A CONCENTRATION METHOD FOR ACID FAST BACILLI IN SKIN BIOPSIES FROM LEPROSY PATIENTS

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A skin biopsy of approximately 5 x 3 x 5mm. is obtained from a selected area in persons suspected to be suffering from leprosy. The tissue is immediately dropped in about 3 c.c. of one per cent acetic acid and left there between four to eight hours. It is then taken on a clean dry glass slide and the epidermis is easily scraped off from the dermis, by means of a sterile scalpel.

The tissue, now free from epidermis is dropped into a pyrex glass homogeniser tube (Fig. 2), with an inner ground glass surface, in fresh 3 c.c. of one per cent acetic acid and crushed at about 2,000 r.p.m. with an electrically operated mechanical glass crusher (Fig. 1) for about five to ten minutes. The crushing process is con-

tinued until a homogenous milky emulsion of the tissue particles is obtained while a residual cake of the tough dermal tissue sinks to the bottom of the tube. The tissue particles adhering to the sides of the stirrer and the tube are washed down with about one to two c.c. of one per cent acetic acid, bringing the contents to a total volume of about 5 c.c.

Twenty drops of petroleum ether sulphuric ether mixture (1:10) are added to the turbid fluid with a drop bottle. The tube is shaken vigorously to ensure thorough mixing, corked and allowed to stand in a test tube rack for about 15 to 20 minutes.

10 c.c. of distilled water is let into the tube, along its side, washing down any particle adhering to the wall of the tube. From the white or amber coloured ring formed on the surface after two to five minutes, eight drops are carefully picked up with a sterile 3 m.m. platinum loop and deposited on a clean glass slide. The next eight drops are placed on another clean slide. The drops are then spread over an area roughly 2 x 2 cms. The slides are kept in a covered Petri dish and are allowed to dry in an incubator at 37°C.

The dried smears are fixed by flooding the slides with Carnoy's fixative which is poured off after 15 minutes and the smears dried in air. These dried smears are stained by a slightly modified Ziehl-Neelsen method. Instead of warming the Fuchsin stain on the slide, a test tube containing the Carbol Fuchsin solution is warmed in boiling water in a water bath and poured through a filter paper on the slide. The stain is kept on the slide for ten minutes. The decolourisation is then carried out with 33 per cent Hydrochloric acid for 20 to 30 seconds and the slide, carefully washed in a Coplin jar with running water for at least five minutes. A very dilute solution of Azur II is used as a counter stain. The smears are allowed to dry completely by leaving the slides in such a manner as to prevent the accumulation of dust on the smear surface.

The entire surface of the stained and dried smear is carefully examined for acid fast bacilli under an oil immersion objective.

Carnoy's Fixative (E. V. Cowdry—Laboratory Technique in Biology and Medicine).

†Absolute Alcohol	...	...	...	...	6 parts
Chloroform	...	...	...	...	3 parts
Glacial Acetic Acid	...	...	...	...	1 part

†Rectified spirit (not methylated spirit) can be used instead of Absolute Alcohol.