Production of Metabolic Benzpyrene Derivatives *in vitro*

It has been shown by Weigert and Mottram¹ that, independent of its mode of introduction into an animal, benzpyrene passes through a series of chemical changes prior to its final excretion. The following evidence suggests that the first derivatives—provisionally termed X_1 and X_2 —are produced locally in those organs (liver, kidney, lung) where the benzpyrene is concentrated after intravenous or intraperitoneal injection, and also in the blood. The same happens in the subcutaneous tissues and in the skin of mice after the injection or painting of benzpyrene.

A series of mice were decapitated immediately after the intravenous injection of a colloidal suspension of benzpyrene. The shed blood and the carcasses were then stored at 0° C. and examined at intervals of a few hours for metabolic products. The amount of X_1 present in the liver, lung, kidney and the clotted blood increased slowly. Further, human blood was mixed with a benzpyrene suspension and the presence of X_1 was detected after a few hours by a sensitive fluorescence method.

The following technique has been adopted for the study of benzpyrene metabolism *in vitro*. Mice are clipped, killed and the whole of the skin between the fore and hindquarters is removed and placed on filter paper moistened with Ringer-Locke solution. Measured areas are painted with solutions of benzpyrene in acetone and then excised and floated on to Ringer-Locke solution in Petri dishes, the hair surface being uppermost. These are placed in the incubator at 37° C.

After incubation for periods of three hours or more the presence of X_1 can be shown. On the basis of its absorption and fluorescence spectra and its behaviour towards various solvents, the X_1 formed under these conditions would appear to be identical with that formed in the mouse skin after painting the living animal and after intravenous or intraperitoneal injection. Quantitative estimations (full details of the methods used will be published elsewhere) indicate that when a dilute solution of the benzpyrene (0.002 per cent in acetone) is applied, up to 40 per cent is recoverable in the form of X_1 .

The use of this *in vitro* technique with the mouse skin shows definitely that the first stage of the metabolic conversion is the same as *in vivo* and takes place at the site of application of the benzpyrene.

Records of detailed studies using this *in vitro* system and the corresponding *in vivo* system are now in course of preparation for publication.

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¹ Weigert, F., and Mottram, J. C., *Cancer Res.*, **6**, 97 (1946).

Relation of Crystal Size and Shape to Contact Toxicity of D.D.T. Suspensions

It is frequently found that in two-phase disperse insecticidal systems in which the toxic compound constitutes part or whole of the disperse phase, toxicity bears an inverse relation to the particle size of the disperse phase. Recent work here has shown, however, that in the case of aqueous suspensions of pure *p,p'*-D.D.T. [1-trichloro 2,2-bis(*p*-chlorophenyl) ethane], this relationship is reversed.

p,p'-D.D.T. normally crystallizes in needles¹, but in certain circumstances can be made to form plate-like crystals. In the preparation of suspensions the overall size as well as shape of crystal can be varied. This does not involve any changes in the fundamental habit of the crystal, but merely variations in the external form produced by different growth conditions. In this way, suspensions of six distinct types, each of characteristic and fairly uniform crystal size and shape, have been prepared and tested here against *Tribolium castaneum* Hb. (red-rust flour beetle), using, chiefly, a recently developed dipping technique². Within the range of crystal sizes tested, namely, from colloidal D.D.T. to a suspension of needle-shaped aggregates 350 μ long, toxicity is associated primarily with overall length, and increases with length; breadth is of lesser importance, but it seems definite that increasing the breadth of a crystal from a needle to a plate shape subtracts somewhat from its toxicity. Thus, of all the sizes tested, colloidal D.D.T. is least, and 350 μ needles most, toxic. The ratio of the median lethal doses of these extreme sizes is of the order of 12:1.

There must, of course, be an upper limit to the toxicity, for it is obvious that an increase in the crystal dimensions beyond certain limits would not be accompanied by a corresponding increase in toxicity. As the choice of 350 μ needles as a standard size was purely one of convenience, it is scarcely likely that such a suspension does represent maximum toxicity.

These results are in line with those of Parkin and Green³, who showed that the toxicity to houseflies of residual D.D.T. films increases with the age of the film and that this is related to slow crystallization of D.D.T. from a "poorly toxic gum-like residue".

The origin of these differences is not clear, but they are certainly not due to the few small differences in the media of the separate types of suspension. That they do not have their origin in any operation peculiar to the dipping technique is shown by the fact that similar differences are obtained with the Potter spraying apparatus⁴. However, it may be that with smaller particles there is a greater "run off" of insecticide from the insects' bodies, that is, larger particles are retained more readily by irregularities on the cuticle surface. This would be equivalent, in fact, to a higher dosage; hence the higher kill.

Further work on this subject is in progress here and complete details will be published elsewhere at a later date.

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¹ Gooden, E. L., *J. Amer. Chem. Soc.*, **67**, 1616 (1945).

² McIntosh, A. H., in the press.

³ Parkin, E. A., and Green, A. A., *Nature*, **155**, 668 (1945).

⁴ Potter, C., *Ann. Appl. Biol.*, **28**, 142 (1941).

An Insect Vector of the Turnip Yellow Mosaic Virus

In a recent communication¹ we described a new virus attacking turnips, which we had isolated in crystalline form. At that time we had found no insect vector for the virus, all the experiments with aphids having proved negative. However, since the virus attacks an annual crop like the turnip it seemed highly probable that some insect vector was involved, so further investigations were undertaken. A small plot of turnips was sown out-of-doors and a few infected plants placed at random in the plot. It was soon evident from the rate and manner of spread of the virus that some insect was transmitting it.

Examination of the insect fauna of the plot showed the only insects present in any number to be aphides and flea-beetles (*Phyllotreta* spp.). Since the aphides had already been tested with negative results attention was directed to the flea-beetles and the following experiment was carried out. Two insect-proof cubicles in the glasshouse were filled with healthy young turnip and Chinese cabbage plants and two infected plants were placed in each cubicle as a source of virus. In one cubicle a large number of flea-beetles, mostly *Phyllotreta cruciferae* and *P. vittula*, were liberated; the other cubicle which acted as a control was kept free of insects. Ten days after the introduction of the flea-beetles into the cubicle, the first plant became infected; the following day three more plants developed the disease and during the next two days, five more. In the control cubicle there was no spread of the virus.

This seems fairly conclusive that the flea-beetle is, in fact, the vector of the turnip yellow mosaic virus, and if confirmed will be the first instance of virus transmission by a biting insect in Great Britain. Whether the transmission is a purely mechanical process is not yet certain, though it seems probable that this is so.

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¹ *Nature*, **157**, 300 (1946).

Carrot Fly Control

THE control of carrot fly by the application of insecticides direct to the plant rows has shown interesting anomalies when compared with that of root flies of somewhat similar habits. Calomel, for example, effective against cabbage root fly, does not appear to work against carrot fly.

It is of interest to record in this connexion the results of trials made in 1945 comparing the efficacy of a D.D.T. (Geigy) 5 per cent dust and a benzene hexachloride dust containing 0.25 per cent gamma isomer as crude benzene hexachloride (formulated as P.F. flea-beetle dust). It is seen from the accompanying table that the control given by the benzene hexachloride (B.H.C.) is of a quite remarkable order, whereas only moderate control was effected by D.D.T.

CARROT FLY TRIALS 1945. SUMMARY OF REPLICATES					
	Total number of carrots examined	% Clean	% Slight attack	% Moderate attack	% Unsale- able
Control	236	12.7	18.7	11.9	56.7
D.D.T.	419	44.2	21	9.3	25.5
B.H.C.	350	99.7	0.3	—	—

Results obtained this year have, so far, been confirmatory.

The reason for this difference is not yet apparent. The characteristic odour of B.H.C. might suggest that a deterrent effect is involved, but this is scarcely borne out by the way protection so exactly followed the limits of distribution of the dust; in addition, a fumigant effect may not be out of the question. It is hoped to get further evidence on these matters.

One other point regarding phytocidal action may be mentioned. Unnecessarily heavy dressings of B.H.C. do not appear to have a hurtful action on the carrot, whereas with the Brassica even moderate dressings may seriously affect the stem at the point where contact is made—possibly a point of interest in regard to the mode of action of benzene hexachloride.

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Powdery Mildew of Potato

POWDERY mildew on potato was first recorded in Great Britain in 1932, when the oldial stage was found on the leaves of seedling potatoes in a glasshouse at Cambridge¹. It was again observed at Cambridge in the late summer of 1945, and a field survey was made to determine its prevalence. The mildew was found not only on seedling plants in the greenhouse, but also on potatoes in trial plots in the open. A search was then made for it in the immediate neighbourhood, and later this was extended to cover an area within a ten-mile radius of Cambridge.

Mildew was prevalent throughout the area inspected. Crops in the vicinity of the trial plots were most heavily infected, but elsewhere infection was greatest in the south-west of Cambridgeshire. Mildewed plants were found twelve miles away, namely, at Burwell, where a mild attack had developed in a garden.

In the field the disease was observed on the following varieties: Majestic, Dunbar Rover, Abundance, Dunbar Standard, Arran Victory and President. A number of hybrids, derived from parental stock of King Edward, were also affected. Majestic was the variety most noticeably attacked, but mildew was not observed on Kerr's Pink, Gladstone and Red Skin, or on any other Solanaceous plant.

Inoculation experiments were made on plants related to the potato, and on species known to be susceptible to *Erysiphe cichoracearum*.