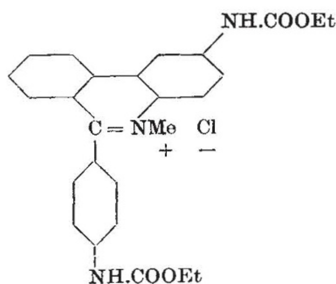
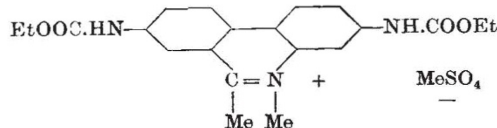


Formula I. *S*1544



Formula II. *S*1582

The fact that cured animals and also those showing suppression of infection failed to develop an obvious infection when reinoculated, even many months later, indicates that with this trypanosome immunity phenomena play a considerable part in conducing to the therapeutic effect. But treatment is more successful at an early stage of the infection than later, when parasites are numerous in the blood, the behaviour in this respect differing from that with *T. congolense*[3,4]. The results in the case of the higher range of dosage both of the phenanthridine compounds and Bayer 7602 (*Ac*) are highly significant. (We are indebted to Dr. R. A. Robb, Mathematics Department, University, Glasgow, for statistical information on this point.)

The above phenanthridine compounds which influence *T. cruzi* infections do not act on *T. brucei* or *T. congolense* except in high doses. Others of the phenanthridine series have also shown some chemotherapeutic influence on *T. cruzi*; and the relations between such action and chemical constitution will be discussed in a later publication. The question of possible influence on the intracellular stage of the parasite has not been examined.

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Production of Diphtheria Toxin by the Submerged Culture Method

THE success which has attended the submerged culture method for the production of penicillin has led us to inquire into the possibilities of producing diphtheria toxin by similar methods. The first step was to see if diphtheria toxin could be produced in flasks which were continually shaken so as to obtain growth throughout the medium instead of merely on the surface.

We took, therefore, our routine papain digest broths and weak tryptic digest broths prepared essentially as described by Pope and Linggood[1], and sterilized them in 300 ml. amounts in one-litre conical flasks. They were then inoculated with our routine strain of *C. diphtheriæ* (Park Williams No. 8), and placed on a shaking machine whereby the contents of the flasks were rotated at approximately 140 oscillations per minute. Prolific growth occurred throughout the medium and, after four days growth, 30–40 units per ml. of diphtheria toxin were found to be present in the culture filtrates when tested by the usual flocculation test. The *p*H of such filtrates was, however, abnormally high, being often 9 or greater. Therefore in subsequent experiments we added much more maltose to our broths, namely, 0·9 per cent instead of our usual 0·3 per cent, and found growth and toxin production to be greater and the *p*H filtrates to be more normal. On a weak tryptic digest broth (total nitrogen content of 3·0 mgm. per ml., maltose content of 0·9 per cent) we have obtained 50 units per ml. of diphtheria toxin after only three days growth in such shaking cultures.

This fact strongly suggests that, as is the case with penicillin, large-scale production of many bacterial toxins may be carried out much more conveniently and much more quickly by submerged culture methods.

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Absorption Spectra of the Exocrine Cells of the Pancreas

IN 1941 Caspersson, Landström-Hyden and Aquilonius[1] demonstrated that the chromidial substance contained in the pancreatic exocrine cells and in other serous secreting elements had a typical absorption spectrum in the ultra-violet region with a peak in 2600 A. As this substance showed a negative Feulgen reaction, they concluded that it is a nucleoprotein with a high content of nucleotides of ribonucleic type. In the apical part of the cell containing the granules, they found absorption curves of a protein type, but with a concentration much lower than in the basal zone; they explain this difference as due to the dilution of the secretion.

I used an ultra-violet microscope with a monochromator similar to that described by Gersh and Baker[2] to re-examine the results obtained by Caspersson *et al.*; the absorption was determined microphotometrically on especially calibrated plates. Details of the technique and full description of the results are reported elsewhere[3].

I wish to refer here particularly to the differences observed in the absorption curves of the apical zone of the pancreatic cells as compared with the results of Caspersson *et al.*

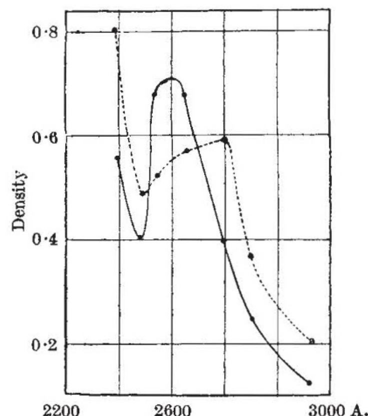

Fig. 1. ULTRA-VIOLET ABSORPTION CURVES OF A FREEZE-DRIED AND DENATURED SECTION, 3μ THICK, OF GUINEA PIG PANCREAS. Full line: absorption curve of the basal part of an exocrine cell (cytoplasm containing chromidial substance). Broken line: absorption curve of the apical part of an exocrine cell (cytoplasm containing zymogenic granules).

As seen in Fig. 1, the basal part of the cytoplasm of the pancreatic cell contains a substance with a maximum absorption at 2600 A., corresponding to the nucleotides. The cytological distribution of this portion of the cell corresponds exactly to the location of the chromidial substance. That these nucleotides are of the ribonucleic type was determined not only by the finding of a negative Feulgen reaction but also by digestion with the crystalline ribonuclease of Kunitz[4] which hydrolyses specifically the ribonucleic acid and thus causes the disappearance of all the basophile staining substances of the cell. (I wish to express my thanks to Dr. M. Kunitz for supplying me with the crystallized nuclease.)

The apical zone containing zymogenic granules gives the typical curve of a protein with intense absorption in 2400 A. and 2800 A. These maxima are much higher than in the basal zone. From this

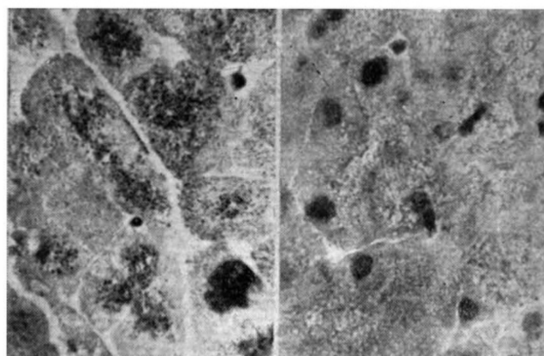

Fig. 2. Fig. 3.

Fig. 2. FREEZE-DRIED DENATURED SECTION OF THE PANCREAS OF A WHITE MOUSE; STAIN: IRON HÆMATOXYLIN. (OB. 90, OC. 6, CL. 55.)

Fig. 3. FREEZE-DRIED SECTION OF THE PANCREAS OF A WHITE MOUSE TREATED FOR AN HOUR WITH GLYCEROL AND AFTERWARDS DENATURED. THE ZYMOGENIC GRANULES ARE DISSOLVED. STAIN: IRON HÆMATOXYLIN.

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