CORRESPONDENCE

The Editor, 6th November, 1948.
"Leprosy Review."
Sir,

There have been suggestions in the literature of the sulphone treatment of leprosy that sulphones may be concentrated in the skin (1, 2). The importance of the study of such an observation is obvious and has been emphasised at the recent International Conference of Leprosy (3).

Some months ago in this laboratory a biopsy specimen of skin was submitted for analysis of sulphone concentration. The result was 35 mg. of sulphone per 100 g. of skin. This result was not in agreement with other values obtained previously, and investigation revealed that the biopsy had been performed under Planocaine local anaesthesia, whereas previous biopsies had been made from nodular material not necessitating the use of a local anaesthetic. A repeat biopsy of this same patient shewing a reported concentration of 35 mg. per 100 g. of skin was made under cocaine local anaesthesia. This second biopsy was made within a few hours of the first and at a site as near to the first as was possible. Analysis of this specimen shewed a concentration of < 2 mg. sulphone per 100 g. of skin, a figure in accordance with the values obtained from previous skin biopsies.

DISCUSSION.

The routine method in general use for quantitative estimations of the proprietary group of substituted diaminodyphenylsulphones is substantially that of Bratton and Marshall (4). The principle is that of diazotisation of the amino grouping and subsequent reaction with a coupling component to produce a compound capable of colourimetric estimation. The method was devised originally for the sulphonamides, but a colour reaction is obtained with any compound having a diazotisable amino grouping. The aromatic anaesthetics, e.g., Procaine and Planocaine, possess such a grouping and will therefore give a colour under the conditions of the test. Infiltration local anaesthesia with this group of anaesthetics is contra-indicated when biopsies of skin for analysis of sulphone concentration are required.

Cocaine is an anaesthetic not possessing a diazotisable amino group and is therefore satisfactory for biopsy work.

We have learnt (5) that in one other leprosy centre high skin concentrations of sulphones have been found to be due to the use of the type of anaesthetic indicated above. In view of this it has been thought desirable to publish our experience together with a method for the estimation of skin concentrations that we have found to work satisfactorily. The whole question of skin concentrations of sulphone will be dealt with more fully in a further article.

METHOD.

Anaesthesia. 1% aqueous solution of cocaine hydrochloride. 1 ml. of this will produce a satisfactory degree of anaesthesia in from 5-10 minutes.

Biopsy. With dark skins it is advantageous to outline the biopsy area with a skin pencil. An elliptical area 2 cm x 0·5 cm extending down to the subcutaneous fat will afford about 0.3 g. of skin, and this amount is the minimum that it is convenient to work with.

Estimation. The excised skin should be rapidly washed in physiological saline to remove any adhering blood, and then pressed dry between several thicknesses of filter paper. Weigh on an analytical balance and transfer to a glass mortar of about 5 cm. diameter. Add 2 or 3 grammes of acid-washed sand and one-third of the calculated volume of N/1 HCl. The calculated volume of N/1 HCl is ten times the weight of the skin excised. Grind until the skin is entirely macerated. With a Pasteur pipette remove the supernatant HCl from the sand and tissue debris, and transfer the HCl to a 10 ml. graduated centrifuge tube. Add to the mortar

the second one third volume portion of N/1 HCl and grind, transferring the supernatant acid to the centrifuge tube as before. The remaining one third volume of N/1 HCl is added to the mortar and transferred, after grinding, as before. In effect the skin is extracted with three portions of HCl. To the centrifuge tube is then added a 12% trichloracetic acid solution to bring the final volume to 15 times the weight of the skin taken.

Example: Weight of skin ... ... 0·5 g.
Calculated volume of
N/1 HCl = 5·0 m. (10 x 0·5 g.)
1/3 calculated volume = 1·6 ml
(skin extracted 3 times with 1·6 ml. of N/1 HCl each time)
Trichloracetic acid added to make final volume up to
7·5 ml. (15 x 0·5 g.)

The tube is then centrifuged, the supernatant fluid poured off into a 10 ml. comparator tube, diazotised and coupled by the method described by Brownlee et al. (6). The above method for skin estimations is applicable to any of the proprietary sulphones, but for each proprietary compound a different standard is necessary. For Sulphetrone, the Lovibond comparator disc is available, and the colour produced by the skin extract may be read directly on the disc as milligrammes per 100 grammes of skin. Promin and Diasone may be estimated approximately by reading in the Lovibond Comparator calibrated for Sulphetrone and applying the following correction factor.

$$\text{mg\% Promin} = \frac{\text{Sulphetrone disc reading}}{1{\cdot}1}$$

$$\text{mg\% Diasone} = \frac{\text{Sulphetrone disc reading}}{1{\cdot}7}$$

Using the above technique we have found no evidence that the sulphones are concentrated in the skin.

Yours faithfully,

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