phatic esterase of the flies treated with TOCP was inhibited to a large degree. The cholinesterase was uninhibited. Treatment with TEPP of the sampel of TOCP-treated flies resulted in inhibition of the cholinesterase at prostration. Treatment of the control flies with TEPP alone caused high inhibition of the ali-esterase and inhibition of the cholinesterase. This latter result is somewhat similar to that obtained by Van Asperen[2] for flies treated with DDVP, but since TOCP-treated flies can tolerate, without showing apparent symptoms of poisoning, a high level of aliphatic esterase inhibition, it is doubtful that this enzyme alone is important in organophosphorus poisoning. The evidence in Table 1 appears to refute Van Asperen's[2] suggestion in this respect. The present work strongly indicates that the inhibition of the aliphatic esterase is of minor importance when compared to the inhibition of cholinesterase. At the same time, it shows that the level of cholinesterase inhibition causing knock-down of the flies is important when evaluating cholinesterase on this basis.

This work will be published more in detail elsewhere.

D. Stegwee*

Pesticide Research Institute,
University Sub-Post Office,
London, Ontario.

* Postdoctorate Research Fellow of the National Research Council of Canada, on leave from the Laboratory of Entomology, Agricultural University, Wageningen, Holland.

[1] Winteringham, F. P. W., and Lewis, S. E. *Ann. Rev. Ent.*, **4**, 30 (1959
[2] Van Asperen, K., *Nature*, **181**, 355 (1958).
[3] Hopf, H. S., and Taylor, R. T., *Nature*, **182**, 138 (1958).
[4] Mounter, L. A., and Whittaker, V. P., *Biochem. J.*, **54**, 551 (1953).
[5] Augustinsson, K. B., *Acta Physiol. Scand.*, **15**, suppl. 52, 1 (1948).
[6] Metcalf, R. L., March, R. B., and Maxon, M. G., *Ann. Entomol. Soc. Amer.*, **48**, 222 (1955).

BACTERIOLOGY

Enhancement of Pigment Production by Pseudomonas

One of the principle factors to be considered in the preparation of media for the detection of fluorescent pigments by *Pseudomonas* is the content of available iron. When Totter and Moseley[1] tested oxine (8-hydroxyquinoline) in an effort to lower the metal content of media by chelation they failed to demonstrate an increase in pigment formation. Despite this rather unexpected result the following method was evolved which, on testing with a wide range of pseudomonads, proved highly successful in stimulating pigment production from many organisms which were previously considered negative or very weakly positive in this character.

The constituents for 1 litre of the medium were :

Dihydrogen ammonium phosphate	1·0 gm.
Potassium chloride	0·2 gm.
Crystalline magnesium sulphate	0·2 gm.
Potassium or sodium gluconate	5·0 gm.

These were dissolved in 400 ml. of glass-distilled water with 2 ml. of 0·5 per cent w/v 8-hydroxyquinoline in redistilled chloroform[2] and agitated vigorously in a separating funnel. The chloroform layer was allowed to settle out and then removed. This process was repeated three further times with fresh chelating agent. Finally the solution was washed twice with 5 ml. quantities of redistilled chloroform to remove the excess oxine. After the removal of the visible traces of chloroform the solution was made up to 1 litre with glass-distilled water, adjusted to *p*H 7, dispensed in normally cleaned tubes and sterilized at 22 lb. pressure.

If a solid medium was desired, shred agar was subjected to a similar chelating process. The unmelted agar was treated at the same time with two solutions; the oxine-chloroform (as above) and a 0·1 per cent solution of ethylenediaminetetraacetic acid (sodium salt) in water. The reagents were replaced every day for 5 or 6 days and the agar finally washed thoroughly with chloroform and water. When dried the treated agar was used in normal concentration to solidify the gluconate medium.

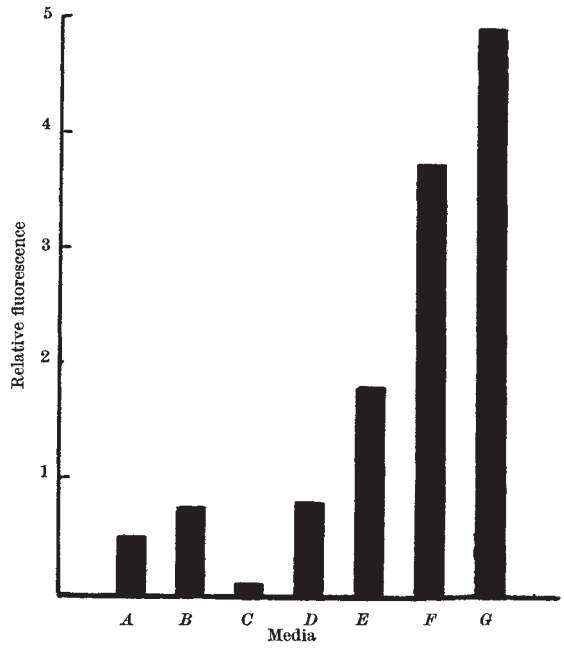

Fig. 1. A fluorimetric comparison of the ability of various solid media to support fluorescent pigment production by a *Pseudomonas* (arbitrary scale).
A, peptone Lemco agar (uninoculated); *B*, peptone Lemco agar (inoculated); *C*, gluconate medium (nutrients and agar treated; uninoculated); *D*, gluconate medium (untreated; inoculated); *E*, gluconate medium (nutrients treated; inoculated); *F*, gluconate medium (nutrients and agar treated; inoculated); G, gluconate medium (as *F* with addition of 0·05 per cent 'Yeastrel'; inoculated).

An ultra-violet light source with a Wood's glass filter was necessary when examining cultures as a brilliant fluorescence was not necessarily accompanied by a pigment visible to the naked eye. Neither the liquid nor solid medium showed any fluorescence without the growth of pseudomonads.

Fig. 1 shows the relative intensity of fluorescence produced in various solid media with or without the growth (48 hr. at 27°C.) of a culture of *Pseudomonas mors-prunorum* Wormald as determined by a Locarte fluorimeter (Locarte Co., 24, Emperor's Gate, London, S.W.7) standardized by a suitable aqueous solution of fluorescein. On ordinary nutrient agar this culture showed no visible fluorescent pigment even under ultra violet light and only a trace was detectable by the fluorimeter. This trace of pigment was amplified about 25 times by the use of the suggested medium (*F*) and more than 30 times when 'Yeastrel' was added at a concentration of not more than 0·05 per cent (medium *G*).

The liquid medium could be used, in addition, for the detection of keto-acid formation[3,4], a property of many *Pseudomonas*.

A. M. Paton

Bacteriology Department,
University of Aberdeen.
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[1] Totter, J. R., and Moseley, F. T., *J. Bact.*, **65**, 45 (1953).
[2] Waring, W. S., and Werkman, C. H., *Arch. Biochem.*, **1**, 303 (1942).
[3] Haynes, W. C., *J. Gen. Microbiol.*, **5**, 939 (1951).
[4] Stewart, D. J., *Nature*, **183** 1133 (1959)