

These results will be reported more fully elsewhere. This work was done while one of us (S. S. C.) was in receipt of a grant from the Commonwealth Fund.

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<sup>1</sup> Smith, J. E., and Wyatt, G. R., *Biochem. J.*, **49**, 144 (1951).

<sup>2</sup> Weed, L. L., and Cohen, S. S., *J. Biol. Chem.*, **192**, 693 (1951).

<sup>3</sup> Marshak, A., *Proc. U.S. Nat. Acad. Sci.*, **37**, 299 (1951).

<sup>4</sup> Weed, L. L. (personal communication).

<sup>5</sup> Wyatt, G. R., *Biochem. J.*, **48**, 484 (1951).

### Cytoplasmic Polyhedral Virus Diseases

IN the literature on the polyhedral virus diseases of insects, apart from the many nuclear polyhedroses of the fat-body (and cuticular tissues), only one case of a nuclear polyhedrosis of the gut and one cytoplasmic polyhedrosis of the fat-body have been described. Previously, no cytoplasmic polyhedroses of the gut has been reported.

Several different cytoplasmic polyhedroses of the gut have now been found, and cross-infection to other species established with them. For example, cytoplasmic gut polyhedroses have been obtained on infecting *Vanessa io* larvae with the polyhedra of *Rhyppara purpurata*. The silkworm, *Bombyx mori*, develops similar polyhedroses on infection with the polyhedra of *R. purpurata*, *Aretia villica*, or the cytoplasmic gut polyhedra of *Sphinx ligustri*. Such polyhedroses have also been obtained in *Lymantria dispar* and *Aglais urticae*, etc. It has been established that *villica* polyhedra give rise to a gut cytoplasmic polyhedrosis in their host species, *A. villica*. It is suspected, though not demonstrated, that this is also the case with *purpurata* virus in its natural host.

In all these cases, as with others, the cytoplasmic gut polyhedra have been found to be blue-staining with methylene blue even after only a very light heat treatment of gut smears. Nuclear polyhedra do not stain after such treatment. It has been previously established<sup>1</sup> that *villica* and *purpurata* polyhedra show an absence of membrane and virus rods when examined under the electron microscope after being treated with weak alkali. The same is true for *purpurata* polyhedra in the silkworm, and for the naturally occurring cytoplasmic gut polyhedroses of the privet hawk (*S. ligustri*), the only ones of those mentioned so far examined<sup>2</sup>. All polyhedra of this type which have been examined showed the 'honey-comb' structure described by Smith and Wyckoff<sup>3</sup>.

Provisionally, then, it can be suggested that two distinct types of polyhedral diseases exist. The polyhedra from nuclear polyhedroses do not stain with methylene blue, are readily soluble in alkali, and possess membranes. The polyhedra from cytoplasmic polyhedroses are readily stainable with methylene blue, are less readily soluble in alkali, are without membranes and leave a pitted 'honey-comb' structure after alkali treatment.

It is hoped that further research will establish the conditions favouring the development of one or

other type of polyhedrosis, and whether under appropriate conditions a given polyhedral virus may give rise to a polyhedrosis in a different cytological and histological site from its original one.

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<sup>1</sup> Smith, Kenneth M., and Wyckoff, R. W. G., *Nature*, **166**, 861 (1950).

<sup>2</sup> Smith, Kenneth M., Wyckoff, R. W. G., and Xeros, N. (in the press).

### Tainting of Tobacco by a Dichloropropene-Dichloropropane Soil Fumigant

ONE of the most serious pests of tobacco in Southern Rhodesia is the eelworm, *Heterodera marioni*. Control of this organism is effected by injecting the soil, by means of a plunger-type injector, with ethylene dibromide or a dichloropropene-dichloropropane mixture. During the 1951-52 growing season, it has been estimated that the latter fumigant has produced 'off-odours' in approximately five per cent of the tobacco crop. These odours first become apparent during the curing process, and persist through all subsequent stages of processing. Impurities in the fumigant mixture have been shown to be responsible for this tainting and have been isolated and characterized by the methods described below.

Green tobacco leaf was extracted twice successively with its own weight of ether, the combined extracts filtered and the ether distilled off. The residue was extracted with ethanol and chromatographed on aluminium oxide, using ethanol to develop the column. Spectrophotometric examination of the primary colourless eluate, collected before any pigment bands had passed through the column, showed that the tainted tobacco contained material absorbing in the deep ultra-violet that was not present in untainted leaf.

Fractionation of the primary eluate yielded two components, boiling at 69° (fraction F) and at 148° (fraction X). Comparison of the spectrophotometric absorption curves, boiling points, refractive indices and the melting points of the S-alkylisothiourea picrate derivatives (prepared according to Brown and Campbell<sup>1</sup>), together with mixed melting point determinations, have led to the identification of these two fractions with two of the components of the soil fumigant mixture, fraction F being tentatively identified as 2:2-dichloropropane and fraction X as 1:2:3-trichloropropane.

The concentration of the contaminating substances in the green tobacco leaf was found to be approximately nine parts per million. No such compounds could be isolated from untainted tobacco.

Paper chromatography of the leaf pigments, using methanol-hydrochloric acid-water (50:25:25) and ethanol-acetic acid (98:2) as the solvents, followed by elution of the separated pigment spots and their spectrophotometric examination, showed differences between tainted and normal leaf, several of the pigments from the tainted leaf exhibiting a shift of absorption maxima towards the red end of the spectrum.

It is thought that certain of the components of the soil fumigant mixture may be entering into the