed.

RESULTS

Figs. 1, 2 and 3 represent diagrammatically chromatograms of the various urine specimens compared with standard solutions of compounds I and III.

Administration of compounds III and IV to rats was not followed by the appearance of the demethylated product I in the urine. This confirmed the observations of Giraldi (1959) on the metabolism of compound III in the rat.

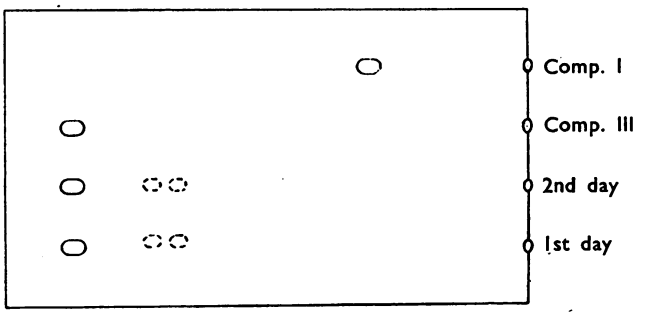


Fig. 1. Chromatogram of urine from human subjects treated with compound III.

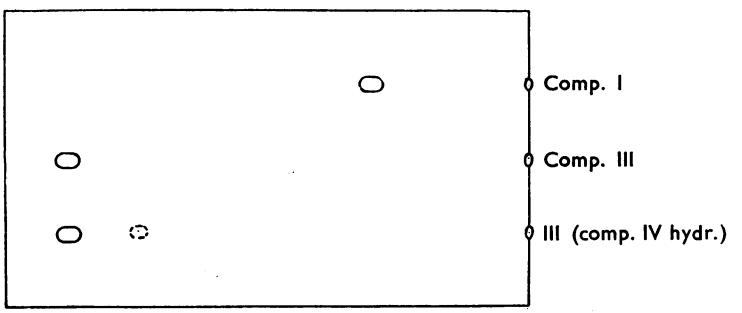


Fig. 2. Chromatogram of urine from rats treated with compound IV and successively hydrolysed.

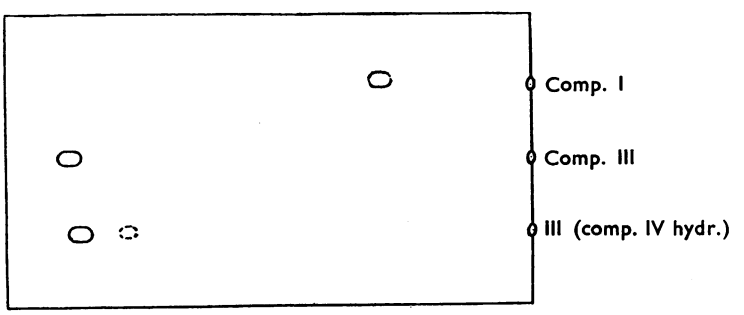


Fig. 3. Chromatogram of urine from human subjects treated with compound IV, successively hydrolysed, extracted with ethyl acetate, subjected to chromatography on a preparative scale and extracted with acetone.

The chromatogram of the urine of human subjects treated with compound III showed, under the stain due to compound III, two faint stains denoting the presence of extremely small amounts of metabolites which are presumably hydroxy derivatives of compound III (compare Giraldi, 1959).

The urine of rats treated with compound IV, after hydrolysis, shows under the stain due to compound III the presence of a single derivative of the metabolite which is perhaps identical with one of those isolated in the urine of rats treated with compound III (Giraldi, 1959). The presence of the stain due to the second metabolite is not observed, probably because of its low concentration. The urine of human subjects treated with compound IV, hydrolysed and subjected to chromatography as described in the method, shows the same chromatogram.

DISCUSSION

These studies clearly show that demethylation of compounds III and IV does not occur in the rat and man. Thus, these compounds can exert no action on carbonic anhydrase.

These compounds behave similarly to chlorothiazide with regard to urinary elimination of sodium, potassium, chloride, and bicarbonate ions. It may be concluded that compounds III and IV act by the same mechanism as chlorothiazide, and that, in the case of chlorothiazide, the carbonic anhydrase inhibition is of secondary importance for diuresis.

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