LETTERS TO THE EDITORS

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Ferroverdin, a Green Pigment containing Iron produced by a Streptomycete

DURING a search for antifungal antibiotics, a streptomycete was isolated from a sample of soil from Leopoldsville (Belgian Congo) which produced an intense green non-diffusible pigment on yeast agar. The streptomycete producing this pigment is not identical with any of the following species stated to produce green pigments: *S. verne*, *S. viridans*, *S. virgatus*, *S. flavovirens*, *S. viridis*, *S. viridochromogenes*. Its morphological and cultural characteristics indicate that it may be a new species.

The green pigment can be produced in submerged culture in shake flasks and in stirred fermenters on a medium containing 0·5 per cent yeast autolysate and 1 per cent glucose, *p*H 7; good aeration is essential for its production.

The pigment is extracted into ethanol from the wet mycelium, transferred into butyl acetate and the butyl acetate solution percolated through a column of alumina (Merck). The column, which retains the pigment adsorbed at the top, is developed with 95 per cent ethanol followed by absolute ethanol.

Three green bands separate, one remaining immobile at the top, one moving quickly down the column and one, representing the main fraction, following the fast-moving band. The pigment from the main fraction has been obtained in the crystalline state after repeating the chromatographic procedure and concentrating the final ethanolic solution. The yield of the crude crystalline product is about 100–200 mgm. per kgm. (wet weight) of mycelium. After recrystallization from methanol, elementary analysis showed a composition in good agreement with the formula $C_{30}H_{24}O_8N_2Fe$ (found: C, 60·0; H, 4·1; N, 4·7; Fe, 9·3 per cent; calculated: C, 59·8; H, 4·05; N, 4·73; Fe, 9·3 per cent). In view of its colour and its iron content, the pigment has been named 'ferroverdin'.

Ferroverdin is insoluble in water, benzene, petroleum ether, chloroform and carbon tetrachloride, but is soluble in methanol (about 5 mgm./ml.), ethanol (about 2 mgm./ml.), acetone (about 15 mgm./ml.) and glacial acetic acid (about 15 mgm./ml.). It is readily reduced by hydrogen gas, in the presence of a palladium – barium sulphate catalyst, absorbing 6 mol. of hydrogen. The reduced solution is colourless and does not develop colour on shaking with air.

In the visible spectrum ferroverdin absorbs at 4300 and 6700 A. ($\varepsilon_{4300} = 1{\cdot}10 \times 10^4$, $\varepsilon_{6700} = 1{\cdot}10 \times 10^4$; it also absorbs strongly in the ultra-violet region with peaks at 2765 and 3000 A. ($\varepsilon_{2765} = 6{\cdot}18 \times 10^4$; $\varepsilon_{3000} = 5{\cdot}12 \times 10^4$. After reduction it shows peaks at 2340, 2710 and 3125 A. ($\varepsilon_{2340} = 3{\cdot}33 \times 10^4$, $\varepsilon_{2710} = 1{\cdot}37 \times 10^4$, $\varepsilon_{3125} = 7{\cdot}2 \times 10^3$ (solvent methanol).

The iron is firmly bound in ferroverdin, but appears in ionic form after catalytic reduction. It is gradually released by hydrolysis at 100° C. in 2 *N* hydrochloric acid and in 2 *N* sulphuric acid. Investigations of its chemical structure are in progress.

We thank Dr. G. Gualandi and G. Serlupi for the spectrophotometric measurements and acknowledge with thanks the collaboration of Dr. A. Ballio in the initial stages of this work. We also thank Mr. F. Zanchi and the staff of the fermentation pilot plant of this Institute for their co-operation in the production of ferroverdin, and Mr. J. Daymond for technical assistance.

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July 26.

A Brain Factor influencing the Viability of Neurotropic Rift Valley Fever

DURING experiments on the viability of Rift Valley fever it was noticed that crude extracts of virus-infected brain tissue in 10 per cent rabbit, sheep or human serum were less stable than the virus partially purified by differential ultra-centrifugation and suspended in the same diluents. In Fig. 1 this difference in viabilities is shown. Curve (*a*) shows the survival at 37° C. of virus in a crude or unpurified suspension of four virus-infected adult mouse brains in 40 ml. of 10 per cent rabbit serum saline. Curve (*b*) shows the survival of partially purified virus stored under the same conditions. It will be noticed that the disappearance of the virus in the partially purified material followed the exponential law, whereas the unpurified material showed an increasing rate of inactivation with time.

The factor responsible for the rapid inactivation of the virus is present not only in emulsions of virus-infected tissues but also in 10 per cent serum saline extracts of uninfected or normal mouse brain. In Fig. 2, viability curves are shown of purified virus suspended in normal brain extracts (4 brains in 40 c.c. rabbit serum saline) in two separate experiments (*a*), and that of purified virus in 10 per cent rabbit serum saline (*b*).

The factor is probably not a protein as no fraction capable of inactivating the virus was precipitable from brain emulsions by ammonium sulphate between 0 and 100 per cent saturation.

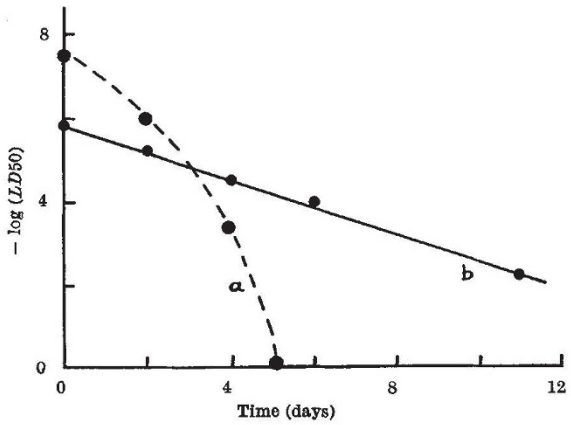

Fig. 1. Survival or viability curves of neurotropic Rift Valley fever at 37° C. (*a*) Crude infected brain suspension in 10 per cent serum saline; (*b*) partially purified virus in 10 per cent serum saline