Paper Electrophoresis of Trypanosomal Extracts

ELECTROPHORESIS has been of value in determining the physico-chemical constitution of cell-free extracts of micro-organisms[1,2]. These studies have been confined mainly to the bacteria and no such investigation of trypanosomal extracts has been made. Moving-boundary electrophoresis has been the method most frequently employed to analyse microbial extracts although the simplicity of paper electrophoresis would be of obvious advantage. The purpose of this communication is to describe the technique for paper electrophoresis and the resultant electrophoretic patterns of trypanosomal extracts.

Trypanosomes were obtained from citrated heart-blood of heavily-infected rats by differential centrifugation. After the third washing with physiological saline in a graduated centrifuge tube, the trypanosomes were re-suspended in distilled water to the proportion of 0·5 ml. water to every 0·1 ml. packed trypanosomes. This suspension was shaken with ballotini beads in a Mickle disintegrator for half an hour. The extract contained approximately 20 mgm. protein/ml. The type of buffer used appears to be a critical factor in electrophoresis of the extracts. Longsworth's veronal buffer at *p*H 8·6, Sørensen's phosphate buffer at a *p*H range of 6·0–8·2 and McIlvaine's phosphate-citric acid buffer all failed to effect adequate migration and demarcation of the several fractions. The buffer described by Bodman[3] gave excellent results. This buffer of *p*H 8·7 is composed of: barbitone soluble 40 gm., sodium acetate 26 gm., magnesium sulphate 2 gm., *N*/10 sulphuric acid 256 ml., and distilled water to make a final volume of 5 litres. The buffer is always discarded after use. The extracts were applied to strips of Whatman 3 *MM* paper (no separation occurred on bacterial-membrane filters) with a Pasteur pipette using a ruler as a guide across the horizontal electrophoresis tank. A potential difference of 130 V. was applied for 20 hr. after which the strips were fixed in a solution of 9 parts methanol and 1 part glacial acetic acid and then stained with bromophenol blue. Electrophoretograms of the patterns were constructed with an 'EEL' scanning unit.

Fig. 1 shows a typical electrophoretogram of an extract of *Trypanosoma rhodesiense*. It will be seen

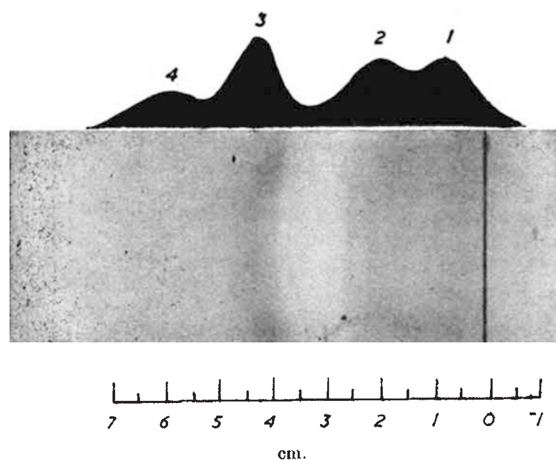

Fig. 1. Paper electrophoretic analysis of a cell-free extract of *Trypanosoma rhodesiense*. Electrophoresis performed in Bodman's veronal-acetate buffer of *p*H 8·7 at 130 V. for 20 hr.

that the extract is composed of four fractions. Fraction 1 and the closely associated fraction 2, both of low mobilities, are 28·75 per cent and 28 per cent of the total respectively. Fraction 3 which appears as a distinct band of greater mobility contains 26·65 per cent of the total. Fraction 4 which appears as a 'trail' is present in most, but not all samples; in this instance it amounts to 16·6 per cent of the total. There is a slight variation in the proportion of fractions from sample to sample but the number of fractions, except for fraction 4, and their respective mobilities seem to be constant.

Work is now in progress to determine the chemical nature of the individual fractions and to compare the electrophoretograms derived from various species of pathogenic African trypanosomes. It is also foreseen that the isolation of the trypanosome's antigens and the application of immuno-electrophoretic techniques may shed some light on the perplexing problem of the apparent antigenic variation occurring during the course of some trypanosome infections.

This work will be published in detail elsewhere.

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June 15.

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Hydrolysis of 'Heated' Hæmoglobin

A DIMINISHED rate of alkaline denaturation of hæmoglobin is not confined to fœtal hæmoglobin only, as was already found by Singer *et al.*[1]. Künzer[2] in a survey on the occurrence of 'fœtal' hæmoglobin in various blood disorders found an alkali-resistant fraction in the anæmia developing after burns. Our observations have confirmed Künzer's and it has been found that this minor hæmoglobin abnormality develops during the first few hours after the burn and persists for some time. This abnormality develops before that of clinical anæmia and involves the patients' own and transfused cells. The detailed results of this work will be published elsewhere.

Heating to 52°C. for four minutes followed by incubation at 37°C. in glucose acid citrate in an atmosphere of nitrogen did *in vitro* produce a similar lesion.

Hæmolysates were rendered stroma free and concentrated by ultra-filtration.

Aliquots were hydrolysed with 1·5 *N* hydrochloric acid at 110°C. for periods of 5, 10, 15, 20, 25 and 30 minutes. The hydrolysis products in the supernatant were separated by drying measured aliquots in polythene caps *in vacuo* over phosphorus pentoxide and potassium hydroxide at approximately 4°C. The dried residues were quantitatively applied to Whatman 3 MM. filter paper squares and the peptides separated by combined electrophoresis and chromatography[3]. Parallel experiments were run simultaneously. Fifteen spots could be located after 30 minutes hydrolysis and these were arbitrarily numbered. To investigate the rate of liberation of the peptides, the colour intensities of the spots 1, 2, 3, 9 and 10 were determined according to the method described by Meyer[4]. The readings were expressed as

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