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LABELLED INSECTIDE STUDIES: TECHNIQUES
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Co-operation of the United
SOME APPLICATIONS OF RADIOISOTOPES TO THE STUDY OF THE CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS*

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Abstract — Résumé — Anotarion — Resumen

SOME APPLICATIONS OF RADIOISOTOPES TO THE STUDY OF THE CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS. Preliminary work was carried out using di-iodo-octadecane-214 as a tracer in solution in oils. This substance proved useful for investigating the creep of oil films over insect epidermis, but was unsatisfactory for critical work on oil absorption through the cuticle, being converted in the tissues to one or more water-soluble derivatives.

In subsequent investigations, diethyl-214 has been used in solution in oils labelled with tritiated hexadecane. In comparative experiments with Tribolium castaneum exposed to these solutions of different viscosity, appreciable differences in both the rates of diffusion over the insects and in absorption through the cuticle have been found. After an initial period, diethyl is absorbed relatively faster than solvent, the magnitude of the differential absorption varying with viscosity.

QUELQUES APPLICATIONS DES RADIOISOTOPES DANS L’ÉTUDE DE LA CONTAMINATION DES INSECTES PAR DES INSECTICIDES EN SOLUTIONS. Les travaux préliminaires ont été exécutés en utilisant, comme indicateur, le di-iodo-octadécane marqué par 214I en solution huileuse. Cette substance s’est révélée utile pour l’étude de la dispersion des pellicules d’huile sur l’épiderme des insectes, mais elle n’a pas donné de résultats satisfaisants lors de travaux sur l’absorption de l’huile à travers la cuticule de fait qu’elle se trouvait transformée dans les tissus en un ou plusieurs dérivés solubles dans l’eau.

Au cours d’études ultérieures, la diéthylène marquée par 214C a été utilisée en solution huileuse marquée à l’aide d’hexadécane radioactif. Des expériences comparatives sur le Tribolium castaneum, exposé à trois solutions de viscosités différentes, ont révélé des différences sensibles en ce qui concerne les vitesses de diffusion sur l’Insecte et l’absorption à travers la cuticule. Après une période initiale, la diéthylène est absorbée à une vitesse relativement plus grande que le solvent, la différence étant fonction du degré de viscosité.

ВОЗМОЖНЫЕ ПРИМЕНЕНИЯ РАДИОИСТОПОВ ДЛЯ ИССЛЕДОВАНИЯ НАСЕКОМЫХ ГРУЩИВОЙ РАСТВОРЯМИ ИНСЕКТИЦИДОВ. Были выполнены предварительные работы с использованием ди-кетотриамидина, метанового 214I, в качестве индикатора в растворах в маслах. Это вещество оказалось удобным для исследования скоростей миграции ионов по капилляризации насекомых, но оно не было открытым для критической работы по определению аборбции масла через кожу насекомого, так как оно превращалось в тканях в одно или несколько водорастворимых производных.

В последующих исследованиях использовался метановый 214I, в растворах в маслах, смертным показаны трофические эффекты. Экспериментальные опыты с использованием Tribolium castaneum в виде трох растворов различной вязкости привели к следующим результатам в отношении скоростей диффузии у насекомых, так и в отношении аборбции через кожу насекомого. После начального периода диффузии аборбционная способность менялась в зависимости от вязкости раствора.

EMPLEO DE LOS RADIOISÓTOPOS PARA ESTUDIAR LOS EFECTOS CONTAMINADORES DE LAS SOLUCIONES INSECTICIDAS. Las primeras investigaciones se hicieron utilizando como marcador diodo-octadecano-214 en solución oleosa. Esta sustancia ha resultado útil para estudiar la infiltración de las películas de

* This work was supported by the Tropical Pesticides Research Committee of the Department of Technical Co-operation of the United Kingdom Government.
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Abstract — Résumé — Аннотация — Resumen

SOME APPLICATIONS OF RADIOISOTOPES TO THE STUDY OF THE CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS. Preliminary work was carried out using di-isooctadecane-14C as a tracer in solution in oil. This substance proved useful for investigating the creep of oil films over insect epicuticle, but was unsatisfactory for critical work on oil absorption through the cuticle, being converted in the tissues to one or more water-soluble derivatives.

In subsequent investigations, dieidrin-14C has been used in solution in oils labelled with utilised hexadecane. In comparative experiments with Tribolium castaneum exposed to tritiated solutions of different viscosity, appreciable differences in both the rates of diffusion over the insect and in absorption through the cuticle have been found. After an initial period, dieidrin is absorbed relatively faster than solvents, the magnitude of the differential absorption varying with viscosity.

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Au cours d'études ultérieures, le dieidrine marqué par 14C a été utilisée en solution huileuse marquée à l'aide d'hexadécane. Des expériences comparatives sur le Tribolium castaneum, exposés à trois solutions de viscosités différentes, ont révélé des différences sensibles en ce qui concerne les vitesses de dispersion sur l'insecte et l'absorption de l'huile à travers la cuticule. Après une période initiale, le dieidrine est absorbé à une vitesse relativement plus grande que le solvant, la différence étant fonction du degré de viscosité.

ВОЗМОЖНОСТИ ИСПОЛЬЗОВАНИЯ РАДИОИСТОТОПОВ ДЛЯ ИЗУЧЕНИЯ ПОДВОДНОГО ПОДПАСУНЬЯ НАСЕКОМЫХ РАСТВОРАМИ ИНСЕКТИЦИдов. Были выполнены предварительные работы по использованию диизооктадекана, метиленового 14C, в качестве индикатора в растворе в масле. Это вещество оказалось удобным для изучения скорости образования полимерных пленок эпидермиса насекомого, но оно непригодно для критического изучения по определению скорости впитывания через кутикулу, так как оно превращается в тканях в одно или несколько водорастворимых производных.

В последующих исследованиях использовался диизооктадекан-14С для растворов в масле, метилированный тяжелым водородом. Применялись для опытов с использованием Tribolium castaneum в виде трех растворов различной вязкости были обнаружены заметные различия в отношениях скоростей диффузии у насекомых, так и в отношении впитывания через кутикулу. После начального периода диизооктадекан быстро впитывается, в отличие от растворов.

EMPLEO DE LOS RADIOISÓTPOS PARA ESTUDIAR LOS EFECTOS CONTAMINANTES DE LAS SOLUCIONES INSECTICIDAS. Las primeras investigaciones se hicieron utilizando como marcador diisooctadecano-14C en solución oleosa. Esta sustancia ha resultado útil para estudiar la difusión de las películas de

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acces to the epicuticle of the insects, was made in order to investigate the absorption of acetone by the cuticle, once a small amount of acetone was selectively injected into the insect. Comparing the experiments conducted with Tribolium castaneum treated with acetone solutions, no significant differences were observed in the rate of diffusion in the insects and the absorption rates. After a period of time, the rate of absorption of the acetone was found to be different, indicating that the difference was due to the viscosity.

**INTRODUCTION**

When an oily solution of an insecticide is picked up by an insect, the oil facilitates the action of the insecticide in at least two ways: firstly, by spreading the insecticide over the epicuticle of the insect and thus presenting it very intimately to the insect; secondly, by facilitating the diffusion of the insecticide through the cuticle. These processes have not hitherto been studied quantitatively, and indeed it is difficult to see how they could be without the employment of radioactive tracer techniques, as no chemical method of analysis for small quantities of hydrocarbon oils is known.

Two series of experiments on this subject have been carried out. The first series was principally concerned with the spread of the oil over the cuticle of active insects, for which purpose iodine-131 in the form of an iodized hydrocarbon was employed as a tracer. The second series was particularly concerned with the penetration of both oil and solvent and an insecticide, dieletrin, through the cuticle. In this series the oils and insecticide were labelled with tritium and carbon-14 respectively.

1. **EXPERIMENTS WITH DI-IODO-OCTADECANE AS AN OIL TRACER**

**Materials and methods**

Traces of di-iodo-octadecane-131, prepared by the addition of iodine-131 to the unsaturated hydrocarbon octadecene, were added to a highly refined mineral oil, Shell Risella 17, which consists of hydrocarbons of chain length varying from 17 to 20 carbon atoms. The specific activity of the oil sample was adjusted to 2 mc/ml. This tracer was selected because of its suitability for liquid and solid counting techniques using Geiger-Müller tubes, the only methods of radio-assay available in the department at that time.

The oil deposits used in the experiments were always of 3 μl/cm² evenly deposited on Whatman No. 1 filter papers. Blowflies of the species Phormia terrinovae R-D were exposed to the oil substrate at 25°C for varying periods of time up to 60 min, by a method described previously [1].

Immediately after removal from the active substrate, some of the flies were rapidly dissected to enable assays of oil associated with different parts of the body to be carried out. The films of oil adhering to the epicuticle of whole or part insects were washed off by three successive rinses of paraffinic hydrocarbons, boiling range 60-100°C. This treatment was shown by control experiments with untreated flies to remove the epicuticular waxes. It is therefore clear that the adherent oil was totally removed, together with the fraction taken up in the epicuticular wax.

The rinsed insects were washed and extracted, first with X-ray film for aeration of oil on the compound, then gauged qualitatively.

**Results**

A brief summary illustrates the scope of oily films on insect where [2].

Two processes on an oily deposit transfer from the surface allow a diffusion of the oil.

Initially, oil was per fly, the rate of fly was actively carried away from the tarsi of the body.

The wings accumulate the surface area of the fly, calculated for gas-a mean thickness of oil that if the oil molecule layer is e.

Autoradiograph shows that the rate of radioactivity, in the legs, especially the legs, than the upper segments.

If the oil were s, the insects would cease before the removal of change, as seen with the facts that up the oil proceeds over the epicuticle of the cuticle.

When the insect is k, the oil, advanci
The rinsed insects were then ground up in anhydrous sodium sulphite and extracted, first in ether, then in 10% NaOH to obtain the absorbed tracer. Washings and extracts were assayed with a Geiger–Müller liquid counter tube. The ground residue was finally dried and checked for any residual activity with a thin-end-window Geiger–Müller counter.

Other individuals were dissected and mounted between 2 layers of thin adhesive tape weighing 8.06 mg/cm², and the preparations were exposed on X-ray film for one week. From the resulting autoradiographs, the location of oil on the body surface could be determined and its rate of spread gauged qualitatively.

Results

A brief summary of the more significant results only will be given, to illustrate the scope and limitations of this tracer substance in the study of oily films on insects. Some aspects have been partially described elsewhere [2].

Two processes were distinguished in the contamination of an insect on an oily deposit. Firstly, there is the primary uptake of oil by direct transfer from the substrate to the tarsi of the insect; secondly, there follows a diffusion of oil away from the tarsi up the legs to the general body surface.

Initially, oil was taken up by the tarsi at a mean rate of 3×10⁻⁷ ml/min per fly, the rate of uptake remaining substantially constant so long as the fly was actively walking. The oil diffused over the surface of the insect away from the tarsi at the rate of approximately 1.5×10⁻⁷ ml/min to all parts of the body.

The wings accumulated oil at 15×10⁻³ ml/min. Since the molecular surface area of the wings of this strain of the species is known, having been calculated by gas–adsorption methods [3], it is possible to calculate the mean thickness of oil film on the wings for any given treatment. It appears that if the oil molecules are randomly oriented on the surface, a monomolecular layer is established in 5–15 min from the initial contact of insect with deposit.

 Autoradiographs confirm that the wing membranes are covered with tracer molecules after this period. They also reveal higher concentrations of radioactivity, indicating oil, along veins and at the confluence of wing veins. Similarly, they show uneven distribution in other parts of the body, especially the legs, the tarsi being, of course, more heavily contaminated than the upper segments.

If the oil were spreading by normal surface-tension forces, these differences would certainly be lost and an even film would be established quite soon after the removal of fly from deposit. But the differences persist without change, as successive autoradiographs show. This evidence, coupled with the fact that uptake varies with the activity of the fly, suggests that the oil proceeds over the cuticle aided by the very small elastic deformations of the cuticle which occur during active movements of an insect [4]. When the insect is killed, the movement of the very thin oil film ceases.

The oil, advancing at first as a mono-molecular layer, is simultaneously being absorbed. When we consider the absorption of oil, the limitations
of this tracer compound as a guide to oil movement become apparent, for the halogenated hydrocarbon tracer may well be differentially absorbed from the oil. But a second disadvantage also became apparent when measurements of the rate of absorption of tracer were made. Not all the $^{131}$I could be recovered in ether extractions; for example, after 60 min only 12.8% of the $^{131}$I was extracted in ether, 55.6% of the residual $^{131}$I was recovered with boiling water and 30% of the remainder was extracted with 10% NaOH solution. Thus it is clear that the $^{131}$I moiety of the tracer molecule is converted after absorption to one or more water-soluble compounds.

To sum up, di-iodo-octadecane-$^{131}$I has proved useful as a tracer for oil movements on the surface of an insect, but is not suitable as a tracer for oil movements across the cuticle or within the tissues.

2. EXPERIMENTS WITH DIELDRIN-$^{14}$C AND HEXADECANE-1:2-$^3$H

Materials and Techniques

Dieldrin-$^{14}$C of specific activity 6.54 mc/mM, obtained from the Radiochemical Centre, Amersham, was dissolved in three solvents of differing viscosity to form 1% solutions. The three solvents were n-hexadecane and the Shell oils Risella 17 and Risella 35, having viscosities of 2.8, 22.1 and 167 cP, and hydrocarbon chain lengths of 10, 17-20 and 20-35, respectively. The labelled hydrocarbon n-hexadecane-1:2-$^3$H of specific activity 207 mc/mM was also added to each solution to allow the tracing of the hydrocarbon solvent molecules. The specific activity of the n-hexadecane-1:2-$^3$H in each oil sample was adjusted to 27.5 mc/ml.

Filter-paper slips were treated as before to provide a deposit of 3 ml/cm² of solution, and batches of insects of the species Tribolium castaneum Herbst, were allowed to crawl over the deposits of 25°C for periods ranging from 30 min to 24 h.

After removal from the deposit, batches of ten insects were washed with three rinses of petroleum hydrocarbons (boiling range 40-60°C) to remove the adherent solution. The washed insects were then ground up with anhydrous sodium sulphite and extracted first with hexane for 2 h, then with water to recover absorbed oil, insecticide and any water-soluble metabolites of the insecticide which might be formed.

The quantities of carbon-14 insecticide and tritiated hydrocarbon in each sample were assayed and discriminated by means of internal liquid scintillator techniques. The scintillation liquids, obtained already formulated from Nuclear Enterprises (G.B.) Ltd., were NE213 based on xylene for the hexane extracts and external washes, and NE220 based on dioxane for the aqueous extracts. In the xylene-based scintillator, the counting efficiency was 5.5% for $^3$H and 73% for $^{14}$C, under the conditions of test. In the dioxane-based scintillator, the maximum efficiency for $^{14}$C was rather lower, though it should be mentioned that the presence of relatively high concentrations of sodium sulphite caused the formation of a flocculant precipitate when the sample was added to the scintillator solution. This introduced much noise of a very low energy level, possibly due to chemiluminescence, which would have interfered with tritium assay, but could be discriminated from the $^{14}$C response.

Results

Adherent film of oil

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In contrast, at f contamination with Rist many hours exposure two solutions were:

The faster rate 16 h. At this time of uptake of all thr
CONTOHINTION OF INSECTS BY INSECTICIDE SOLUTIONS

Results

The insects became contaminated more rapidly with the hexadecane solution than with the more viscous oil solutions, differences becoming apparent from the first measurement, taken after 30 min, when less than $1 \times 10^{-4}$ g of dieldrin had been accumulated.

In contrast, at first no significant differences between the rates of contamination with Risella 17 and Risella 33 solutions were found. Even after many hours exposure, differences between the adherent quantities of these two solutions were relatively small.

The faster rate of uptake of hexadecane solution is maintained for about 18 h. At this time the insects are moribund or very feeble, and the rate of uptake of all three solutions falls to a much lower level (Table I). As

<table>
<thead>
<tr>
<th>Duration of</th>
<th>Hexadecane</th>
<th>Risella 17</th>
<th>Risella 33</th>
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<tbody>
<tr>
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<td>(x10^4 ml)</td>
<td>solution</td>
<td>solution</td>
</tr>
<tr>
<td>0.5</td>
<td>0.92</td>
<td>0.28</td>
<td>0.34</td>
</tr>
<tr>
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<td>2.28</td>
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<td>9.31</td>
<td>3.19</td>
<td>2.66</td>
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<th>Dieldrin (x10^6 g)</th>
<th>Dieldrin (x10^6 g)</th>
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<td>exposure (h)</td>
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<tr>
<td>0.5</td>
<td>0.25</td>
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<tr>
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<td>2.15</td>
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<tr>
<td>24.0</td>
<td>9.24</td>
<td>3.3</td>
</tr>
</tbody>
</table>

TABLE I

ADHESIVE FILMS: QUANTITIES OF SOLVENT AND DIELDIN RECOVERED FROM THE EXTERIOR OF TRIBOLIUM CASTANEUM AFTER EXPOSURE TO 3-µl/cm² DEPOSITS OF 1% SOLUTIONS ON FILTER PAPER

...
would be expected, the ratio of insecticide to solvent in the adherent films does not differ from that in the original solutions applied to the substrate.

Absorption of solvent and insecticide

The three solvents were absorbed through the cuticle at a rate of approximately 2 × 10⁻⁷ ml/h at first, becoming progressively slower. There were no appreciable differences between the rates of absorption of the three solvents (Fig. 1).

![Graph showing absorption rates](image)

**Fig. 1**

Quantities of solvent and dieldrin extracted from the times of *Tribolium castaneum* Herbst. after exposure to 3-µl/cm² deposits of 1% solutions on filter paper

- O Dieldrin absorbed
- □ Solvent absorbed

However, the rates of absorption of dieldrin from the three solutions varied considerably. In the first hour, the average rate of uptake of dieldrin was relatively slower than that of the solvent. Subsequently, the insecticide was absorbed relatively faster than the solvent, the rate of uptake of dieldrin being approximately linear for each formulation (Fig. 1).

The magnitude of the differential absorption of dieldrin from each solution is illustrated by Table II, where the differential is expressed as the ratio (absorbed dieldrin over absorbed solvent) × 100. This ratio would be 1.0 for uniform absorption of both components of a 1% solution, but it will be seen that it rises steadily within 16 h to values of 8.36, 4.14 and 2.86 for solutions in hexadecane, Risella 17 and Risella 33 respectively.

Water-soluble metabolites

No evidence of water-soluble derivatives of dieldrin was found in the extracts.

**Discussion**

Although octade for oil movements, in particular, the import the uptake and spread. However, the phasic take, at least for fil. (*Tribolium castaneum* labelled with n-hexane cially faster than th.

The effects of these insecticide solutions *Aedes aegypti* L. an work permit the fac.

The rates of abs were not significan (Fig. 1). This result trate isolated pieces no such differences absorptive capacity o rate of solvent absor in solvent absorptio phenomenon.

The concentrati the rate of solvent a before, if the solvent f
The adherent films were taken up by the substrate.

The films were dried at a rate of approximately 0.5 mm per day. There was no significant difference in the rate of uptake of the three solutions with the exception of the three

<table>
<thead>
<tr>
<th>Treatment (h)</th>
<th>Hexadecane solution</th>
<th>Rissler 17 solution</th>
<th>Rissler 33 solution</th>
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<tr>
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<td>1.09</td>
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<td>0.82</td>
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<td>16.0</td>
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<td>24.0</td>
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Discussion

Although octadecane di-iodide has certain limitations as a tracer for oil movements, significant results were obtained from its use; in particular, the importance of active movements of the cuticle in facilitating the uptake and spread of very thin oil films over an insect was demonstrated. However, the physical properties of the solvent also affect the rate of uptake, at least for films several molecules thick, for when crawling insects (Tribolium castaneum) were exposed to three solutions of different viscosities labelled with n-hexadecane-1,2-H\(^2\), the least viscous was picked up appreciably faster than the more viscous (Table I).

The effects of solvent viscosity on the relative toxicities of different insecticide solutions has been noted previously from biological assays with *Aedes aegypti* L. and *Musca domestica* L. [6]. The results of the present work permit the factors concerned to be analysed in greater detail.

The rates of absorption of the three solvents through the living cuticle were not significantly different, despite the differences in molecular size (Fig. 1). This result contrasts with earlier observations that light oils penetrate isolated pieces of cuticle faster than heavier oils [6]. The fact that no such differences were revealed in the present work suggests that the absorptive capacity of the hypodermal cells exercises a limiting effect on the rate of solvent absorption through living cuticle. The progressive decline in solvent absorption rate with time provides evidence of a saturation phenomenon.

The concentration of dieldrin in the film adhering to each insect and the rate of solvent absorption are the same for each formulation. Therefore, if the solvent facilitated the entry of dieldrin principally by carrying
the insecticide molecules in association with solvent molecules through the cuticular barriers, the quantities of dieldrin absorbed would be the same for each formulation.

But the quantities of dieldrin absorbed vary with solvent viscosity, and are much greater than would be expected on the basis of a direct solvent carrier effect (Fig. 1, Table II). Indeed, the capacity of the tissues to absorb dieldrin is considerable, and is not diminished by death, for the uptake of insecticide varied linearly with time and, unlike the solvent absorption, showed no appreciable indication of a saturation effect within a period of 24 h. Differential absorption of dieldrin from the adherent film was made good by diffusion from the substrate.

If neither concentration gradients nor direct carrier effects can explain the differences between the rates of dieldrin absorption from the different solvents, the facilitated absorption must depend almost entirely upon a modification of the cuticular barrier by the intercalation of solvent molecules, permitting faster transference of insecticide molecules than would otherwise occur. WIGGLIESWORTH [6] has shown that the wax layer delays the absorption of pyrethrin solutions by Rhodnius nympha. One likely effect of the oily solvents in the present experiment is to disturb the organization of the epicuticular wax. The smaller are the molecules of the solvent hydrocarbons dispersed in the wax layer, the faster the diffusion of dieldrin would be expected to take place.

In this context, it would be of interest to compare the effects of different solvents on insecticide absorption by a species having a structurally different type of epicuticle, e.g. blowfly larvae. Indeed, much remains to be done before the mechanism of insecticide absorption are fully understood; it is certain that radioactive tracer techniques will be of great value in such studies.

REFERENCES


DISCUSSION

J. Halberstadt: The author reports no significant difference in the cuticle absorption rates as between hexadecane, Risella 17 and Risella 33, using tritiated hexadecane as tracer for all three solutions. But is he not in fact measuring the absorption of hexadecane in all three cases, so that there may be a difference of absorption between hexadecane and the two Risella oils? If that were the case, then there might be a much smaller difference in spreading rates between hexadecane and the two Risella oils, which could be explained if Risella oil by absorption of the Risella oil active or inactive, hexadecane.

C. T. Lewis: Absorption of the hexadecane led to the conclusion a modification of the cuticular barrier by the intercalation of solvent molecules. The wax layer delays the absorption of pyrethrin solutions by Rhodnius nympha. One likely effect of the oily solvents in the present experiment is to disturb the organization of the epicuticular wax. The smaller are the molecules of the solvent hydrocarbons dispersed in the wax layer, the faster the diffusion of dieldrin would be expected to take place.

However, I still proportionally with f is certainly the case your second point, w the Risella oils won slowly than the hexane was absorbed faster: the results of Fig. 1.

Moreover, I think for the three solvents taken up on the oills. Thus the B³⁻ is dissolved.

D. A. Crossley: Caused in 1957 on the which ingested ¹²³¹ really a site of activity with ¹²³¹.

C. T. Lewis: I experiments on absorption T. Phillips: the effects could be obinium layer of oil is (this true for the win in any way by a res in experiments with experiments that are the body).

C. T. Lewis: I several groups of oil have no information of oil. It is possible
which could be explained by assuming that the tracer disappears out of the Risella oil by absorption in the cuticle. That would convey the impression that the Risella oil spreads more slowly, while in fact the oil, now less active or inactive, might go on spreading at much the same rate as hexadecane.

C. T. LEWIS: The tracer must be giving a faithful measure of the absorption of the hexadecane solvent as it is chemically identical, and we are led to the conclusion that the facilitated absorption of dieldrin depends on a modification of the cuticular barriers and on this result alone; for much more dieldrin is absorbed than could be transported directly in the volume of solvent absorbed. The smaller absolute quantities of dieldrin-\textsuperscript{14}C absorbed from the Risella oils show that the cuticular barrier is less affected by the longer oil molecules, independently of the effect on the volumes of oil absorbed. Thus my principal conclusions still hold.

If, as you suggest, the tracer is absorbed differentially from the Risella oils, the longer but otherwise similar Risella molecules must be penetrating more slowly than the tracer. In that event, the differential absorption of dieldrin relative to Risella oils would be greater than Table II suggests.

However, I still favor the view that the hexadecane-\textsuperscript{3}H tracer moves proportionally with the oils in which it is dissolved, or very nearly so. That is certainly the case for movements of \textsuperscript{127}I on the cuticle surface, to take your second point, which was that a differential absorption of \textsuperscript{3}H tracer from the Risella oils would give an impression that the oils were spreading more slowly than the hexadecane solvent. That could be true only if the \textsuperscript{3}H tracer was absorbed faster from the Risella oils than from the hexadecane; but the results of Fig. 1 show that this is not the case.

Moreover, if the totals of \textsuperscript{3}H tracer, absorbed plus adherent, are added for the three solvents, it will be seen that the total for the hexadecane solvent taken up on the insect is much greater than the total for the Risella oils. Thus the \textsuperscript{3}H tracer is not behaving independently of the oils in which it is dissolved.

D. A. CROSSLEY: I recall reading a paper published by Cumpel and Casida in 1957 on the metabolism of \textsuperscript{127}I by the cockroach Periplaneta, in which ingested \textsuperscript{127}I was found to be concentrated in the cuticle. If that is really a site of iodine concentration, it might explain some of your difficulties with \textsuperscript{131}I.

C. T. LEWIS: Yes, I think that the use of the iodized hydrocarbon for experiments on absorption is not acceptable.

F. T. PHILLIPS: I would like to ask the author whether any humidity effects could be observed. The evidence suggests that movement of the monolayer of oil is caused by small elastic deformations of the cuticle. Is this true for the wings, or could the spread of oil be facilitated or affected in any way by a presorbed layer of moisture? This could perhaps be shown in experiments with different humidities. Also, was it possible in your experiments that some of the oil was picked up on the wings by contact with the body?

C. T. LEWIS: Elastic stresses do occur in the wings, which possess several groups of campaniform sensilla which respond to such stresses. I have no information about the influence of absorbed moisture on the spread of oil. It is possible that some of the oil passing to the folded wings may

...
have been transferred by incipient contact of the membranes proximally with the dorsal thorax, but the autoradiographs indicate the oil movement is principally by way of the articulations. If we assume oil was transferred by contact with the body, that still leaves unexplained the creep of oil over the whole of the wing. For that, one must still accept some suggestion of active cuticular movements which are on a sub-microscopic scale. Working with Dysdercus, which has much more rigid forewings than hindwings, I found that the spread occurs much more readily on the forewings than on the flimsy hindwings and this might very well be correlated with the elasticity of two types of wings.

K. van ASPEREN: Could the difference in absorption of dieldrin possibly depend on a difference of solubility in the oils used?

C. T. LEWIS: No; dieldrin has practically the same solubility in all three solvents used. Moreover Hadaway and Barlow (1958) found that the relative toxicities of solutions of DDT, and, I believe, of dieldrin also, did not vary with solubility in different solvents when topical applications were applied to Musca and Aedes.

W. KLOFT: The ideal subject for this type of studies might be coccids (scales) of the type of Diaspididae, and this for two reasons. First, the females are more or less immobile, since they have no legs to allow screening movements and spreading of the insecticide over the cuticle in that way. Secondly, Diaspididae are well protected and covered by their scutum (scale) and must be controlled with oily solutions (or preparations in the vapour phase) since they suck in the plant’s parenchyma and do not therefore pick up systemic insecticides. Your interesting method could be used to study how the oily film spreads from the scutum over the scale-body.

C. T. LEWIS: Your suggestion is very appropriate. A study of the uptake of insecticide from oily solutions and from the vapour phase by a scale insect is now in progress in my department. You mentioned that the oil could not be spread around by the legs. I would like to clear up any misunderstanding here. In the experiments on the creep of oil over the blow-fly, the insects were exposed only to oil and iodized tracer. There was nothing irritant in the solution and therefore no cleaning movements were performed. If a fly did perform random cleaning movements during the course of the experiment then it was noted and the results were not used for determining the rate of spread of oil, so that the spread that I refer to is not interfered with nor in any way assisted by the cleaning movements of an insect. Of course, with an insecticide solution in the field cleaning movements would be important.

J. R. OGLE: Like an earlier questioner, I would be inclined to doubt the validity of using hexadecane-\(^3\)H to trace the rate of passage of a much heavier oil through the insect cuticle. Could you check this point by repeating the experiment with another tracer of rather higher or lower molecular weight? If the ratio of uptake of activity were then identical for the three oils, but different from those obtained with hexadecane, it would in fact suggest that you were measuring the rate of uptake of the tracer rather than the oil as a whole, and conversely, if the results were all in agreement, that would be confirmatory evidence that the nature of the solvent had little effect on its rate of uptake.
CONTAMINATION OF INSECTS BY INSECTICIDE SOLUTIONS

C.T. LEWIS: I would have liked to carry out such experiments. But a hydrocarbon of lower molecular weight than hexadecane would be too volatile for accurate work. No tritium-labelled paraffinic hydrocarbon of higher molecular weight is at present available, and the cost of synthesizing one would be considerable.
THE APPLICATION OF CHEMICAL STUDIES. The be conveniently studied if it for a simple measuring scale. Methods of application suitable for the distribution SS-em circular area. Some measurements of surfaces are included.

APPLICATION ET ME PHYSICO-CHIMIQUES. Po sur les végétaux et d'autres à la radiance suffisamment élevée. L'étude des méthodes mieux au point d'un vaporisateur radioactif sur une surface cir.

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THE APPLICATION AND MEASUREMENT OF LABELED RESIDUAL INSECTICIDES IN SOME PHYSICO-CHEMICAL STUDIES

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Abstract — Résumé — Anotación — Resumen

THE APPLICATION AND MEASUREMENT OF LABELED RESIDUAL INSECTICIDES IN SOME PHYSICO-CHEMICAL STUDIES. The disappearance of residual films of insecticides from plant and other surfaces may be conveniently studied if the insecticide is labelled with a radioactive isotope of sufficient radioactive energy to allow for a simple measuring technique.

Methods of application of insecticide solutions on different surfaces led to the design of a spray chamber suitable for the distribution of very small amounts (a few drops) of radioactive liquid formulations over a 30 cm² circular area.

Some measurements of the rates of volatilization of C₁⁴-labelled dichlordrin and aldrin crystals from glass surfaces are included.

APPLICATION ET MESURE DES INSECTICIDES RÉMANTS MARQUÉS DANS CERTAINES ÉTUDES PHYSICO-CHEMIIQUES. Pour étudier la disparition des pellicules des insecticides, éparpillés sur les végétaux et autres surfaces, on a avantage à les marquer au moyen de radionucléides ayant une énergie radiante suffisamment élevée pour pouvoir utiliser une méthode de mesure simple.

L'étude des méthodes d'application des insecticides en solutions sur diverses surfaces a conduit à la mise au point d'un sprayeur permettant de répartir de très faibles quantités (quelques gouttes) de liquide radioactif sur une surface circulaire de 30 cm².

Le méthodes donnent les résultats de quelques mesures des vitesses de volatilisation de cristaux de dichlordrin et d'aldrine marqués au C₁⁴ déposés sur des surfaces de verre.

ЦИКЛЫЗИС И ОПРЕДЕЛЕНИЕ ОСТАТКОВ ИНСЕКТИЦИДОВ ПРИ НЕКОТОРЫХ ФИЗИКО-ХИМИЧЕСКИХ ИССЛЕДОВАНИЯХ. Исследование остатков пленок инсектицидов на растениях и других поверхностях может быть легко осуществлено, если инсектициды метить радиоактивным изотопом и достаточным электрическим насыщением, позволяющим применять простые методы измерений.

В результате исследований методом измерения растворов инсектицидов на различных поверхностях были изучены некоторые инсектициды, позволяющие распределять очень малые количества (несколько грамм) радиоактивных веществ на площадь поверхности около 30 cm².

Приведены некоторые результаты измерения скоростей улетучивания инсектицидов с различных поверхностей, метенных C₁⁴-хлоралдирин микрокристаллами и олеандером.

APLICACIÓN DE INSECTICIDAS MARCADOS Y MEDICIÓN DE SUS RESIDUOS EN ALGUNOS ESTUDIOS FISICO-CHEMÍFICOS. La desaparición de las películas residuales de insecticidas que quedan en la superficie de las plantas y en otras superficies puede estudiar fácilmente si los insecticidas se marcan con radionúclidos de una energía radiante que permita utilizar una técnica de medición sencilla.

Los métodos de aplicación de soluciones insecticidas sobre superficies planas han llevado al desarrollo de una cámara de pulverización capaz de distribuir cantidades numerosas pequeñas de solución (unas gotas) sobre una superficie circular de 30 cm².

La medicion reproduce los valores hallados para los índices de volatilización de cristales de Diclorodrín y Aldrin marcados con C₁⁴, aplicados sobre superficies de vidrio.

* Work financed by a grant from the Tropical Pesticides Research Committee, Department of Technical Co-operation of the United Kingdom Government.
INTRODUCTION

In the study of the persistence of residual films of insecticides it is an obvious advantage to be able to follow the variation by a method which does not destroy or disturb the sample which is being measured. This may be done if a radiotracer is incorporated in the insecticide molecule, provided that this tracer truly follows the course of the molecule and does not exchange with similar atoms in different types of molecules and that further conditions are observed, namely, that the radioisotope has a half-life of sufficient duration to enable practical measurements to be made of the amount of radiation present, and that the type and strength of the radiant energy allows for a simple measuring technique. Thus, the chlorinated hydrocarbon residual insecticides may be conveniently studied if they are labelled with Cl$^{35}$, which has one of the longest half-lives known (8 x $10^5$ yr) and whose $\beta$-radiation (0.714 MeV) can be measured simply and with high efficiency by means of a G-M end-window counter.

The laboratory application of thin and even films of insecticides on to a variety of surfaces may be accomplished in several ways with varying degrees of difficulty, but when the insecticides are radioactive (and in addition may have a high chemical toxicity to humans) either the difficulty of the method or the contamination hazards involved may preclude its use. For example, the sublimation of an insecticide from a warm to a cool surface [1], the controlled deposition of which would be especially difficult when using plant surfaces, or the spraying of the insecticide in an apparatus such as the Potter Tower [2], where special precautions would have to be taken, cannot be wholly satisfactory even under the best of conditions. The laboratory methods described in this paper involve a simple spreading method which has often been used but is suitable only for smooth surfaces, and a new spraying method which has been evolved for the distribution of a radioactive insecticide over a leaf surface or other plant surface.

THE VOLATILIZATION RATES OF PURE CRYSTALS OF DIELDRIN AND ALDRIN FROM GLASS SURFACE

Both the dieldrin and aldrin samples, containing Cl$^{35}$ as radiotracer, were of specific activity 100 $\mu$Ci/g. The insecticide was dissolved in a suitable solvent (as described later) at a concentration of 4 mg/ml, and usually a 10 $\mu$l portion of this solution (containing 40 $\mu$g of insecticide) was spotted with a micro-pipette on to a 20-cm$^2$ circular area at the centre of a glass plate and spread evenly over this area with the edge of a thin glass cover-slip. With experience, reproducible results giving an even coverage of 2 $\mu$g/cm$^2$ of insecticide crystals after evaporation of the solvent could be obtained by this method. Attempts to obtain an even coverage by wetting the glass surface with the pure solvent and then adding the radioactive solution dropwise, allowing it to spread by diffusion, usually resulted in an uneven coverage.

The crystalline insecticide film was measured radiometrically by placing the glass plate in a specially constructed Perspex castle containing an end-window G-M counter with a window area of 4.5 cm$^2$. The whole was enclosed within lead 10 counts/min. The end-window, so that the specimen was close to the counter, was achieved. A fine layer of graphite was placed over this layer to minimize contamination of radioactive insecticide.

Some results obtained from glass slides of different size and weight are shown below. In general, the results were obtained using a glycol monomethyl ether crystals.

(i) Dieldrin crystal at an initial deposit of 0.05 - 0.08 $\mu$g/cm$^2$
(ii) Dieldrin crystals at a long (average, appr. $5\mu$g/cm$^2$, gav)
(iii) Aldrin, former length approx. 0.03

Thus, a reduced a hundredfold gave volatilization rates in more, the volatilities. This shows that the surface agreement with a sp. of the initial weight ps levels. The results 15 times more volatilization: movement and time; a 2 $\mu$g/cm$^2$ deposit took 3 - 4 weeks to fall was accomplishe of 2.0 - 2.5 mile/h.

It was noticed that were linear, at very increased progress; approach the time a segment with the observer.
Insecticides it is an important method which does not work. This may be the case for the molecule, provided that it is not toxic and does not exert an action, and that further contaminates occur. It is a half-life of 20 years, which can be made of the growth of the radiating elements. The chlorinated hydrocarbons are studied if they are known (3 x 10^5 yr) to be used in high amounts.

Insecticides on to the radiochemicals with varying radiations, and are inactive (and in some cases, and in either the difficulty of the target, which precludes its use. They are difficult to a cool surface, which is generally difficult when they are used in an apparatus such as in the experiments. The laboratory spreading method is to be used on the surfaces, and a radiotracer in the solution of a radiochemical.

EVALUATION OF DIELDROIN AND ALDRIN

Dielethrine, as radiotracer, dissolved in a suitably dilute solution (mg/ml, and usually 0.5% alcohol), was spotted in the central area of a glass dish. The excess glass cover was removed while uniform coverage of the surface was obtained by carefully spreading the radioactive solution. This resulted in an even distribution of the insecticide on the dish. The whole was enclosed within lead shielding to reduce background counts to approximately 10 counts/min. The upper surface of the glass plate was 3 mm from the end-window, so that the central portion of the 20-cm² treated area was presented close to the end-window and a counting efficiency of over 20% was achieved. End-windows made of thin mica are often covered with a fine layer of graphite to eliminate photosensitivity. It is important to remove this layer (by wiping with cotton-wool moistened with alcohol), otherwise contamination of the counter-window by sorption of vapour from the radioactive insecticide will ensue.

Some results obtained for the rates of volatilization of these insecticides from glass surfaces are summarized below. The two ranges of crystal size were obtained by choosing a suitable solvent and allowing crystallization to proceed at room temperature (circa 20°C). "Cellosolve" (ethylene glycol monomethyl ether) gave large crystals and dioxan solvent gave small crystals.

(i) Dielethrine crystals, forming needles 1 - 10 mm long (average 5 mm long) at an initial deposit density of 2 μg/cm², gave a rate of volatilization of 0.06 - 0.08 μg/cm²d at 20°C.
(ii) Dielethrine crystals, forming a milk-white deposit of needles <0.01-0.05 mm long (average, approx. 0.02 mm long) at initial deposit densities of 2 μg/cm² or 5μg/cm², gave rates of volatilization of 0.12 - 0.14 μg/cm²d at 20°C.
(iii) Aldrin, forming a milk-white deposit of rod-shaped crystals of average length approx. 0.01 mm at an initial deposit density of 2 μg/cm² lost most of this deposit at 20°C. after 1 d.

Thus, a reduction in the crystal size of dielethrine of the order of a hundredfold gave a twofold increase in the volatilization rate, although the volatilization rate was independent of the initial deposit density. Furthermore, the volatilization rates were linear, except for very low deposit densities. This shows that the rate of volatilization appears to be partly dependent on the surface area of the crystals forming the deposit, and also, in general, the crystalline deposits of aldrin to be at least 15 times more volatile from glass surfaces than a similar deposit of dielethrine.

Volatilization rates of these crystalline deposits were sensitive to air movement and temperature change. To give extreme examples, whereas a 2 μg/cm² deposit of dielethrine crystals (1 - 10 mm long) at 20°C in still air took 3 - 4 weeks to fall to a deposit density of 0.2 μg/cm², the same tenfold fall was accomplished in 4 - 5 h at a temperature of 40°C and a wind speed of 2.0 - 2.5 mile/h.

It was noticed that although the volatilization rates of dielethrine and aldrin were linear, at very low deposit levels (circa 0.2 μg/cm²) these rates decreased progressively, the curves of deposit density/time tending to approach the time axis asymptotically. This again is apparently in agreement with the observations of Hoskins.
THE APPLICATION OF SMALL AMOUNTS OF RESIDUAL RADIOACTIVE INSECTICIDES TO SURFACES BY A LABORATORY SPRAYING TECHNIQUE

The apparatus, shown in Fig. 1, consists of a glass tube of length 23 cm, internal diameter 6.6 cm and wall thickness 3 mm, with flat ground edges. The tube is gripped by nylon screws which allow for adjustment of its position, and the whole is supported by a stand. A brass plate, which bears the spray nozzle and 6 brass absorption tubes, is positioned on the top of the tube by a locating groove which is packed with a suitable grease (e.g. silicone high-vacuum grease) to form a seal. A brass platform at the bottom is fitted to a ball and socket joint which moves the platform into the spraying position. A ball and socket joint which can be clamped in any position and joins the platform to the rack allows for adjustment of any tilt in the platform.

![Diagram](image)

**Fig. 1**

Apparatus for laboratory spraying technique

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The atomizing nozzle consists of a brass body with an inlet for air under pressure and a 1.5-mm-diameter aperture into which a monel-metal liquid jet of external diameter 1.25 mm and internal diameter 0.75 mm fits concentrically. The tip of the liquid jet, which has a flat edge, is flush with the surrounding air orifice. A stainless-steel liquid tube of internal diameter 0.7 mm leads from the bottom of a small glass reservoir to the centre axis of a stainless-steel tube of internal diameter 1.75 mm. This latter tube allows an air bleed controlled by a needle valve to pass across the mouth of the liquid tube, and thus some atomization of the liquid occurs before it reaches the main spray. To prevent the formation of droplets, the nozzle is clamped to the plate by two screws and an air-tight seal between tubes, each of 3 cm spacing, is achieved by using silicon grease. The glass plate contains cotton-wool.

To obtain the desired results, a solution of insecticide is fed into the glass plate and a filter-paper is placed on top of the plate. A ground edge of the glass plate and the solution escapes past the needle valve, which is adjusted to 52 cm above the line and set to the required pressure.

The needle valve is 400-500 cm² air/min and resistance of the plugs is obtained by using a manometer. Records are taken or a sucticameter placed in a well-ventilated room. The spraying is quickly carried out by racking the apparatus up and down repeatedly, with liquid to the liquid tubes being placed in a well-ventilated room.

As is common with volatile solutions, caused by air turbulence of spray to the top of the resistant solution, cotton-wool plugging the orifices is entrained in the apparatus, as fine droplets, and the area at the bottom of the platform and the solution are found to be the central part of the insecticide. The solution of the dieletrin depositing long, somewhat sput, sprayed in the Potts.
reaches the main atomizing nozzle. This was found to be essential in order to prevent the formation of coarse liquid droplets in the spray chamber. The atomizing nozzle is inserted through a conical hole in the top brass plate and is clamped to the plate by screws which are also used for slight tilt adjustment in the nozzle. Silicone high-vacuum grease is again used to form an air-tight seal between the nozzle and brass plate. The six brass sorption tubes, each of 6-cm length and 8-mm internal diameter, slide into holes spaced equidistantly around the central atomizing nozzle and are soldered to the brass plate. They serve as exit tubes for the air during spraying and contain cotton-wool plugs which are used to trap any spray solution.

To obtain the optimum spraying conditions, the following procedure is adopted: a glass plate is placed on the platform so as to cover it completely and a filter-paper of somewhat larger diameter than the glass tube is placed on top of the plate. The platform is racked firmly against the lower flat-ground edge of the tube so that the filter-paper forms a seal between the glass plate and the edge of the glass-tube. Tests have shown that no spray solution escapes past this seal. The air pressure to the atomizing nozzle is adjusted to 52 cm Hg (10 lb/in²) by means of a mercury manometer in the air-line and cotton-wool plugs are inserted in the sorption tubes. The needle valve is adjusted so as to give an air-bleed flow-rate of 400 - 500 cm³ air/min, adjustment being made at the same time in the resistance of the plugs to the air issuing from the spray chamber. This is achieved by teasing out or compressing the cotton-wool so that a water manometer records a positive pressure of 7 cm water inside the spray chamber or a suction pressure of 12 cm water in the liquid tube.

These spraying conditions are established quite easily, and spraying is quickly carried out by first racking down the platform, placing the leaf or other plant surface on the filter-paper (fixing with adhesive if necessary) and racking up again, and then presenting the reservoir containing the spray liquid to the liquid tube. As a precaution the apparatus, when in use, is placed in a well-ventilated fume chamber.

As is common with all spraying procedures, there were spray losses caused by air turbulence on the walls of the chamber and also entrainment of spray to the top of the chamber. Using both aqueous dye solutions and radioactive solutions of dieldrin in "Cellosolve," it was shown that the cotton-wool plugs in the sorption tubes were completely successful in holding back entrained spray solution. When water or "Cellosolve" was sprayed in the apparatus, approximately 15% of the spray solution showed a very fine droplet size and good coverage was obtained over the 35-cm² circular area at the bottom of the chamber. When small glass discs were placed on the platform and sprayed with radioactive solutions of dieldrin in "Cellosolve" and the crystalline deposits were assayed radiometrically, it was found that the central circular area of 5 cm² received an even coverage of the insecticide. The mean deposit density of the surrounding area was found to be slightly more than half that of the central area. The crystal sizes of the dieldrin deposits were in the range 0.1 - 1.5 mm (average 0.3 mm long), somewhat smaller than those obtained when similar solutions were sprayed in the Potter Tower (range 0.1 - 1.5 mm, average 1 mm long).
This apparatus has been developed for spraying small amounts of liquid; reproducible results have been obtained with as little as 0.05 ml liquid in the reservoir.

ACKNOWLEDGEMENTS

I wish to thank Dr. C. Potter, Head of the Department of Insecticides & Fungicides, for helpful advice and Mr. A. J. Arnold of the same Department for valuable technical help and the manufacture of the nozzle.

The samples of radioactive dieldrin and aldrin were a gift from Shell Research Ltd.

REFERENCES


DISCUSSION

G. F. Burnett: Your deposit on the spray chamber is described as about 15% of that expended. How reproducible was this?

F. T. Phillips: For five consecutive sprayings the amount of spray solution deposited ranged from 13 1/2 to 16 1/2% approximately (the approximate mean being 15%).

G. F. Burnett: Did the deposit increase with repeated sprayings in the chamber?

F. T. Phillips: Except for the initial spraying, which usually appeared to be less, there was no visible increase in the amount of spray deposited when 0.05 ml aqueous dye solutions were sprayed consecutively on to consecutive filter-papers until eventually run-off of spray solution occurred down the inside of the chamber.

D. F. Heath: Did you analyse the evaporation rates from glass slides in terms of the formula: rate = evaporation area x wind-speed, the effect of the temperature being exponential as usual? Your results seem on the whole to indicate that a formula of this sort might apply.

F. T. Phillips: No, I have not used this or any similar formula showing correlations with wind-speed, because at the moment insufficient data have been obtained using different wind-speeds. Experiments using a range of wind-speeds are, however, to be conducted in the near future.

D. F. Heath: What I had in mind was that you might have analysed your curve by determining, for example, the surface areas of crystals under the microscope.

F. T. Phillips: No, I have not really done a proper analysis, though I can say that under the microscope at the very beginning, at a high deposit density, the crystals tend to be all joined together like fern-trees in arrangement. They touch at certain points. As time goes on, in a few weeks perhaps, these slowly disappear, until you get a very dispersed crystal system. The crystals themselves diminish in size. I did not measure the

diameters of the cry...
Labelled Residual Insecticides

I measured the amount of liquid; to 100 ml. 0.05 ml liquid in

F. T. Phillips: No. Experiments using leaf or other plant surfaces
were held up because of lack of a suitable method of applying insecticide
solutions to these surfaces. With the design of the spray chamber

G. F. Burnett: It is relevant, I think, to Mr. Miles' remarks that
regular work is being done by Hadaway and Barlow on the disappearance
of spread on mud surfaces*. They have obtained much bigger discrepancies
in the rate of disappearance of crystals of different sizes than you have at
Rodhamsted. They graded the crystals into 1 μm, 10 μm and, I think, 100 μm.
There the vapour phase disappears into the mud, which suggests that the
removal of the vapour is extremely important in determining the rate of
volatilization. You may find that if you increase your wind-speed above
two miles an hour and completely remove your extracted air from contact
with your surfaces, you considerably increase your rates of volatilization.

F. T. Phillips: That is certainly true. At the moment I have only
used two rates of air movement but, as you say, the evaporation rate
increases quite remarkably with the rate of change of air movement.

Again, when we place the radioactive plates in still air, we have to be
careful that although we shield the plates from draughts, we do not cover
them: otherwise the concentration of vapour builds up in a covered
container. Although there is some egress into the atmosphere, there is a
considerable concentration above the plates and this diminishes the rate of
volatilization. We have to find a happy medium between the two.

The suggestion that increased wind-speed might increase not only the
evaporation rate but also the difference in evaporation rates due to different
crystal sizes is a most interesting one. It seems also to agree with the
formula cited by Dr. Heath and I shall certainly put it to the test when I come
to do the work on variations in wind-speed.

K. von Asperen: In our own and other laboratories evidence has been
obtained that chemical changes may occur under normal atmospheric
conditions. Did you consider or exclude this possibility?

F. T. Phillips: These possibilities were considered. Any degrada-
tion by ultraviolet light could be excluded because of the conditions of the
experiments: the glass plates with the insecticide deposits were held in a
dark constant-temperature room at 20°C. Any oxidation of aldrin to
dieldrin would probably be shown by a change in the rate of volatilization
as the experiment proceeded. This did not occur, except for the progressive decrease in rates at very low deposit levels which was noted for both aldrin and dieldrin. Also, most of the aldrin in this experiment volatilized during a short time (one day). Thus it was considered that no measurable amounts of aldrin oxidized to dieldrin.

K. von ASPHERN: I believe that other conversions could occur, but even then the occurrence of straight lines must be an indication that there is no chemical change in your experiment; is that right?

F.T. PHILLIPS: Not necessarily, but I do not think that there could be any other chemical changes except, as I said, a very slight change of aldrin into dieldrin. These are very stable compounds compared with most insecticides — that is why they are residual insecticides. The only strong instability is the fact that aldrin oxidizes to dieldrin, but that occurs generally when we spray into soil or any organic material; on glass plates there is very little opportunity for oxidation to take place.

RADIOAUTOGRAPH

CONTROL. In return for a radioautography offer great sensit of singly or doubly radiolabeled seedling of residues in far are other phases which can especially, are being studied in the presence of the quantity of plant tissues or even parts per cent in contrast to one sample. Standard radiography of very weak emitters such as radiocarbon apparatus has been used.

The technique places uronium and C14 will register nearly every insecticide with radioautographs and have no difficulty. Long exposure specific-activity insecticides are tagged at such a pace it is desired to study. Once it standard histological technics can then be deparaffinized a radioactive compounds in the loss or stabilization of the tissue. Staining can be done of the stain desired. In genetically processed. With sufficient graphed, in which case histo- compared with the film in.

The most convenient is into sliced bulk emulsion in a dark chamber for a day, at least. At the expiration and cover slips are mounted, magnification. A slight up can be thus easily studied in the available laboratory equipment.

L' Auto-Radio-Graphie

Utilisée dans la lutte contre l' infection et la propagation de plantes des recherches.
RADIOAUTOGRAPHY IN THE STUDY OF RADIOISOTOPICALLY-TAGGED SUBSTANCES IN INSECT CONTROL

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Abstract — Résumé — Anmerkung — Resumen

RADIOAUTOGRAPHY IN THE STUDY OF RADIOISOTOPICALLY-TAGGED SUBSTANCES IN INSECT CONTROL. In return for a relatively small expenditure of effort, the newer, simplified techniques of radioautography offer great sensitivity and precision at the microscopic level in many phases of the investigation of singly or doubly radioisotopically-tagged insecticides: e.g., uptake, translocation, metabolism and determination of residues in insects, plants and animals. The physiology and biochemistry of irradiated insects are other phases which can be studied by radioautography. Radiation effects on the generative organs, especially, are being studied with tritiated thymidine. Whereas counting techniques yield gross information on the presence and quantity of radioisotopes, radioautography can provide information on actual sites of deposition in tissues or even parts of cells. This resolution is available on ordinary permanent histological preparations, in contrast to counting techniques in which accuracy of counting depends on the destruction of the sample. Since radioautography is especially useful for conveniently following fractional minute amounts of very weak emitters such as radioisotopes (C14) and tritium (H3), as opposed to the very expensive, highly sophisticated apparatus which is required for counting techniques when very weak emitters are employed.

The technique places no restriction on the isotope used for labelling, since even the radiation from tritium and C14 will register on the nuclear emulsion. In fact, tritium and C14 (which are desirable since nearly every insecticide will contain carbon and hydrogen) have the advantage of yielding high-resolution radioautograms and have long half-lives, so that if very long exposure times are necessary, there would be no difficulty. Long exposure times may be required since only tiny amounts of radioactivity, even of high-specific-activity insecticides, can be expected to be taken up. It is necessary to be certain that the insecticides are tagged at such a point in the molecule that the radioactive tag remains bound to the moiety which it is desired to study. Once the insecticide has been taken up or administered, the specimen preparation follows standard histological techniques of fixation, embedding, cutting and attaching to glass slides. The tissues can then be deparaffinized and hydrated and are then ready for application of the nuclear emulsion. If the radioactive compounds in the tissues are in a soluble state, freeze-drying techniques can be used to prevent loss or mobilization of the radiotope. Dry-emulsion can be used for these preparations.

Staining can be done either before or after application of the nuclear emulsion, depending on the nature of the stain desired. In general, it is preferable to stain after the emulsion has been applied and photographically processed. With sufficiently energetic isotopes, whole mounts of plants or plant parts can be radioautographed, in which case histological preparation can be eliminated; the part to be radioautographed is simply compressed with the film in a plant press or other suitable device.

The most convenient method of applying nuclear emulsion on histological preparations is to dip them into melted bulk emulsion in the dark and then clean the bottom of the slides. The slides are then placed in a dark chamber. In a dry, inert atmosphere and allowed to expose for a time which must be adequately determined. At the expiration of this period, the slides are photographically processed and thoroughly washed and coverslips are mounted. The slides are then ready for study just as regular histological specimens at all magnifications. A slight upward adjustment of the focus brings into view the radioautographic pattern which can be easily studied in relation to the tissues. The recommended techniques and the simple, generally available laboratory equipment required are described.

L'AUTOGRAPHIE RADIOMÉTRIQUE DANS L'ÉTUDE DE SUBSTANCES MARQUÉES AU MOYEN DE RADIOISOTOPES UTILISÉES DANS LA LUTTE CONTRE LES INSECTES. Les nouvelles techniques simplifiées d'autographie radioisotopique sont très sensibles et précises au niveau microscopique dans de nombreuses phases des recherches sur les insecticides marqués au moyen de un ou deux radioisotopes: recherches
sur l'absorption, la translucence, la métamorphose et les réactions chez les insectes, les végétaux et les animaux. L'autoradiographie peut aussi servir à étudier la physiologie et la biochimie d'insectes irradiés. On étudie notamment les effets des rayonnements sur les organes reproducteurs au moyen de thyamine radiée. Alors que les méthodes du comptage fourni des renseignements approximatifs sur la présence et la quantité de radiotraceurs, l'autoradiographie permet d'obtenir des données sur l'emplacement exact du dépôt dans les tissus, voire dans des parties cellulaires. Cette détermination peut se faire par des préparations histologiques normales permanentes, alors que les méthodes du comptage impliquent la destruction de l'échantillon pour obtenir des résultats exacts. L'autoradiographie cortante est particulièrement utile pour repérer des parties de l'œuf d'une manière plus ou moins aisée que le radiocarbone (14C) et le tritium (3H); le comptage des symétries émanées du grâce aux appareils très coûteux et hautement perfectionnés.

L'autoradiographie s'applique d'aujourd'hui à une large gamme de problèmes de la chimie et de la physique. En fait, 14C et 3H (qui sont particulièrement intéressants du fait que tous les insecticides contiennent du carbone et de l'hydrogène) ont l'avantage de donner des autoradiographies à haute résolution et d'avoir de longues périodes; ils s'entendent à peu près de difficultés lorsqu'un très long temps d'exposition est nécessaire. C'est souvent le cas, parce que même pour des insecticides ayant une forte activité spécifique, les quantités de radiotraceurs que l'on peut employer absorber sont généralement très faibles. Il faut veiller à ce que l'insecticide soit marqué au point de la molécule tel que le radiotracer soit enlevé à la position que l'on désire étudier. Une fois que l'insecticide a été marqué ou admis, on prépare les échantillons en appliquant les méthodes histologiques courantes: fixation, encrage, découpage et pose sur latine de verre. Les tissus sont ensuite déparaffinés et hydratés; ils sont alors prêts pour l'application de l'émaillage nucléaire. Si les composés radioactifs se trouvent à l'état soluble dans les tissus, on peut faire appel aux méthodes de désamination par congélation pour éviter toute perte ou tout mouvement de radiotraceurs. Des films à émission d'énergie peuvent être utilisés pour ces préparations.

La coloration peut être faite avant ou après application de l'émaillage nucléaire, selon la nature de la couleur désirée. Il est préférable, en général, que la coloration intervienne après l'application et le traitement photographique de l'émaillage. Si l'on utilise des radiotraceurs ayant une énergie suffisante, on peut procéder à l'autoradiographie de plans entiers ou de parties de plans, et supprimer ainsi la préparation histologique: la partie à autoradiographie est simplement complétée avec le film une à deux semaines après l'application appropriée.

La méthode la plus pratique pour appliquer les émissions nucléaires aux préparations histologiques consiste à placer l'échantillon dans une émulsion fine et à nettoyer ainsi le pourtour inférieur de la lamelle. Les émulsions sont ensuite placées en chaleur noire, dans une atmosphère riche et inerte, et exposées pendant une durée qui doit être déterminée empiriquement. Après avoir, elles ont l'objet d'un traitement photographique, sont soigneusement lavées et revêtues d'une protection. On peut alors les étudier comme des échantillons histologiques normaux, avec le grossissement voulu. Un léger déplacement du foyer vers le haut permet de voir l'autoradiographie, que l'on peut ainsi facilement étudier en regard du tissu. Le motif est décrit en détail les méthodes recommandées ainsi que le matériel nécessaire et des laboratoires.

**APPLICATIONS RADIOGRAPHIQUES EN PHYSIQUE ET MÉDECINE**

Vraiment, pour les cardiologues et les physiciens, l'utilisation de l'autoradiographie est une méthode très précieuse. Elle permet d'étudier les tissus et les organes dans des conditions naturelles, sans intervention chirurgicale. Les échantillons sont disposés sur des support spéciaux, et exposés à une longue période. Les images sont ensuite développées et examinées sous une loupe ou un microscope. Les résultats obtenus peuvent être utilisés pour étudier les processus biochimiques, les mouvements cellulaires, les interactions moléculaires, etc.

**APPLICATIONS EN BIologie**

Dans le domaine de la biologie, l'autoradiographie est utilisée pour étudier les interactions moléculaires à l'échelle cellulaire. Elle permet de visualiser les processus de transduction de l'information, tels que la réplication du génome, la translation de la protéine, la transcription de l'ARN, etc. Les échantillons sont généralement placés dans des conditions contrôlées, et exposés à des rayonnements de haute énergie. Les images obtenues sont ensuite analysées pour déterminer les processus biochimiques en cours.

**APPLICATIONS EN PHYSIQUE**

En physique, l'autoradiographie est utilisée pour étudier les interactions atomiques et moléculaires à l'échelle atomique. Les échantillons sont généralement exposés à des rayonnements de haute énergie, tels que les rayons X ou les rayons gamma. Les images obtenues sont ensuite analysées pour déterminer les interactions atomiques et moléculaires en cours.

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LA AUTO RADI GrAPHIE EN EL ESTUDIO DE LAS SUBSTANCIAS MARC A DAS CON RADIÓSTÓPS UTILIZADAS EN LA Lucha CONTRA LOS INSECTOS. Las nuevas técnicas autoradiográficas simplificadas requieren cierto esfuerzo pero permiten obtener una gran sensibilidad y exactitud al nivel microscópico en el estudio de las insecticidas marcados con uno o dos radiótopos y, en particular, en el de la absorción, traslación, metabolismo y desaminación de residuos en plantas, insectos y otros animales. Con ellas se pueden estudiar también la fisiología y la bioquímica de los insectos tratados. Se están investigando con tintas radiactivas los efectos de las radiaciones sobre los órganos reproductores. Las técnicas de recuen deducen información general sobre la presencia y la cantidad de los radiótopos, mientras que la autoradiografía proporciona datos sobre el lugar exacto de fijación en los órganos e incluso en partes del estómago. El poder de resolución se alcanza con preparados histológicos ordinarios que pueden conservarse, comparativamente a la que suele con las técnicas de recuento que exigen, si se quiere lograr mayor exactitud, la desecación de la muestra. La autoradiografía continúa siendo particularmente útil para analizar por métodos muy sencillos fracciones de microcuras de emisoras muy débiles como el radiocarbono (14C) y el tritio (3H), mientras que las técnicas de recuento requieren en estos casos un instrumental muy complicado y caro.

Esta técnica no impone limitaciones alguna en cuanto a los insectos que pueden emplearse como indicadores, ya sea en condiciones de laboratorio, en las cuales la radiación no es un factor, o las condiciones de campo, en las cuales las radiaciones pueden ser de gran importancia. Las técnicas autoradiográficas han sido usadas con éxito para determinar la absorción y el almacenamiento de insecticidas en plantas, insectos y otros animales. Con ellas se pueden estudiar también la fisiología y la bioquímica de los insectos tratados. Se están investigando con tintas radiactivas los efectos de las radiaciones sobre los órganos reproductores. Las técnicas de recuen deducen información general sobre la presencia y la cantidad de los radiótopos, mientras que la autoradiografía proporciona datos sobre el lugar exacto de fijación en los órganos e incluso en partes del estómago. El poder de resolución se alcanza con preparados histológicos ordinarios que pueden conservarse, comparativamente a la que suele con las técnicas de recuento que exigen, si se quiere lograr mayor exactitud, la desecación de la muestra. La autoradiografía continúa siendo particularmente útil para analizar por métodos muy sencillos fracciones de microcuras de emisoras muy débiles como el radiocarbono (14C) y el tritio (3H), mientras que las técnicas de recuento requieren en estos casos un instrumental muy complicado y caro.
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INTRODUCTION

Participants in previous symposia [1, 2] which were closely related to this one in subject matter have repeatedly mentioned the usefulness of radioautography in entomological research. In spite of this, examination of publications reveals that only a few investigators are taking advantage of this tool. Perhaps this is due to the fact that the past radioautography had been difficult to perform and often yielded equivocal results. Recent development of more sensitive and dependable emulsions, which are commercially available, and simpler techniques and apparatus now place radioautography in the category of a routine tool which should be considered in any research problem where the fate of tracers in an organism is of interest. This is especially true if the research is to be done in the field or in areas remote from the amenities of highly-refined counting equipment and the repair personnel and other support items which such equipment requires. Furthermore, the amounts of radioactivity required are smaller than for other procedures, and so handling the tagged compounds can be more convenient.

Even in laboratories where all the refinements are available, radioautography offers a type of information which cannot be obtained in any other way. While it is now possible to detect extraordinarily small quantities of even tritium and radiocarbon by liquid scintillation counting, only radioautography makes it possible to visualize the sites of deposition within a tissue (or with tritium-tagging, within cells) of a radioisotopically-tagged compound. The purpose of this communication is to provide general background information necessary for using radioautography.

Within the area of the special interests of those attending this Symposium, radioautography is applicable in many ways. It is ideally suited to studies of the uptake, translocation and metabolism of tagged insecticides in insects, animals and plants; in studies of the physiology and biochemistry of insects under normal conditions and following radiation; the response of the reproductive processes in insects in response to radiation; the transport of
radioactive parasites from host to host through the insect vector; location of radioactive parasites in the tissues of the host, including insect parasites such as *Empusa muscae*; sites of deposition in studies of the influence on resistance of radioactive trace elements in plants; movements of ions across membranes; and many others.

**SELECTION OF THE RADIOISOTOPE TO BE USED**

Radioautography itself does not place any restrictions on the radioisotope to be used, since the nuclear emulsions now available respond to even the very low energies emanating from tritium, the weakest of the radioisotopes. The decision as to which isotope will be used then is made on the basis of the physiological, biochemical and metabolic factors under study and the chemical constituents of the compound it is desired to investigate. The effective half-life and the mean path length in tissue and emulsion must also be considered, as well as type of radiation - alpha, beta or gamma – and degree of avidity of the organism for the labelled compound. The radiation dose delivered to the organism should be kept as low as possible. The possibility of an isotope effect where the mass of the radioisotope is very different from that of the non-radioactive sister, as in the case of tritium, which has three times the mass of hydrogen, must also be taken into account [3].

**LABELLING**

Insects have been labelled in many different ways. They have been exposed to radioactive gas, to artificial nutrients contaminated with radioisotopes, dipped into, injected, perfused, sprayed or painted with radioactive solutions, or allowed to feed on their natural food which has been made radioactive. Radioactive insecticides can be produced by substituting a radioactive isotope in the original synthesis [4], or by chemical manipulation of the insecticide, or by exchange, as with tritium [3].

In using a labelled insecticide, there are several factors to consider: half-life, decay products produced, isotopic effects, position in the molecule, specific activity attainable, radiochemical purity, radiochemical stability and the radiation dose to the animal. The half-life is important because when the radioisotope decays to a daughter product the chemical composition changes, and the insecticide may change its properties or the insecticide will be metabolized differently, or may become undetectable if the daughter product is not radioactive. The position on the molecule should be known, since the metabolism of the insecticide may break up the molecule and it may be necessary to know which moiety of the original molecule is being followed. In such a case, the insecticide per se may disappear, but the toxic metabolite may persist for weeks, and be demonstrated by the radioautogram. Reversible or irreversible exchange may also occur, particularly with tritium or radiocarbon. If the insecticide is not radiostable, the analysis of results will be confused because the original material will have changed. Very high specific activities are ordinarily desirable.
unless they significantly reduce radioactivity and/or deliver radiation doses to the insect sufficient to cause undesired radiation effects. Radioactivity can sometimes be preserved by storing the insecticide in a solvent which helps absorb some of the radiant energy.

HISTOLOGICAL PROCESSING

The method of administration of the radioisotope or of the tagged insecticide will be determined by the experimental work which is being done and the kind of information being sought, so little can be said about this. However, the time allowed between administration of the isotope and fixation of tissue for radioautography and the time spent in histological processing must be kept as short as possible relative to the half-life of the radioisotope. Except for reagents which dissolve the radioisotope-bearing compounds out of the tissues, ordinary fixatives and embedding techniques can be used. Alternative techniques can be found if necessary; for instance, if the compounds of interest are soluble in organic solvents but not in water, one can use water-soluble paraffin instead of paraffin or celloidin for embedding [5]. If the compounds are water-soluble, freeze-dry techniques may be employed.

The thickness at which the sections are cut is determined by the cytological resolution required. Of course, thin sections will contain less isotope than thicker ones, but then there are problems with absorption within the specimen, so, in general, one simply cuts at the same thickness as for non-radioautographic work.

Since the stains used may also dissolve the radioisotope-bearing compounds and since some stains are decolorized by the photographic processing which the radioautogram must undergo, it is generally preferable to stain after the photographic processing. However, there is considerable leeway in selecting stains. In our laboratory, we have found [6] that some stains, such as Feulgen-fast green, chromium hematoxylin phloxine and aldehyde fuchsin-PAS, work best when applied before application of the nuclear emulsion. Others, such as celestin blue-Mayer's haemalum, metanil yellow-iron hematoxylin, lithium carmine-picric acid, Weigert acid-iron haematoxylin, alun cochineal, methyl green pyronin, indigo carmine-picric acid, methylene blue-azure A, toluidine blue, Nissl and Cason, are best when applied after the photographic processing. It was also found that some combination stains, such as trichrome-PAS, Luxol-fast blue-PAS, haematoxylin-eosin andaflox blue-carbol fuchsin, worked best when part of the staining was performed before application of the nuclear emulsion and the remainder applied after the photographic processing.

NUCLEAR EMULSIONS

Photographic emulsions intended for use in radioautography contain up to 95% silver halide, mostly bromide, by weight, with an average grain size of 0.2 to 0.3 μm and a maximum of 0.5 μm. X-ray film has much larger grain size, up to 6 μm, which yields high sensitivity but poor resolution.

The response characteristics of the radioisotope mass and move relative to their path but do not course. Therefore, in a fairly straight line at the origin than at a cylinder composed of the medium and the indicated track. The track of a little mass is a more tortuous and the indirect volume, X or gamma, register in nuclear tracks similar to betas.

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The response of the emulsion to radiation depends upon the characteristics of the radiation traversing it. Alpha particles, which have a large mass and move relatively slowly, give up their energy to atoms in or near their path but do not tend to be deflected from an approximately straight course. Therefore, passage of an alpha particle through the emulsion results in a fairly straight line defined by a series of silver granules further apart at the origin than at the end of the path. The track may actually consist of a cylinder composed of granules if the local ionization is sufficient in quantity. The track of a beta particle is much more complicated, since it has little mass and is easily deflected by atoms in its path. The beta track is tortuous and the individual granules composing it are relatively far apart. It is impossible to unravel a group of beta tracks if they pass through a small volume. X or gamma rays, which are very fast and of negligible mass, register in nuclear emulsion by eliciting secondary electrons, which yield tracks similar to beta particles.

Nuclear emulsions are available from Eastman Kodak in the United States of America and Kodak, Ltd., and Ilford, Ltd., of England in a variety of forms. American Kodak provides nuclear emulsions in a variety of sensitivities and thicknesses coated on glass plates the size of microscope slides in several thicknesses, with and without a gelatin protective coating. In order of ascending sensitivity these emulsions are designated: NTA, NTB, NTB2 and NTM3, NTB2 and NTB3 are the most commonly used in biological work. These emulsions may be obtained in bulk, also. Kodak also supplies stripping film in several forms. British Kodak supplies several types of emulsion, of which their AR10 stripping film is quite popular in the United States and is now being distributed by the American branch, but it is not available in bulk. Ilford also provides a variety of emulsions as stripping film or in bulk. When not in use, nuclear emulsion should be stored at 5°C. It will keep up to six months or longer if not exposed to too much radiation. The ways in which the various forms of nuclear emulsion are utilized will be discussed below. HERZ [7] and YAGODA [8] have discussed the fundamental interactions of radiation and nuclear emulsions.

**Radioautographic Procedures**

Dozens of modifications of the few basic methods of radioautography have been evolved, and many of them have been described by BOYD [9]. There are also extensive bibliographies gathered by PASSALAQUA [10] and JOHNSTON [11, 12]. A few of the most generally applicable methods will be described to indicate the possibilities which in practice are limited only by the ingenuity of the investigator.

The two most widely used methods have come to be known as the dipping technique [13, 14] and the stripping technique [15]. Both yield excellent results with characteristic high resolution and dependability. Of the two, the dipping technique, which was developed in this laboratory, is the simpler and more convenient. All techniques have many manipulations in common, and the dipping technique will be described in some detail as a model for the others.
In our laboratory the procedure is as follows. If it is feasible, the mounted tissues should be rehydrated after paraffin or other embedding medium has been removed. This step will aid in obtaining even layers of emulsion which adhere to the tissue and glass much better than if the slides are not rehydrated, and it should be performed unless there is danger of loss of the radioisotope. While the tissue slides are going through the last stages of processing, the light-tight [14] container of bulk nuclear emulsion is taken from the refrigerator and placed in a water bath which is set to bring the emulsion to a temperature of approximately 60°C, in the darkroom. If thinner emulsion layers are desired, the temperature may be raised but not above 50°C (to avoid heat sensitization artifacts). When the emulsion is at temperature and the slides are ready, the slides are taken from the water in which they were rehydrated and arranged in a convenient array where they may be easily grasped at the label end in the dark. If too many slides are removed at once, the last ones to be done will be dried out and may lose some cytological detail. Groups of ten are convenient. The lights are shut off and the series 1 or 2 safelight turned on. Nuclear emulsions are quite insensitive to red light. The cover is then removed from the emulsion container and a clean glass rod is used to stir the emulsion thoroughly but gently without introducing bubbles. Stirring is unnecessary because during standing of the melted emulsion silver halide settles out of the gelatin. Once the stirring is completed, the first slide is picked up and immersed long axis vertical so that the tissue goes about half an inch below the surface. It is immediately withdrawn and allowed to drain for a few seconds to a minute on to a gauze pad held in the other hand. Within limits, the longer the drainage, the thinner the emulsion layer and the better the resolution. With 10-sec drainage, the dry layer thickness will be about 5 μm. Emulsion must not be allowed to drain back into the container. After draining, the gauze pad is used to remove all emulsion from the bottom of the slide. The slide is then placed horizontally in a tray to gel and be exposed. The slides need be kept horizontal no longer than the ten to twenty minutes it takes the emulsion to gel, after which any position of the slide will be all right. When the tray is full, it is placed in an exposure chamber specially made for the purpose* and other trays used if the number of slides require it. The proper compartments of the chamber are then charged with Drierite and solid CO₂, and the cover is sealed on. The chamber seals itself by means of an oil valve after all the CO₂ has sublimed. As the gas evolves, it is led through the chamber in such a manner that the air is completely swept out. If precautions to eliminate oxygen and reduce moisture below 15% relative humidity are not taken, serious losses of latent image may occur during the exposure period.

There has been much discussion about the proper temperature in the exposure chamber [13], but the best temperature to use is between 18 and 24°C. The argument that less background or fogging occurs at low temperatures has been advanced. In reality, the reduction of fog is due to the reduced sensitivity of the emulsion in the cold and the ratio of useful image
to fog probably remains seldom a problem if a microgram per 100 μm² perfectly amenable prefer to do so, or box may be placed in.

Exposure times for a dose of isotope and the incubation, the pattern of the tissue slices a cult to reduce to a few has been found simple value of several days annual cycle. When the initial is performed with a developing solutions rack for immersion developer, Kodak S. grain, finely stable and appear in use also. The probability of dimen hardens the emulsion size, of the emulsion.

The development to ten minutes or more, but increasing the development without rapid image processing is not desirable.

Kodak Kodak Kodak

Was

All solutions should be kept at 18°C, in temperature, the tures increase the impossible to achieve used. After the slid light may be used to the slides shou twice the clearing t: removes all the fixe hypo will occur if thorough washing (x: mounted in the usual as the mounting med
to fog probably remains the same. With the dipping technique, fog is very seldom a problem and, therefore, it is preferable to expose at room temperature to obtain a more useful image. The fog level averages three to five grains per 100 \( \mu \text{m}^2 \) in this laboratory. However, the dipping technique is perfectly amenable to low-temperature exposure, should an investigator prefer to do so, or if there is an experimental reason for it, the exposure box may be placed in a cold room after it has been sealed.

Exposure time varies according to sensitivity of the emulsion, the total dose of iodide administered, its effective half-life, the length of time between administration and sacrifice of the animal, the degree of concentration, the pattern of distribution in the tissues under study, the thickness of the tissue slices and the energy of the radiation. These factors are difficult to reduce to a formula which could indicate actual exposure times. It has been found simpler to use replicate slides and to develop a few at intervals of several days until optimum exposure times become apparent. One soon gains experience enough to estimate the initial exposure period.

When the initial exposure period has elapsed, photographic development is performed with ordinary glass or plastic staining dishes containing the developing solutions. Ten to twenty slides are placed in the usual staining rack for immersion in the solutions. The solutions used are Kodak D 19 developer, Kodak SB5a stop bath and Kodak Fixer. D 19 is a rapid, fine-grain, fairly stable developer in wide use in radiography, although others are in use also. The SB5a stop bath is non-swelling, thereby reducing the probability of dimensional changes and silver grain shifting. The acid fixer hardens the emulsion layer as it fixes, which helps prevent damage to, or loss of, the emulsion layer during washing or subsequent handling.

The development time can be as short as one minute or less or prolonged to ten minutes or more. The longer the time, the more granules appear, but increasing the development time too much will frequently increase the background without increasing the useful image proportionately. The photographic processing schedule is as follows:

- **Kodak D19 developer**: 1 to 10 min
- **Kodak SB5a Stop Bath**: 15 s
- **Kodak Fixer**: 2 X clearing time
- **Wash, running tap water**: 1 h

All solutions should be 18 ± 1°C. If the individual solutions vary appreciably in temperature, the likelihood of reticulation increases. Higher temperatures increase the likelihood of the emulsion layer washing off. If it is impossible to achieve 18°C or lower, tropical processing methods can be used. After the slides have been in the fixer for a minute, sufficient white light may be used to observe the time it takes to clear the emulsion, and then the slides should be left in the fixer for an additional period equal to twice the clearing time. Thorough washing in running tap water at 18°C removes all the fixer and ensures that no image loss or crystallization of hypo will occur if the preparations are stored for long periods. After thorough washing (or staining) has been completed, cover-glasses can be mounted in the usual way and the preparations can then be studied as soon as the mounting medium is hardened.
The radioautograms prepared this way can be studied under the microscope as though they were ordinary histological preparations. Under low and high dry magnification, only slight adjustment of the fine focus enables one to correlate the histologic with the radioautographic image. Oil immersion requires somewhat greater adjustment of focus, but it is still quite convenient. Similarly, photomicrography is quite simple under the lower magnifications by using a compromised focus, but it is necessary to make two negatives if oil-immersion magnification is required because the focal plane is too thin to focus clearly on both the tissue and the emulsion layers at once. A very simple registration frame suggested by Hoekker and described in Boyd [9] permits very accurate registration of the negatives for printing. Thus, study and recording of the data obtained by radioautography are simple.

Baserca [16] has very cleverly modified the dipping technique to permit radioautography with doubly-labelled material using tritium and radiocarbon. The modification consists of proceeding essentially as described above but adding a layer of celloidin thick enough to absorb the weak tritium emanation over the developed radioautogram and redipping and developing to obtain a second radioautogram over the first, consisting of the granules exposed only by the radiocarbon.

Hampton and Quastler [17] use fluid emulsion in conjunction with electron microscopy.

Stripping-film technique differs (as do the other techniques to be described) from dipping primarily in the method of application of the nuclear emulsion to the specimen and also because a film of emulsion is used rather than a fluid emulsion. Specimen preparation and photographic development are similar to the dipping technique. The emulsion is applied by detaching or "stripping" the nuclear emulsion from the temporary glass or acetate support upon which it is placed by the manufacturer and floating it on a water bath. The specimen on the glass slide is then dipped under the floating film and brought up out of the bath with the film adhering to it. When the emulsion has dried, it usually adheres quite tightly to the slide and specimen because it shrinks as it loses water. In this method resolution is excellent, and the radiographic image retains its original relationship to the specimen. The only drawback to this technique is the difficulty most people encounter in handling the thin unsupported emulsion: it is quite time-consuming and there is a possibility of radioisotope loss in the water bath.

Stripping films in its dry state has been used by Fitzgerald [18] to apply to dry specimens where it was desirable to avoid loss of water-soluble isotopes. While this technique may create some artifacts due to the rather cavalier handling of the emulsion film which is necessary, no other method will serve when very soluble compounds are involved.

The methods described so far are used for high-resolution work with sections of specimens. For radioautography of whole mounts, for instance of a whole plant or a leaf or of a whole small insect, the simple apposition technique is very useful. In this technique the specimen is positioned on a cut film or a plate film protected from light and exposed, usually under pressure (as in a plant press or other device) to obtain close contact. After exposure, the specimen is separated from the film and the film is developed. The main drawbacks to this method are that it is very difficult to correlate the radioautographic the two are separate gross survey work, book [9].

CONTROLS AND AR

Each slide should of developed silver gas in areas relatively background is subtract tissue slides in order the presence of radio reducing reagents where other sources of beta rays, naturally occur irradiation of emulsion radiation from adjacent and heating. From the fog level of the exposed discarded.

CONCLUSION

It is hoped that this investigator who wish information. The technique, and the variety radioautography in most be emphasized that it is a new tool and should not the be the cytological post- radioautography is a very important new, this Symposium was c

[1] Radioisotopes and Radiations
[7] HERZ, R. H., Photographic Art
[8] YAGUDA, H., Radioactive
the radioautographic image with the specimen at the microscopic level once the two are separated, and resolution is not good, but it serves well for gross survey work. Several apposition methods are described in Boyd's book [9].

CONTROLS AND ARTIFACTS

Each slide should be used as its own control by observing the number of developed silver granules in natural or artificial tissue spaces as well as in areas relatively remote from the tissue. In evaluating the slides this background is subtracted. It is also necessary to use non-radioactive control tissue slides in order to account for silver granules which may be due to the presence of reducing substances such as sulphhydryl compounds or even reducing reagents which have not been completely washed out of the tissues. Other sources of background are: accidental exposure to white light, cosmic rays, naturally occurring radioisotopes in the containers, slides and tissues, irradiation of emulsion during immersion of the radioisotope-bearing tissue, radiation from adjacent slides in the exposure chamber, mechanical factors and heating. From time to time blank slides should be included to check the fog level of the emulsion; when it gets too high the emulsion should be discarded.

CONCLUSION

It is hoped that this short review will serve as a general guide to any investigator who wishes to take advantage of radioautography to obtain unique information. The techniques are not difficult if the fundamentals are understood, and the variety of techniques available make it possible to utilize radioautography in many types of research problems. Perhaps it should be emphasized that radioautography, as are radioisotopes in general, is a tool and should not be considered as an end in itself. However, for detecting the cytological positions of very small quantities of radioisotopes, radioautography is a very powerful tool, and it can serve the purposes for which this Symposium was convened admirably.

REFERENCES

DISCUSSION

K.K. NAIR: Is it not true that a mere blackening of the autoradiographs need not be an indication of radioactivity in the sections, but could be an artefact resulting from the precipitation of silver halide in certain niches in the section?

D.L. JOFTES: Yes, certainly. The emulsion is heir to many ills. There are usually radioisotopes in the structures which we use to manipulate the emulsion. Even the glass slides frequently contain some K_40, which then interferes, and mechanical manipulations heat it up too.

There are many many sources of error in this sort of thing and it is necessary for that reason to use adequate controls, to be very ruthless in analysis and to eliminate radioautograms which seem to be questionable. We have been doing radioautography now extensively for about eight years, and generally there is no question about really good positive radioautographs. The questions always arise when they are powerfully negative. Non-radioactive control tissues should always be included in the early runs to any new experiment involving radioautography. I have previously published a report on the type of artefact to which you refer. I believe it to be the result of differential drying of the emulsion, which is thicker over these tissue interfaces; this differential drying causes mechanical strain in the emulsion and so "exposes" it.

M. HASCOET: What limits of definition can one hope to obtain by using dry stripping films?

D.L. JOFTES: We have not used this technique in my laboratory but I believe the resolution achievable should be only slightly less than with standard stripping film techniques.

M. HASCOET: Is it possible to work at the cellular level?

D.L. JOFTES: With low-energy isotopes such as H_2 or C_14, probably. It seems to me that one cannot achieve an intimate contact between tissue and a dry film as one can by dipping or standard stripping techniques, and this will adversely affect the resolution.

M. HASCOET: I have had great difficulties in using a similar method with S_35. It is quite difficult to localize sulphur finally at the cellular scale and I would have been happy to know what methods you used to facilitate this.
D. L. JOFTES: That is partly a function of the mean path length of the radiation in the tissue and in the emulsion. I would expect you to get results with S^{35} comparable to those of C^{14}. Unfortunately I myself have no experience with the dry-film technique.

M. FRIED: I wondered if you had any experience of using film slides with fresh frozen sections, and the kind of artefact you get under these conditions.

D. L. JOFTES: We have not done this ourselves but we collaborate with another laboratory which has done work on these lines, and my guess is that the main problem would be associated with the melting, if you will, of the tissue fluid unless care was taken to dry the tissue into position. In the dipping system we put these things immediately into a chamber where humidity is very low and, the sections of tissue and the emulsion being very thin, there was very rapid drying, whereas in the fresh-frozen section there is a fair amount of water; and if you have a soluble isotope there, in compound, when this is warmed you may get some motion or some passage into the emulsion at some distance from the tissue.

The reason that I suggest drying and then rewarming is that you lower the sensitivity of a film considerably by chilling it. With the dipping technique and frozen dry sections one sometimes gets a build-up of emulsion in the interstices which occur there and then in the differential drying of the emulsion film, we often see streaks of silver granules running around the edges of the tissues and in the interstices, but this is such a fine kind of artefact that I am sure you would have no trouble.
III.

LABELLED INSECTIDE STUDIES:
TOXICOLOGY AND RESIDUES
RADIOISOTOPES IN T？”
The use of radioisotopes has increased in both plants and animals, and the selection of new materials than would have been possible. Information gained in chemicals are relatively non-toxic to mammals than with some insect pests. This analysis have been developed for use with insects. Isotopes have made it possible to study the absorption and movement of chemicals in animals. More than 50% of the isotope should be of systemic insecticides.

EMPLI DES RADIOISOTOPES AUX ANIMAUX ET AU POINT D’INSECTICIDES Enfin, on a déterminer plus tôt l’emploi, emplacement et a utilisation supérieure.

Les renseignements obtenus ont été utilisés et pour certaines mammifères que par métaméthodes de l’extinction des insectes pénétrants jusqu’à présent. L’emploi d’insecticides sur les proies des mammifères et le métabolisme des insecticides dans les laboratoires et leur exécution.

En utilisant des produits d’insecticides endo-éthérapiques insecticides endo-éthérapiques
RADIOISOTOPES IN THE STUDY OF THE FATE OF INSECTICIDES APPLIED TO ANIMALS AND PLANTS

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Abstract — Résumé — Anotaciones — Resumen

RADIOISOTOPES IN THE STUDY OF THE FATE OF INSECTICIDES APPLIED TO ANIMALS AND PLANTS. The use of radioisotopes has been of great value in the development of systemic insecticides for the control of insect pests of both plants and animals. General patterns of absorption, circulation and localization, metabolism, and the site and nature of residues have been determined more rapidly through the use of labelled materials than would have been possible by other available methods of analysis.

Information gained in studies with labelled insecticides has enabled entomologists to learn why some chemicals are relatively more toxic to insects than to mammals and why some are safer for use with certain types of mammal than with others. Results of these studies have led to the development of new methods for controlling insect pests. Hitherto unsuspected metabolite pathways have been elucidated and new methods of analysis have been developed.

Labelled insecticides have been essential in developing basic information on plant systems. Radioisotopes have made it possible to follow the absorption, translocation and metabolism of systemic insecticides in plants. Also, the chemicals can be followed into insects that feed on treated plants and their metabolism and excretion can then be studied in the insects.

By utilizing labelled materials it has been shown that cotton plants grown from seed treated with systemic insecticides absorb less than 5% of the applied dose. Other studies have demonstrated that systemic insecticides are not readily translocated from treated leaves to new growth.

The use of radioisotopes in studying systemic insecticides will increase in the future. More emphasis is being placed on basic physiological studies on the fate of insecticides in plants, animals, and insects. Another area where isotopes should be used rather extensively is in studies designed to improve methods of application of systemic insecticides.

EMPLOI DES RADIOISOTOPIES DANS L’ÉTUDE DE LA TRANSFORMATION DES INSECTICIDES ADMINISTRÉS AUX ANIMAUX ET AUX VÉGÉTAUX. L'emploi de radioisotopes s'est révélé très utile pour la mise au point d'insecticides endothermiques pour la lutte contre les insectes nuisibles aux végétaux et aux animaux. On a pu déterminer plus rapidement divers processus généraux - absorption, circulation, localisation, métabolisme, emplacement et nature des résidus - grâce à des produits marqués que par les méthodes d'analyse utilisées auparavant.

Les recherches obtenues à la suite des études faites au moyen d'insecticides marqués ont permis aux entomologistes de découvrir pourquoi certains produits chimiques sont plus toxiques pour les insectes que pour les mammifères et pour quelles raisons l'emploi de quelques-uns d'entre eux présente moins de danger pour certains mammifères que pour d'autres. Les résultats de ces études ont pu nous montrer de nouveaux méthodes de lutte contre les insectes nuisibles. On a pu aussi expliquer certaines transformations métaboliques inattendues jusqu'à présent et élaborer de nouvelles méthodes d'analyse.

L'emploi d'insecticides marqués a joué un rôle essentiel dans l'assemblage des données fondamentales sur les propriétés endothermiques des plantes. Les radioisotopes ont permis, en effet, de suivre l'absorption, la translocation et le métabolisme des insecticides endothermiques dans les plantes. On peut aussi suivre les produits chimiques dans les insectes qui se nourrissent sur les plantes traitées et étudier ainsi leur métabolisme et leur excrétion.

En utilisant des produits marqués, on a pu montrer que les contaminants tels, de nombreuses unités au moyen d'insecticides endothermiques absorbent moins de 5% de la dose appliquée. D'autres études ont prouvé que les insecticides endothermiques ne sont pas facilement transportés des feuilles traitées aux nouvelles pousses.
INTRODUCTION

Entomologists have long been interested in the effects of insecticides on insects, particularly how they affect the growth and development of insects. The use of radioactive tracers has been a valuable tool in studying insect behavior and physiology. These tracers allow researchers to track the movement and distribution of insecticides within the insect, providing valuable insights into their effectiveness and potential areas for improvement.

1. FATE OF INSECTICIDES

A. Introduction

Within the context of entomology, the study of insects, the fate of insecticides is a critical area of investigation. Understanding the fate of insecticides is essential for developing effective and environmentally friendly control strategies. In this chapter, we will explore the various pathways through which insecticides are degraded and eliminated from the environment.

B. General patterns

Early in their development, insecticides were primarily categorized as being "persistent" or "volatile." Persistent insecticides are those that remain active in the environment for a long period, while volatile ones are rapidly broken down or absorbed by insects. Over time, this classification scheme has evolved, and modern insecticides are often designed to exhibit specific degradation patterns tailored to specific pest management strategies.

Extensive work has been done to understand the fate of insecticides in the environment, focusing on their degradation pathways and the impact on non-target organisms. This knowledge is crucial for developing sustainable pest management practices that minimize environmental impact while effectively controlling pest populations.
INTRODUCTION

Entomologists have used radioisotopes in many areas of research. One of the major fields in which this research has been productive has been that of the fate of insecticides in various biological systems. Through the use of radioactive samples, our understanding of the metabolic fate of insecticides under in vivo conditions has been greatly broadened.

The use of radioisotopes has given entomologists a tool of unparalleled sensitivity for measuring factors such as the rate of penetration or uptake of insecticides by plants, animals and insects. The various and complex reactions, whether intoxications or detoxifications, undergone by pesticides in biological tissues have been studied most effectively in efforts in which labelled samples of insecticides have been used. The determination of general patterns of the rates at which insecticides are lost by excretion, or stored in the form of residues, has been greatly aided through the use of labelled materials. Differences in toxicity to different forms, i.e. selective toxicity, have become understood in part through the use of isotopes.

In the present paper, we shall attempt a review of the general principles and patterns of the fate of certain insecticides, as determined by means of these studies.

1. FATE OF INSECTICIDES IN ANIMALS

A. Introduction

Within the necessarily brief confines of the present review, the discussion will be restricted largely to comments on the fate of selected organophosphate insecticides in warm-blooded animals. The entire area of this subject matter, particularly as it has to do with the development of systemic insecticides, has been reviewed extensively in recent years [8, 39, 40, 41, 55, 57]. Perhaps the most thorough recent review is that of O'BRIEN in his book "Toxic Phosphorus Esters" [38]. For the most part, the present paper will be restricted to general comments on work published before 1960, with somewhat more detailed comments on certain papers published in the past two years.

B. General patterns of the fate of systemic insecticides in animals

Early in their development, organophosphate insecticides were found to be more satisfactory for use on domestic animals than the chlorinated insecticides. The chief advantages of the organophosphates were the lack of persistent insecticide residues stored in the tissues of treated animals and the low levels or absence of insecticide in milk. Early analyses were generally made by means of chemical methods, each method being specific for the compound studied.

Extensive work with radiolabelled samples of organophosphates, for the most part prepared with 32P, verified the earlier findings. A long series of papers on the fate of labelled organophosphates in large animals has been
published in the United States in the last seven years. These studies have indicated that, in most instances, the organophosphates are rapidly absorbed by animals after dermal, oral, or intramuscular treatment. Following absorption, small amounts of the insecticides may be found in the blood of treated animals for a few hours. Degradation to non-toxic water-soluble compounds, which are then excreted via the urine, is rapid. The entire process is usually 90% or more complete within 48 h. Small amounts of radioactivity may partition into fat or be excreted in the milk. Generally, only a portion of this radioactivity is in the form of non-degraded insecticide, and even this portion is rapidly dissipated.

Within this overall pattern, considerable variation has occurred in the rates at which the insecticides undergo destructive metabolism. The variations observed have, for the most part, been the reflection of the relative solubility of the compounds studied. The more soluble the compounds are found to be in polar solvents such as water, and the lower their solubility in the more inert and non-polar solvents such as chloroform, the more rapidly the compounds and residues associated with them are dissipated.

C. Selected examples

Dipterex (dimethyl 2, 2, 2-trichloro-1-hydroxyethyl phosphonate) is an example of one of the organophosphates which undergo extremely rapid degradation in biological systems. The insecticide is soluble to the extent of about 20% in water. Partitioning data have shown that when a sample of the radioactive insecticide is partitioned with equal volumes of chloroform and water, about 45% remains in the aqueous layer [2]. This result is in contrast to what occurs with most of the organophosphates, which can be recovered from aqueous solvents in a near-quantitative manner by a single extraction with chloroform.

Dipterex was the first of the insecticidal organophosphates to be studied in detail following administration to cattle. In 1956, ROBBINS et al. [49] reported on the fate of F32 labelled Dipterex in a lactating cow following administration of a single oral dosage of 25 mg/kg. Radioactivity equivalent to 15 ppm appeared in the blood at 2 h after treatment and then declined to less than 1 ppm at 18 h. However, of the peak level of 15 ppm in the blood, only 7.5% behaved as Dipterex; the other 92.5% represented material degraded within 2 h of administration. There was no evidence that any unmetabolized insecticide was excreted in the milk, although 0.2% of the administered radioactivity was recovered in it. The major means of elimination of the insecticide was via the urine, the peak of excretion occurring 2.5-5.5 h after treatment. Only 3% of the administered radioactivity was recovered in the faeces, an indication that the insecticide was readily absorbed from the gut.

Similar studies on the fate of Dipterex in a dog and in rats have been reported [2, 3]. In these studies, evidence was obtained that degradation in smaller mammals, as in the cow, proceeds rapidly and is virtually complete within two days of treatment.

Co-ral (O-diethyl O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) is an insecticidal organophosphate with solubility properties much different from those of Dipterex. Unlike Dipterex, Co-ral is almost completely insoluble in many of the more properties of Co-ral different from that of Dipterex.

When radioactive major portions of tagged animals 14 d after treatment.

Poor absorption and faeces for radioactive by the cow quantitatively excreted via the milk. Residue of Dipterex studies were predigested evidence that the coexistence of tissues within two weeks.

In another study following dermal administration of radioactive in Dipterex or Co-ral, following Co-ral studies, considerable evidence has been obtained.

D. Recent studies

Recently published studies as to the nature of the blood of animals treated have been investigated and studied.

In a recent study with (carbamoylmethyl) p-carboxylate which involves non-toxic products obtained which indicate the main site of metabolism in which non-toxic residues of animals sacrificed.

There are no (O-4-tert-buty1-2-chloro-1-phenylethyl) in the eggs of treated [16] has indicated a reflection of different detoxification of the
These studies have shown that most insecticides are rapidly absorbed after administration. Following oral administration, insecticides are found in the blood of animals as water-soluble metabolites, which are rapidly degraded. The entire dose is sometimes excreted in small amounts of urine and faeces. Generally, larger quantities of an applied insecticide, such as Co-ral, are excreted, although it is not known whether this occurs in the urine or faeces. The variable persistence of the relative amount of unchanged compound in the tissues can be studied from the data on co-ral and hexane, the latter being metabolized by green manure crops.

D. Phosphate Degradation

Phosphate compounds (as phosphonic acid) are extremely rapidly degraded; only traces of unchanged material are found in a sample of the faeces of a cow treated with chlorobutyl phosphonate and in the urine of a cow treated with chloroform and phosphoric acid. The main result is in contrast to the findings of Roach et al. [29] who were able to recover 95% of the administered material by a single determination of the urine. Phosphate compounds are rapidly degraded by manure and soil. Results obtained by Roberts and Hill [30] indicate that a significant proportion of the administered radioactivity was found in the urine of the animal. The percentage of unchanged co-ral was low, indicating that the compound was degraded completely in the gut. The results obtained for co-ral were similar to those obtained for chlorobutyl phosphonate, which is rapidly degraded in the gut. The results obtained for co-ral were similar to those obtained for chlorobutyl phosphonate, which is rapidly degraded in the gut.

D. Recent studies

Recently published work has tended to verify the results of earlier studies as to the nature of the metabolism of organophosphorus compounds in warm-blooded animals. The occurrence of interspecific metabolic differences has been investigated and the nature of residues present in other domestic animals studied.

In a recent study, the fate of P32-labelled Ruelene (O-4-(tert-butyl-2-chlorophenyl O-methyl methylphosphoramido) in sheep was determined [11]. However, in those studies involving cattle [12, 13], the insecticide was found to be non-toxic to other animals. In this study, paper chromatographic evidence was obtained which indicated that cleavage of the carbonyl-nitrogen bond was the main site of metabolic degradation. The evidence confirmed previous findings of studies in which ion-exchange chromatographic procedures had been utilized. Residues behaving as unmetabolized insecticide were Practically nil in tissues of animals sacrificed 2 and 4 weeks after treatment.

There are no published papers on the fate of P32 labelled Co-ral in sheep. However, in poultry, it was determined that the insecticide was rapidly degraded after oral administration [10]. No evidence was obtained for the presence of insecticide in the eggs of treated hens. Similar work in poultry with P32 labelled Co-ral [16] has indicated that small insecticide residues may be present in eggs after dust treatment. As in work with cattle, these differences are probably a reflection of differences in solubility properties and ease of metabolic degradation of the two insecticides.
Another material of solubility properties similar to Co-ral is Bayer 22408 (O,O-diethyl O-naphthalimido phosphorothioate). In cattle, following oral treatment with radioactive samples of the insecticide, GATTERDAM et al. [22] found that more P32 was eliminated via the faeces than via the urine. Significant portions of the radioactivity present in the faeces occurred as undegraded insecticide and its oxygen analogue. The authors suggested that the insecticide might be effective against coprophagous insects, although it had proved to be ineffective as a systemic insecticide against several species of livestock pests.

Following dermal administration of the same insecticide to Holstein cows, BUTTRAM et al. [9] determined that residues of less than 0.1 ppm were present in the milk. Curiously, from 22 to 40% of the applied dosage was excreted in the faeces, with as much as 22% occurring as non-degraded insecticide. The residues of insecticide in the faeces were toxic to stable-fly larvae introduced into samples collected 2-7 days after treatment. The toxicity data obtained agreed well with those of radioactive analyses.

E. Future uses of radioisotopes in animal systemic research

The use of labelled samples of insecticides has proved an excellent tool in systemic insecticide research. The methods of analysis developed in these studies and the general pattern of results elucidated have been applicable to other studies involving the fate of labelled insecticides in insects and mammals. Future utilization of labelled insecticides will probably be made in studies involving isotopes other than P32. Thus, the World Health Organization is currently making samples of C14-labelled malathion available to interested entomologists, and a study on the fate of a tritiated sample of Famosphon (O-p-(dimethylsulfamoyl)phenyl O,O-dimethyl phosphorothioate) in mammals and insects has been reported [49].

A new usage of organophosphate insecticides, derived as a direct result of the radioactive studies, was developed for the control of faeces-breeding flies; such control is obtained by feeding the insecticides to the animals so that their faecal deposits will be toxic to insects breeding therein. Thus, both Co-ral and Bayer 22408, materials of such poor solubility that they are never absorbed from the digestive tract, have proved effective in controlling faeces-breeding flies [1, 19]. Similar studies on the control of flies breeding in poultry droppings have been reported [17, 50].

That the use of labelled compounds is not the sine qua non of metabolic studies has been shown recently in work by HOPKINS et al. [26]. These workers have demonstrated through the use of enzymatic rather than radioassay techniques that active antinestastere materials other than the oxygen analogues of phosphorothioates may be produced and be responsible for toxic effects. Working with rotenone (O,O-diethyl O-(2,4,5-trichlorophenyl) phosphorothioate), they found the parent insecticide and its oxygen analogue present in the blood of treated cattle; in addition cattle grubs from the same animal contained rotenone and an unidentified antinestastere other than the oxygen analogue. The authors did not speculate on the nature of the unknown material.

As in plant systemic work, no problems of technique have arisen in the use of labelled systemic insecticides. Not all radioactivity present in tissues is insecticide; index is insecticide. The development of a hexane- even this tool has proven that feeding labelled acetanitride-solid the presence of toxic graphic techniques is necessary. The ex certain instances, a use of labelled sampl

2. Fate of insects

A. Introduction

The portion of tissue limited to a ge. Systemic insecticides compounds which are the plant in large enough quantities to control the growth of wheat plants roots and wheat root systems for control of insects until SCI organic insecticides plant systemic insec

Nearly all of the some degree of systemic called non-systemic This property has been utilized by METCALF [34] by the following chapter to penetrate into the transpiration stream insecticide or its toxic action. These criteria of the insecticide as several excellent insecticides [4, 37, contains much information some of the and metabolism of s. A large portion of the use of radiolabelled would not be available on the basis a
FATE OF INSECTICIDES IN ANIMALS AND PLANTS

is insecticide; indeed, not even all radioactivity soluble in organic solvents is insecticide. These problems of analysis have been solved in part through the use of a hexane-acetonitrile clean-up partitioning procedure. However, even this tool has proved to be less than perfect, since it has been shown that feeding labelled phosphoric acid to animals results in the occurrence of acetonitrile-soluble radioactive residues [13]. Thus, in order to prove the presence of toxic residues the employment of paper and column chromatographic techniques of analysis as well as enzymatic assays has been necessary. The extreme sensitivity of these alternative methods can, in certain instances, negate the advantages of sensitivity obtained through the use of labelled samples of insecticide.

2. FATE OF INSECTICIDES APPLIED TO PLANTS

A. Introduction

The portion of this report dealing with the fate of insecticides in plants will be limited to a general review of organophosphorus systemic insecticides. Systemic insecticides, as used to combat phytophagous insect pests, are compounds which are absorbed by a plant and translocated to other parts of the plant in large enough amounts to be toxic to insects feeding thereon. The first practical demonstration of this method of insect control was reported by HURD-KARRER et al. [27], who found that selenium was absorbed by the roots of wheat plants and translocated to the aerial plant parts in sufficient quantities to control aphids. However, little interest was shown in this area of research until SCHRADER [48] discovered the systemic activity of certain organic insecticides. Since this time, considerable work has been done on plant systemic insecticides.

Nearly all of the organic insecticides used at the present time possess some degree of systemic action. Most of the insecticides which are commonly called non-systemic will penetrate into the leaf, stem or roots of plants. This property has been called local systemic or Tiefenwirkung action.

METCALF [34] has stated that systemic action in plants is controlled by the following characteristics of an insecticide: (1) ability of the compound to penetrate into the plant; (2) sufficient water-solubility to move with the transpiration stream; and (3) sufficient stability in the plant so that the insecticide or its toxic metabolic products can exert the desired insecticidal action. These criteria are related to the physical and chemical properties of the insecticide as interrelated with the physiological action of the plant.

Several excellent reviews have been published recently on systemic insecticides [4, 37, 44]. Recent books by O'BRIEN [38] and HEATH [25] contain much information on systemic insecticides. In the following discussion some of the major findings in the areas of absorption, translocation and metabolism of systemic insecticides by plants will be briefly reviewed. A large portion of the data pertaining to these studies was obtained with the use of radiolabelled compounds. Without radioisotopes, much of these data would not be available because precise quantitative and qualitative chemical analytical methods are also not available. More information is available on
metabolism of systemic insecticides than on absorption and translocation, principally because of the emphasis recently placed on insecticide residues on and in food and fibre. Nearly all countries require residue data of some kind before an insecticide can be marketed.

B. Absorption

As previously mentioned, the absorption of organic insecticides by plant tissue is not uncommon. This absorption is undoubtedly due to the lipid solubility of these compounds. In practice, systemic insecticides are usually applied so that they are absorbed by either the roots or leaves. Some experimental work has been done on stem treatment.

Using P32-labelled compounds, DAVID [13, 14] demonstrated that Schradan (octamethyl pyrophosphoramido) was selectively rejected by bean roots but Dimethox (tetramethylphosphorodiamic fluoride) was selectively absorbed. METCALF et al. [35] reported that P32-labelled Schradan was absorbed by lemon roots at the same rate as H3P32O4 from water cultures. THIEZ in a comprehensive report of studies with P32-labelled demeton (Systox) (O, O-diethyl S-(ethylthio)ethyl phosphorothioate) [54], concluded that the toxicant was absorbed readily by the intact root. The most rapid absorption was observed in plants growing in water culture; slower absorption took place in sand and soil cultures. Using beans grown in water culture, this worker found that the roots selectively absorbed the insecticide during the first day but thereafter absorbed water and the insecticide at about the same rate.

Recent reports by HACSKAYLO et al. [23, 24] with P32-labelled phorate (O, O-diethyl S-(ethylthio) methyl phosphorodithioate) and P32-labelled dimethoate (O, O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate) discussed the absorption of these insecticides by the roots of cotton plants. The absorption of P32-labelled phorate from nutrient solutions by cotton roots was very rapid initially but decreased with time. A leakage of the absorbed phorate from the roots back into the nutrient solution was observed. This leakage indicated that the insecticide caused some damage to the root system of the treated plants. In studies with P32-labelled dimethoate, it was found that this compound was selectively rejected by the roots of cotton plants. The roots of cotton plants grown in complete and phosphorus-deficient water cultures absorbed essentially the same quantities of dimethoate.

LINDQUIST et al. [32] exposed small sections of the roots of intact cotton seedlings to P32-labelled phorate and H3P32O4. They found that the amount of phorate absorbed was in direct proportion to the volume of the root exposed to the insecticide. No evidence was found to indicate that any portion of the root absorbed phorate preferentially. However, H3P32O4 was actively absorbed by the cotton root from 20 to 40 mm above the root tip. The volume of the root exposed to H3P32O4 did not influence the amount absorbed. These workers concluded that the mechanism of absorption of phorate and that of H3P32O4 by cotton roots were not similar.

Seed treatment with systemic insecticide for the control of insects attacking young plants has received considerable attention during the past few years. This method of applying systemic insecticides has been recently reviewed [42]. DAVID et al. [15] studied the absorption of P32-labelled demeton thiol isom er . A seed germinated with the seedling after the dosage was the dosage applied. Similar studies with of experiments with phorate, demeton (ethy1) phosphorothioate toxicant absorbed by the sprout after the dosage applied. Similar studies with of experiments with phorate, demeton (ethy1) phosphorothioate toxicant absorbed by the sprout after the dosage applied. Similar studies with of experiments with phorate, demeton (ethy1) phosphorothioate toxicant absorbed by the sprout after the dosage applied.
and translocation, resulting in insecticide residues near the soil surface. Some experimental data on the uptake of insecticides by plants are available. Some experiments indicated that Schradan is absorbed by bean roots but not by intact seeds. However, it was absorbed by bean shoots. 

TIEZEL and CROOM (1954) reported that the most rapid absorption of P-32 labeled phorate, demeton thiol isomer and Di-systox (O, O-diethyl S-[2-(ethylthio) ethyl] phosphorodithioate) occurred in the root culture, this being about the same as absorption by the shoot. 

Mitchell et al. (1958) absorbed by cotton roots of cotton plants grown from P-32 labeled phorate and P-32 labeled dimethoate (O, O-diethyl S-methyl phosphorothioate) was observed. 

Mitchell et al. (1958) reported that the root system of cotton plants absorbed P-32 labeled phorate and P-32 labeled dimethoate. 

The most rapid absorption of the absorbed phorate was observed. This was followed by the root system of cotton plants. 

Mitchell et al. (1958) reported that the roots of intact cotton plants absorbed P-32 labeled phorate. However, it was observed that the volume of the root system was larger than the volume of the phorate absorbed. This was observed that the amount of absorption of P-32 labeled phorate was larger.

The control of insects during the past few years has been recently reviewed by Mitchell et al. (1958). Some workers have reported that the demeton thiol isomer was absorbed directly into the cotyledons as the broad-leafed grasses germinated and that some of the toxicant was absorbed by the roots of the seedlings after emergence. REYNOLDS et al. (1958) reported on a series of experiments with cotton, alfalfa and sugar beet seed treated with P-32 labeled phorate, demeton thiol isomer and Di-systox (O, O-diethyl S-[2-(ethylthio) ethyl] phosphorodithioate). These workers demonstrated that the amount of toxicant absorbed by the plants following seed treatment was about the same as that following application of granules in the seed row. From 2 to 8% of the dosage applied was recovered in the resulting plants 16 d after planting. Similar studies with P-32 labeled phorate (1958) demonstrated that absorption of this insecticide following seed treatment takes place primarily during the first few days after planting. In standard laboratory germination tests, phorate-treated intact cotton seeds absorbed 0.7% of the applied dose in 5 d. However, with the seed coats removed before treatment, the seeds absorbed 11.9% of the applied dose. Furthermore, more dehydrated seeds germinated in the test, and germinated faster, than intact seeds. For comparison, intact and dehydrated seeds were treated with H2P-32O4- and the radioactivity absorbed by the germinating seeds was measured. The presence of the cotton seed hull did not reduce the absorption of the H2P-32O4- as it did the absorption of phorate. Dimethoate is absorbed by cotton plants grown from dimethoate-treated seeds to about the same extent as phorate (1958). From 4 to 8% of the applied dose of phorate or dimethoate was found in the cotton plant. Bidrin (Shell 3562; 2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) is absorbed by cotton seeds to approximately the same extent (LINDQUIST et al., unpublished data). In conclusion it can be stated that with the aid of radioisotopes it has been found that the seed-treatment method is not a very efficient way to get a systemic insecticide into a plant.

MITCHELL et al. (1958) discussed the absorption of systemic insecticides following stem or bark application. In general, this method of applying the toxicant is as effective or more effective than root treatment. Preliminary results with P-32 labeled Bidrin indicate that the toxicant is very rapidly absorbed by cotton stems (LINDQUIST et al., unpublished data). Data presented by BOWMAN et al. (1958) indicated that phorate and chipman R-6200 (O, O-diethyl S-[2-(diethylaminoethyl) phosphorothioate 2-toluenesulfonate]) were readily absorbed by cacao trees when the materials were implanted into the tree trunks. SANTILLAN (1958) demonstrated that dimethoate (Rogar) with tri-n-butylphosphate added was rapidly absorbed by lemon-tree trunks.

Foliar sprays of systemic insecticides probably are more widely used than any other method of application. However, most of the insecticidal activity of these insecticides is local systemic action, i.e., penetration into the leaf. It is apparent, therefore, that absorption of systemic insecticides by leaves is quite important. MITCHELL et al. (1958) compiled data from several sources relative to the rate of penetration of systemic insecticides into lemon leaves. Dimethoate was absorbed more rapidly than Schradan, demeton, or amiton (O, O-diethyl S-[2(diethylaminoethyl) phosphorothioate hydrogen oxalate]). Several studies have been conducted on the absorption of P-32 labeled systemic insecticides by leaves (5, 13, 14, 52, 53, 54). In general, these authors found that the compounds were not absorbed through the stomata and that light and temperature influenced the rate of absorption.
Although methyl parathion (O, O-dimethyl O-p-nitrophenyl phosphorothioate) is not regarded as a systemic insecticide, recent studies [51] have shown that this compound is readily absorbed by cotton leaves. By using $^{32}$P-labelled material, maximum absorption was found to take place within 3 h, after which the concentration within the leaf gradually decreased. Studies on the penetration of $^{32}$P-labelled Bidrin into excised cotton leaves indicated that maximum absorption was effected after 4 h when the leaves were held in the light, but took 24 h when the leaves were held in the dark (LINDQUIST et al., unpublished data). No difference in the rate or amount of insecticide absorbed was found when leaves were treated on the upper or lower surfaces.

C. Translocation

The translocation of systemic insecticides from the roots to the aerial plant parts appears to be via the transpiration stream in the xylem. Recent reports by HACKSKAYLO et al. [23, 24] supported this observation. These authors also demonstrated that, following the initial rapid absorption of phorate by cotton roots, the insecticide was rapidly translocated upward into the leaves, thus reducing the phorate concentration in the roots. Factors which governed transpiration, i.e., temperature and humidity, also governed the amount of phorate accumulated in the leaves. LINDQUIST et al. (unpublished data) have shown that the translocation of dimethoate to cotton leaves, after application of the toxicant to the soil, follows the transpiration stream. Experiments were conducted in which half of the plant was held under conditions which slowed transpiration and the other half under conditions which were favourable to transpiration. The results of these tests demonstrated that dimethoate accumulated in much larger quantities in leaves held under favourable transpiration conditions.

Translocation of systemic insecticides following seed treatment appears to be similar to translocation following root treatment [37]. However, as previously stated, considerable amounts of the insecticide are absorbed directly by the germinating embryo. In fact, LINDQUIST et al. [33] have shown that phorate-treated cotton seeds absorb most of the toxicant that will eventually be absorbed before the young seedlings emerge. This result indicated that there is little actual translocation of the toxicant via the root system following seed treatment.

The translocation of systemic insecticides following stem treatment has not been investigated very thoroughly. WEEDING [56] demonstrated that $^{32}$S-labelled demeton was translocated upward and downward in lemon seedlings after stem application. Most of the movement was upward. Translocation took place initially in the xylem but gradually diffused into the phloem. LINDQUIST et al. (unpublished data) found that $^{32}$P-labelled Bidrin was translocated almost entirely upward when applied to the stems of cotton plants. METCALF et al. [36] found that $^{32}$P-labelled demeton thiono isomer was translocated to the leaves of lemon seedlings after stem treatment in a pattern similar to that noted after root treatment.

More research has been reported on the translocation of systemic insecticides after foliar application than on translocation after other methods of treatment. In general, enough quantities of insecticide protection, 1 downward translocation and light are important factors, so infection probably is

D. Metabolism

Since the metabolism of systemic insecticides was investigated, a recent report on insect and plant tests have been published [37]. One of the major results was that the metabolites of systemic insecticides were found in a plant after treatment with the parent compound. The degradation of the parent compound was further degraded to the extent of establishing the relationship between the parent and the metabolite.

E. Future uses of radioisotopes

Radioisotope investigations concern the absorption and translocation of metals and the metabolism of a systemic insecticide. It is the writer's belief that this would greatly assist in the study of the systemic behavior of a systemic insecticide. Very little work has been done in the soil after gross method of applicative precision with the greater precision with the above account.

Other studies with radioisotopes include the determination of the absorption and translocation of systemic insecticides.
of treatment. In general, these insecticides are not translocated in large enough quantities to be insecticidal. However, if the entire plant is treated, sufficient translocation takes place to the new growth to give insect protection. Upward translocation takes place more readily than downward translocation. THOMAS et al. [52] reported that both temperature and light are important in translocation. Light appeared to be the most important factor. The translocation of these toxicants following foliar application probably is related to photosynthate movement in the plant [37].

D. Metabolism

Since the metabolism of systemic insecticides has been thoroughly reviewed [20, 21, 25, 38], the discussion of this subject here will be rather brief. Almost all of the metabolism studies have been carried out with radio-labelled insecticides. This research has revealed the relatively complex metabolic degradation pathways of many of the systemic insecticides. In a recent report on dimethoate [7], 11 metabolites were detected in various insect and plant extracts. Without the use of radiolabelled compounds research of this nature would not be possible.

One of the major difficulties in measuring the absorption and translocation of systemic insecticides is the rapid metabolism of many of these compounds by the plant [37]. Care must be taken before assuming that all radioactivity found in a plant after treatment with a labelled systemic insecticide is associated with the parent molecule. BULL et al. [7] found that cotton seedlings degraded more than 60% of the absorbed dimethoate in 5 d following root treatment in solution culture. Insects feeding on the treated plants further degraded the toxicants. Additional work along these lines is needed to establish the relationship between plant and insect metabolism.

E. Future uses of radioisotopes in plant systemic research

Radioisotopes have been used quite extensively in the relatively few investigations concerned with systemic insecticides. In the areas of absorption and translocation, very few critical studies have been done in which the metabolism of a compound has been taken into consideration. This effect of the plant on the insecticide must be known before any real knowledge of the systemic behaviour can be studied. Metabolism, however, is a major study in itself.

It is the writer's belief that the use of radio-labelled systemic insecticides would greatly aid the development of methods of applying these chemicals. Very little work has been done in this area. Nothing is known about the effect of various surfactants on the systemic action of these compounds. Very little has been done to elucidate the movement of systemic insecticides in the soil after granular application. Stem treatment is a very promising method of application. All of these topics can be investigated with much greater precision with radiolabelled compounds than without. In all studies discussed above, however, metabolism of the insecticide must be taken into account.

Other studies which can best be done with the aid of radiolabelled compounds include a determination of the mode of action of the absorption of
systemic insecticides by plants and the physical and chemical processes involved in translocation. Considerably more information must be available in this area before we can understand much about systemic insecticide behaviour in plants.

3. SUMMARY

It should be obvious from the preceding report that radioisotopes have been used quite extensively in studies associated with the fate of insecticides applied to animals and plants. Although residues as such have not been discussed at length, all of the topics covered relate directly to residues. In fact, much of the information cited was obtained during residue studies.

The use of radioisotopes has made it possible to study precisely the absorption, translocation, metabolism and excretion of insecticides by animals and plants. As more data are accumulated on the topics discussed in this paper, it will be possible for chemists to synthesize insecticides for use against specific insect pests. Also, insecticides which are extremely toxic to insects but virtually non-toxic to mammals should be the result of this type of research.

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J. W. MILES: I ment of the support work done with C14 WHO has supplies of bution to laboratories In addition a new C carbamate, labelled is bution. Further infon Section, Division of E W. KLOFT: It labelled with P32 ise then transported into that could be used to;
FATE OF INSECTICIDES IN ANIMALS AND PLANTS

...must be available for the chemical processes which are responsible for the breakdown of the insecticide b... 

Radioisotopes have been used to demonstrate the fate of insecticides applied to crops, a technique which has not been used with sufficient frequency to residues. In general the residue studies have focused on studying precisely the fate of individual insecticides by observing the radioactive isotope, discuss the behavior of those insecticides for which are extremely short-lived, and so may be the result of... 

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W.E. ROBBINS (on behalf of F.W. Plapp and D.A. Lindquist): This is dealt with very fully in the work by Mitchell et al. cited as reference [37] to the paper.

G.F. BURNETT (Chairman): What is the chance of charging a cow with sufficient radioactivity to mark tsetse flies (Glossina spp.) feeding on it when driven through the bush? These flies feed on nothing but blood and are very difficult to raise in the laboratory. There is no convenient way of marking them without capture.

W.E. ROBBINS: It would probably be possible. There are quite a number of studies on the fate of radioisotopes in large animals, and certain of these studies might aid you in selecting the appropriate isotope for high blood levels in the animal and a long biological half-life. About 1952, for instance, Dahn did similar work on a goat. He was comparing parathion and inorganic phosphorus. His work might be of assistance as regards the maintenance of blood levels.

F.T. PHILLIPS: I would like to comment on the use of excised cotton leaves in penetration studies of P³²-labelled Ektin. I have been told that the metabolic processes of excised leaves are different from those of leaves attached to the plant, so perhaps the figures obtained for the excised leaves would not be the same as for leaves which were attached to the plant? Photosynthesis and production of proteins in the plant are not going on, for instance, and that may affect your figures.

W.E. ROBBINS: I think Dr. Casida would like to comment.

J.E. CASIDA: Yes, Dr. Phillips is quite correct, but the intact plant can be treated by injection through the stem to minimize this difficulty. We take a very fine pulled-out glass needle, which is easily inserted into the stem with no apparent damage to the plant. Between 5 and 50 µl of solution put in are taken up by the plant within half an hour to two hours. Then we continue with the study.
SOME PROBLEMS IN THE DETERMINATION OF RESIDUES IN PLANTS AND MAMMALS

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Abstract — Résumé — Аннотация — Resumen

SOME PROBLEMS IN THE DETERMINATION OF RESIDUES IN PLANTS AND MAMMALS. In most instances residues must be determined by chemical or biochemical methods without the use of radioisotopes. These methods assume that the nature of the toxic compounds present is known, and that they can be extracted in known yields. Neither assumption is easily validated except by using radioisotopes. The use of radioisotopes to investigate these problems is described, with examples taken from work on demeton, diazinon and the fungicide triphenyltin acetate.

PROBLÈMES RELATIFS À LA DÉTERMINATION DES RÉSIDUS DANS LES VÉGÉTAUX ET CHEZ LES MAMMIFÈRES. Dans la plupart des cas, les résidus doivent être déterminés par des méthodes chimiques ou biochimiques sans recours aux radioisotopes. Ces méthodes sont basées sur l'hypothèse que l'on connaît la nature des composés toxiques présents et que l'on peut les extraire en quantités connues. Aucune de ces deux conditions n'est facile à réaliser si ce n'est à l'aide de radioisotopes. L'auteur décrit l'utilisation des radioisotopes dans l'étude de ces problèmes et donne des exemples tirés de travaux sur le démétone, le diazinon et un fongicide (l'acétate de triphényltin).

НЕКОТОРЫЕ ПРОБЛЕМЫ ОПРЕДЕЛЕНИЯ ОСТАТКА ИНСЕКТОЦИДОВ В РАСТЕНИЯХ И У ЖИВОТНЫХ. В большинстве случаев остатки инсектицидов должны определяться химическими и биохимическими методами без использования радиоизотопов. При применении этих методов предполагается, что известна природа инсектицидов, и что они могут быть извлечены в известных количествах. Ни один из этих предположений не может быть легко подтвержден, если они не сопровождаются радиоизотопным методом. Описывается применение радиоизотопов для исследования этих проблем и приводятся примеры, как из работы, связанных с деметоном, диазиноном и фунгицидом трифенилтиламинаяцетатом.

DETERMINACIÓN DE RESIDUOS DE INSECTICIDAS EN PLANTAS Y MAMÍFERNOS. En la mayor parte de los casos la determinación de los residuos de insecticidas debe efectuarse por métodos químicos o bioquímicos, sin emplear radioisótopos. Para estos métodos se partan de suposiciones que se conoce la naturaleza de los compuestos tóxicos presentes y que estos pueden extraerse con condiciones conocidas. Ninguno de estos supuestos puede justificarse fácilmente si no es mediante el empleo de radioisótopos. El autor de la memoria examina este empleo de los radioisótopos y cita ejemplos tomados de trabajos efectuados con los insecticidas Deméton y Diazinon, y con el fumigácid acetato de trifenilmetilo.

Much of the work in assessing the safety of a pesticide depends upon analysis of residues in crops and animal tissues. A high degree of reliability in methods is obviously essential — residue analysis is not a field in which speculation can be allowed much part — and this sets problems. Some of these problems, and the use of labelled compounds in solving them, are considered in this paper, with examples from work with which the author has been concerned. Not all of this work is new, but little of it has been described in this context.
A problem of very general interest is that of the reliability of recovery tests as usually carried out. In the instance to be described, an apparently reliable method gave substantially nil residues when used on a treated crop, a result which is very acceptable, but also, naturally, suspect. The example is that of Dimefox in cocoa beans.

Dimefox is the organophosphorus systemic insecticide, \( \text{Me}_2\text{N}_2\text{PO} - \text{F} \). It was proposed at one time for the control of mealy-bugs on cocoa trees (Cacao theobroma) [1]. In view of its high toxicity, only levels below 0.1 mg/kg would be acceptable in the beans at harvest. An adequate analytical method sensitive to 0.01 mg/kg, was developed. The beans were macerated with an oil of high boiling point. The oil was distilled under reduced pressure, and the relatively volatile Dimefox was carried over quantitatively with the first few per cent of the oil, from which it could be extracted, and estimated as phosphate [2]. This method was applied to beans from treated trees and gave residues of 0.05 mg/kg or less, even when the trees as a whole contained enough Dimefox to kill the mealy-bugs feeding on their foliage. It seemed possible that the recovery tests were at fault: Dimefox might be recovered quantitatively when added to beans just before maceration, but might not be recovered when it reached the beans by a systemic route, when it might either become irreversibly adsorbed or enclosed in plant matrices, not destroyed by the distillation process. Pods were therefore left for a few days with their stems dipped in a dilute aqueous solution of Dimefox labelled with \( ^{32} \text{P} \). The pods were then opened, and the beans were divided into seven samples and analysed for Dimefox. Four samples were analysed in the usual way, except that the phosphate was estimated as \( ^{32} \text{P} \) in a liquid counting tube, and were found to contain 0.044 ± 0.002 mg/kg (means ± SD). The remaining samples were macerated with water or refluxed with chloroform, and the macerates were counted. The extracts were then bulked, and the Dimefox was separated by partitioning between water and chloroform and estimated by counting. The Dimefox content of the beans as found by this completely different method was 0.046 mg/kg, in excellent agreement with the results of the distillation method. The distillation method was thus fully vindicated. These experiments also provided an explanation for the very low quantities found. The macerates in water contained \( ^{32} \text{P} \) equivalent to 4 mg/kg of Dimefox, but 99% of this was as ionic compounds of very low toxicity, i.e. fruit tissue metabolized Dimefox very much more rapidly than leaf tissue did. This conclusion was later confirmed by analysing pods from a tree treated with \( ^{32} \text{P} \) Dimefox. In these experiments the residual Dimefox concentration was too low to measure, but ionic decomposition products were found.

In this type of study, the advantage conferred by using the labelled compound is that it can be estimated reliably after a method of extraction which does not separate it from naturally occurring compounds. The necessity for this separation is, of course, the controlling factor in most procedures. When it does not exist, as with labelled compounds, very much more drastic procedures can often be used, and a reliable check to a normal, non-isotopic method can be provided. It would be desirable always to have such checks; and where organophosphorus compounds are concerned, this could easily be done almost always.
Another type of problem may arise when there is an obvious discrepancy between the behaviour of an insecticide and the methods suggested. This was the case when Demeton was first marketed under the names Systox and Bayer 8169. Analysis showed that Demeton consisted of two isomers: diethyl 2-ethythioethyl phosphorothionate, I and O-diethyl S-2-ethylthioethyl phosphorothionate, II [3].

Tests on toxicity to insects and mammals indicated that the thiolate, II, was about ten times more active than the thionate, I. Both compounds could be extracted from vegetable material by hydrocarbon solvents, and the methods of analysis proposed depended on this type of extraction, followed by biological estimation using insects. Such methods gave very low residues within a few days of spraying, although insects feeding on treated plants continued to die for several weeks. In this case the results could not be explained in the same way as the low residues of Dimexox in cocoa beans, as the insects killed by Demeton were feeding on the actual foliage analysed. Some sugar beet (Beta sativa) and other plants were therefore treated with the thiolate isomer, II, labelled with P^{32}. Foliage was taken at various times after treatment and macerated with water. The macerate was then extracted successively with solvents of increasing polarity, and these extracts were in turn subjected to a simplified form of counter-current extraction. Four discoveries were made. Firstly, the thiolate itself rapidly disappeared, as already indicated by other methods. Secondly, two non-ionic compounds were produced. Thirdly, these apparently fitted a metabolic scheme which can be represented as follows:

70%
Thiolate isomer → non-ionic A → non-ionic compound B → ionic compounds

30%

The non-ionic metabolites persisted for a few weeks, so that, if they were insecticidal, their formation could account for the prolonged systemic action of Demeton. Fourthly, neither of the two non-ionic metabolites could be extracted by most hydrocarbon solvents, and would not, therefore, be detected by the methods of analysis suggested. The metabolites were extracted in sufficient quantity for their toxicity to be tested, and as they were nearly as toxic as the parent compound both to insects and mammals it was clear that any worthwhile method of residue analysis must record them. This work, which was reported briefly by Hartley [4], did not show what the metabolites were, nor did it supply a method of residue analysis; these problems were solved later [5, 6, 7, 8]. Nonetheless, the basis of much further work on those phosphorus insecticides which metabolize to active
compounds in plants was laid down in this preliminary study, which occupied only about 10 weeks. It is now common practice to use tracers in order to establish the presence or absence of toxic metabolites in plants, and their use for this purpose constitutes an essential step in the justification of analytical methods for most compounds with systemic properties.

Methods of analysis of animal tissues and, products, meat and milk, do not differ in principle from the analysis of crops, and the value of using labelled compounds is the same. New problems appear, however, in the toxicological studies which are an essential part of the work involved in clearing a pesticide for use.

The initial stage in such investigations consists in determining the acute and chronic toxic effects of the compound in question, and of observations on signs and symptoms of poisoning. After this, some knowledge of the behaviour of the compound in mammals is desirable, with the eventual aim of explaining its mechanism of action. This degree of success is very rare. It has been substantially achieved with organophosphorus pesticides, and is of practical value: recommendations on permissible levels, handling and so on can be made with much more confidence about these compounds than about others whose mechanism of action is largely unknown, such as the chlorinated hydrocarbons. It is clear, however, when the studies on phosphorus anticholinesterases are considered as a whole [9, 10, 11] that tracer studies formed only a small part of the work of establishing the mechanism of action; the contribution of biochemists, pharmacologists and physiologists who did not need to use tracers was far greater. It is in the first stages of an investigation that tracers overcome difficult problems: before one asks "How does this act?", and when the questions are: "Where does this lodge in the body, and for how long; how rapidly is it excreted; and is it metabolized at all?" These questions can be answered only by analyses, and they raise acute problems, because the toxicologist wants to start work with small animals in order to correlate easily measurable effects such as the LD50 values with concentrations in vivo, and the sample sizes are then usually so small that most chemical methods are not sensitive enough. Some work still in progress on the fungicide triphenyltin illustrates these points.

Triphenyltin salts have the structure \((\text{C}_9\text{H}_8)_3\text{SnX}\), where X is an acid group. The acetate, under the name Brestan, has been used to control blight \((\text{Phytophthora infestans})\) on potatoes, and other fungus diseases on celery and sugar beet. The compound is very toxic by intraperitoneal injection, with LD50 values of 10 mg/kg in the rat and 4 mg/kg in the guinea-pig. Oral LD50 values are higher – 450 mg/kg for the rat and 30 mg/kg for the guinea-pig when the compound is given in arachis oil [12] – but the LD50 value in the rat is probably only about 70 mg/kg when the compound is given in dimethylformamide. The animals die 2-7 d after a lethal dose, with no specific symptoms and no obvious pathological lesions. Two questions arose. Firstly, does the compound persist in the body? Delayed death is not good evidence for persistence. Secondly, is the rat less sensitive than the guinea-pig to oral dosing because it absorbs the compound less readily, or because it metabolizes it more rapidly before absorption can take place?

A first attempt was made to answer these questions by means of chemical methods of analysis, which appeared sensitive enough [13, 14]. When, however, triphenyltin acetate was left in tissues for 30 min before extraction was attempted, only macceration with a n- acid \((200/100/2)\) by v lipids [15, 16]. This worked reliably on r

A specimen of the testis obtained from a rat was carried to and reported here. Each was dosed with triphenyltin in arachis oil or dim injection, and special collection from the content was estimated. The efficiency was such that specimens had to be equilibrated between half-life of 164 min.

The results of the in vitro tests were several tissues, and the distribution of concentrations could be found 2 h after injection represent material
Determination of Residues in Plants and Mammals

was attempted, only one method of extraction was found useful, namely maceration with a mixture of chloroform, methanol and conc. hydrochloric acid (200/100/2 by volume) similar to that used for the extraction of proteolipids [15, 16]. This had to be followed by tedious processing, and then only worked reliably on rat brain.

A specimen of triphenyltin chloride labelled with Sn\textsuperscript{113} (500µc/g) was then obtained from the Radiochemical Centre, Amersham, England. Experiments were carried out on rats and guinea-pigs, but only those on the rat are reported here. Essentially, albino male rats (Porton Wistar strain) were dosed with triphenyltin chloride intraperitoneally in arachis oil or dimethylformamide. Animals were killed at intervals after injection, and specimens were taken for analysis. Urine and faeces were also collected from animals given the compounds orally. The total Sn\textsuperscript{113} content was estimated by scintillation counting in a 5-cm well-type crystal.

The efficiency was 27% for a 1-ml specimen and 18% for a 5-ml specimen. Specimens had to be left about 12 h between extraction and counting, to allow equilibration between Sn\textsuperscript{113} and its indium daughter-isotope, In\textsuperscript{113m}, with a half-life of 104 min. Most of the recorded radiation is due to In\textsuperscript{113m}, and the equilibrium is disturbed by many extraction procedures.

The results of distribution studies on rats given triphenyltin chloride intraperitoneally are shown in Table I. The Sn\textsuperscript{113} was distributed throughout several tissues, including the brain, the concentration fell only slowly, and the distribution did not change much with time. Brain tissue measurable concentrations could be found 38 d after treatment. The high concentrations found 3 h after injection in specimens taken from the abdominal cavity probably represent material on the surface from the injection and should be discounted.

### Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>2 h</th>
<th>1 d</th>
<th>2 d</th>
<th>4 d</th>
<th>10 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(A)</td>
<td>(B)</td>
<td>(A)</td>
</tr>
<tr>
<td>Brain</td>
<td>3.2</td>
<td>5.2</td>
<td>3.6</td>
<td>4.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Liver</td>
<td>4.1</td>
<td>8.7</td>
<td>10</td>
<td>13</td>
<td>2.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>15</td>
<td>20</td>
<td>19</td>
<td>14</td>
<td>6.8</td>
</tr>
<tr>
<td>Fat</td>
<td>16</td>
<td>30</td>
<td>23</td>
<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.2</td>
<td>2.7</td>
<td>2.1</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>Heart</td>
<td>8.7</td>
<td>2.2</td>
<td>3.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood</td>
<td>2.3</td>
<td>1.2</td>
<td>0.8</td>
<td>0.14</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* Average of 3 rats

Concentrations are expressed as µg triphenyltin chloride per g tissue.

Columns headed "A" refer to rats given 6.8 mg/kg, column headed "B" to rats given 3.5 mg/kg, and column C to rats given 4.4 mg/kg.

1. *How* this lodge in the world is it metabolized and becomes toxic, and they raise questions about how work with small quantities of such the LD50 value is then usually so low. Some work that these points.

2. Where X is an acid it is oxidized to control light reactions on cellophane since a guinea-pig. Oral dosing being for the guinea-pig and the LD50 value in is given in dimethylformamide with no specific name. Firstly, there is not good evidence from the guinea-pig to the animal, or because it

3. by means of chemistry [13, 14]. When,
The results did not show that the compound recorded was triphenyltin. Two methods have been used to investigate this, isotope dilution analysis and the separation procedure described by Bock et al. [17]. Both agreed in showing that, of the Sn\textsuperscript{113} in rats 3 d after peritoneal injection of 6 mg/kg, 85% in the brain and 25% in the liver and kidney were present as triphenyltin. Bock's method also indicated that 25% in all those tissues was present as triphenyltin 18 d after injection. The method may not achieve complete separation in the presence of animal tissues, and this result is being checked by the isotope dilution method. If it is correct, triphenyltin persists considerably longer in the rat than it was found by Herzog and Götte [18] to persist in the sheep; their evidence for the rapid excretion of triphenyltin was not, however, entirely convincing.

Excretion studies showed that Sn\textsuperscript{113} was retained after oral injection (Table II). The rate of excretion seemed to depend on the dose and medium, but more work is required to establish this. Most of the Sn\textsuperscript{113} excreted was apparently triphenyltin until half of the tin had been excreted. Thereafter some tin was probably excreted as metabolites.

Rats treated orally were also analysed for triphenyltin, with the results shown in Table III. The tin concentrations in most tissues were very similar to those found after equitoxic doses given intraperitoneally, except that high concentrations were found in the stomach.

A feature not brought out in the Tables is that the concentrations in internal organs (except the gastrointestinal tract) of rats that died were always about the same, however the rats had received the dose. In the brain the critical level was about 5 µg/g. The only exceptions were the two rats that received 185 mg/kg orally and were killed two days after injection; these

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after treatment (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>17</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
</tr>
</tbody>
</table>

* The high values are probably due to a sampling error on the sixth day.

The results also preciable concentable than the gain rapidly in the gut. A mouth may well be y

A general view can now emerge. In crops, tracer studies of course, they are essential for routine validity of normal or nearly complete pict of METCALF et al. or collogical studies are likely to yield thgation, to give way su
of was triphenyltin.

The dilution analysis
[17]. Both agreed
with an injection of 6 mg/kg,
was present as triphenyltin.

was present as
with the assumption that most of the tin was present as triphenyltin and is
difficult to explain in any other likely way.

| TABLE III |

| TIN CONCENTRATIONS IN RATS TREATED ORALLY WITH TRIPHENYL Tin CHLORIDE |

The column headings refer to rats treated as follows:
A Given 186 mg/kg in linseed oil and killed 2 d after injection; average
of two rats.
B Same, but killed 9 d after injection.
C Given 67 mg/kg in dimethylformamide, average of 3 rats, which died
2 d after treatment.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (mg triphenyltin chloride per g tissue) A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>11</td>
<td>3.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Liver</td>
<td>25</td>
<td>3.1</td>
<td>21</td>
</tr>
<tr>
<td>Kidney</td>
<td>22</td>
<td>7.0</td>
<td>28</td>
</tr>
<tr>
<td>Fat</td>
<td>9.2</td>
<td>9.7</td>
<td>7.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.0</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Stomach</td>
<td>331</td>
<td>0.6</td>
<td>1230</td>
</tr>
<tr>
<td>Small intestine</td>
<td>331</td>
<td>1.8</td>
<td>38</td>
</tr>
<tr>
<td>Large intestine</td>
<td>542</td>
<td>2.3</td>
<td>61</td>
</tr>
</tbody>
</table>

The results show that probably triphenyltin persists in the rat at appreciable concentrations for several days, and that the rat is not less susceptible than the guinea-pig to oral dosing because it decomposes triphenyltin rapidly in the gut. Under these circumstances, triphenyltin salts taken by mouth may well be very toxic to man.

A general view of the part played by tracers in the study of residues can now emerge. In the development of methods of analysis of residues in crops, tracer studies should in almost all cases be obligatory, although, of course, they are not a substitute for non-isotopic methods, which are essential for routine analysis. Tracer studies remove any doubts as to the validity of normal methods and, even in complicated cases, often give a nearly complete picture of the action of the plant on the pesticide. The work of METCALF et al. on Dieldrin is a particularly good example [19]. Toxicological studies can often be helpful, but in most instances they are likely to yield the most useful results at an early stage in an investigation, to give way subsequently to biochemical, pharmacological and physio-
logical techniques. The main reason for this difference in utility is the difference in the relationship between a pesticide and a plant on the one hand and between a pesticide and a mammal on the other. The plant-pesticide interaction is nearly all in one direction: the plant acts on the compound, and not vice versa. Consequently when the nature, concentration and persistence of the products formed in the plant are known, the problems of interest are solved. By definition, however, in a poisoned mammal the pesticide acts on the mammal as well as the mammal on the pesticide. The problems raised by the first, toxic, action are very much more complex than those raised by the second, metabolic, action, and are not usually to be solved by labelling the pesticide.

ACKNOWLEDGEMENTS

The author wishes to thank the Directors of Eisona Pest Control Ltd., Saffron Walden, Essex, England, for permission to publish work on Dimefox and Demeton; and Mr. J. A. Rose for skilled technical assistance.

REFERENCES


DISCUSSION

G. F. BURNETT (Chairman): I think we will see a good deal of the triphenyltin compounds, both as fumigicides and molluscicides. Do you think we should go with care until the results of your work are fully clarified?

D. F. HEATH: Yes, unless it can be shown that the residues are in fact very low indeed. This possibility could easily be checked by using Sn-113-labelled triphenyltin.
C.H. Schmidt: Is there any evidence that triphenyltin may break down when it is applied on foliage and plants under field conditions?

D.F. Heath: The evidence is not very good. Triphenyltin seems to break down on foliage, but break-down may be mainly due to the action of ultra-violet light. How fast triphenyltin which had run down the stalk into shady places would decompose I do not know—perhaps very slowly.

J.R. Ogles: With reference to your remarks on Demeton, there is, I think, one aspect in which the use of radioisotopes could lead to misleading results. It is a well-known fact that many dialkyl sulphides will undergo facile radiolytic oxidation to sulphones and sulphones: if this occurred in Demeton and related compounds in which the sulphones and sulphones are as toxic as the parent compound or more so, the results obtained might not be representative of the behaviour of the inactive material used under similar conditions. Rate of decomposition varies enormously from compound to compound and even between stereoisomers, and is influenced by external factors such as physical state, temperature, presence or absence of moisture, oxygen, etc. It would seem that in using materials under these conditions it would be necessary to check, if in fact you did in your own work, that there was not an extra effect due to the radioisotope. For example, disopropyl fluorophosphates-P³² at 40 mc/mM undergoes 10% decomposition per week but is stable in arachis oil or propylene glycol. Methionine-S⁷⁵ at 80-100 mc/mM may undergo 20% decomposition within four weeks, depending upon the method of storage.

D.F. Heath: In these experiments we used Demeton less than one hundredth as active as the specimens you refer to. In view of this it is perhaps not surprising that solvent distributions showed that our specimens did not break down measurably on storage. Are you normally producing pest control compounds of specific activities at which decomposition is likely to be important?

J.R. Ogles: We have recently been synthesizing insecticides at 60 to 100 mc/mM for various of our clients.

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J.R. Ogles: Pest Control Ltd., 1956. (J.R. Ogles is a colleague who has done work on Dimefox and its resistance.

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ÉVOLUTION ET DÉGRADATION DES DÉPÔTS ENDOThÉRMIQUES: LE DÉPÔT D'AMMONIAQUE DANS LA PLANTATION DE RAPÉS

The rate of inactivation of the plant material in the foliage, the petals, and the fruits treated with the insecticides, was compared to the rate of inactivation of the fruits treated with the insecticides. A hypothesis is put forward to explain the behavior of the insecticides in the plant material.

ÉVOLUTION DES DÉPÔTS ENDOThéRMIQUES: LE DÉPÔT D'AMMONIAQUE DANS LA PLANTATION DE RAPÉS

The absence of precipitation in the plant material depends on the type of environment: plant, soil, or air. These phenomena are also observed in the plant material, where the insecticides are found to be active at the time of application. The rate of inactivation of the plant material is compared to the rate of inactivation of the fruits treated with the insecticides. A hypothesis is put forward to explain the behavior of the insecticides in the plant material.
ÉVOLUTION DES DÉPÔTS SUPERFICIELS, DIFFUSION ET DÉGRADATION DE DEUX INSECTICIDES ENDOThÉRAPIQUES: LE DÉMÉTON-S ET L'ENDOTHION DANS QUELQUES PLANTES MARAÎCHÈRES

M. HASCÖET
CENTRE NATIONAL DE RECHERCHES AGRONOMIQUES, VERSAILLES, FRANCE

Abstract — Résumé — Аннотация — Resumen

CHARACTERISTICS OF SURFACE DEPOSITS, DIFFUSION AND DEGRADATION IN A NUMBER OF MARKET-GARDEN PLANTS OF TWO ENDOThÉRAPIQUE INSECTICIDES: DÉMÉTON-S AND ENDOThION. In the absence of atmospheric precipitation, desorption of plants treated with endotherapeutic insecticides depends on both inactivation of the surface deposits and degradation of the pesticide within the plant. Both these phenomena have been followed in a number of market-garden plants treated with demeton-s and endosulfan labelled with 3H and 32P respectively.

The rate of inactivation of the surface deposits is comparable between endothion and demeton-s, where external conditions are themselves similar.

In the foliage, the persistence of demeton-s and its active metabolites varies with the plant. Normally, it is higher than that of endothion, whose decompositional pattern includes only non-toxic hydrolysis products.

If fruits treated with demeton-s, apparent degradation seems to be very rapid, whereas for endothion it remains comparable with that observed in the foliage and is apparently not affected by the maturity of the fruits. A hypothesis is put forward to explain this difference in behaviour.

Toxic residues found in the fruits may be due either to diffusion of the insecticide from the foliage or to direct contamination. A comparative study of treated and untreated fruits borne by a treated plant has made it possible to define the relative importance of these two phenomena.

Studies were made on penetration and diffusion of Demeton-S and Endothion in asparagus shoots, in an effort to explain the behaviour of these two insecticides in the control of the fly Platyptera pectinicornis. The importance of the part that may be played by the wetting agent is demonstrated.

ÉVOLUTION DES DÉPÔTS SUPERFICIELS, DIFFUSION ET DÉGRADATION DE DEUX INSECTICIDES ENDOThÉRAPIQUES: LE DÉMÉTON-S ET L'ENDOTHION, DANS QUELQUES PLANTES MARAÎCHÈRES. En l'absence de précipitations atmosphériques, la désorption des plantes traitées à l'aide d'insecticides endotherapiques dépend à la fois de l'inactivation des dépôts superficiels et de la dégradation du pesticide dans le végétal. Ces deux phénomènes ont été suivis sur plusieurs plantes maraîchères traitées au demeton-s et à l'endosulfan, marqués respectivement avec 3H et 32P.

La vitesse d'inactivation des dépôts superficiels est comparable pour l'endosulfan et le demeton-s, lorsque les conditions extérieures sont égales."
On observe alors

La contamination

- la contamination
- la contamination
- la contamination : de pollution dont l'air auraient être identifiées ticales de quelques cas une acuité particu

Nous envisageons

diffusion de ces deux d'expliquer leur com

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Plantes utilisées

L’expérimentati

nains, pois nains, e

Produits et doses

Les insecticides

L’o, o-dimethyl marqué au 6 nous

La synthèse radi

et de S-méthyl 2 mé

Saclay. L’élément

Les deux insecti
da la dose efficace, e

Modalité de traiteme

État donnée la i

manque d’installation placée par un badge
DEUX INSECTICIDES ENDOThÉRAPIQUES

On observe alors l'apparition de nouveaux composés dont la toxicité est très variable selon la nature du pesticide d'origine et l'évolution plus ou moins rapide selon la plante traitée.

Ainsi pour un insecticide endothermique, la contamination superficielle se trouve doublée d'une pollution interne dont l'évolution peut être complètement différente.

Enfin lorsque cet insecticide est télétoxique, les parties non atteintes directement peuvent être polluées par voie vasculaire.

Dans ce dernier cas, qui est celui du démétan-S et de l'endothion,
- la contamination superficielle,
- la contamination interne directe, et
- la pollution interne par voie vasculaire constituent trois sources de pollution dont l'importance respective et l'évolution dans le temps ne sauraient être identiques. C'est ce que nous allons étudier dans le cas particulier de quelques plantes maraichères. Cette question revêt dans ce cas une acuité particulière, soit parce que le feuillage est consommé (saucisses), soit parce que la fructification étant échelonnée, il risque d'y avoir des interférences entre les traitements et les récoltes (c'est le cas en particulier des haricots cuillers en vert).

Nous envisageons ensuite quelques aspects de la pénétration et de la diffusion de ces deux insecticides dans les pousses d'asperges afin de tester d'expliquer leur comportement dans la lutte contre Platysarcus pociploropa.

MATÉRIEL ET MÉTHODES

Plantes utilisées

L'expérimentation a porté sur les espèces suivantes : tomates, haricots nains, pois nains, et laitues. Toutes ces plantes ont été maintenues en serre tempérée pendant la totalité de leur cycle végétatif.

Produits et doses

Les insecticides choisis étaient le démétan-S et l'endothion.
L'o, o-diméthyl-thioléosphates de S (éthyl thio 2 éthyle) ou démétan-S marqué au 32P nous avait été obligeamment fourni par les établissements Bayer.
La synthèse radioactive du phénophosphilate de ortho ortho diméthyl et de S-méthyl 2 méthoxy 5 pyrone 4 ou endothion fut réalisé au CEN de Saclay. L'élément traceur était dans ce dernier cas le 32P.
Les deux insecticides ont été utilisées à une dose quatre fois supérieure à la dose efficace, soit 100 g/hl pour le démétan-S et 200 g/hl pour l'endothion.

Modalité de traitement

Étant donné la haute radioactivité spécifique de ces composés et le manque d'installation spécialisée, la pulvérisation insecticide a été remplacée par un badigeonnage de feuillage.
Lorsque cela était possible, les fruits d’un même plant ont été partagés en deux lots, l’un traité, l’autre protégé par une feuille de cellophane; ceci afin d’évaluer séparément la contamination directe (à partir de la surface traitée) et celle d’origine télétoxique.

Afin de suivre l’évolution des résidus, nous avons divisé les plantes traitées en trois lots dont la récolte était échelonnée selon le programme suivant:
- démétion-S: récolte après 8, 15 et 22 j,
- endothion: récolte après 5, 10 et 15 j.

La récolte des végétaux traités au démétion a été légèrement décalée pour tenir compte de la rémanence plus grande de ce produit.

Dosage des résidus

Dans le cas du démétion-S, le dosage des résidus a été effectué selon une technique voisine de celle décrite par MÜHLMAN et TIETZ [1];
- broyage des plantes en milieu acétonique à 80%,
- évaporation de l’acétone sous pression réduite,
- extraction chloroformique de la phase aqueuse.

La phase chloroformique ainsi obtenue contenait l’insecticide et ses dérivés toxiques (sulfoxide et sulfone).

Dans le cas présent ce traitement était précédé d’un lavage des feuilles et des fruits traités afin d’estimer séparément l’importance des dépôts superficiels. Ce lavage a été réalisé en trois opérations successives, par agitation dans une solution de mouillant à 0,5%. Les eaux de lavage réunies ont été soumises à une extraction chloroformique dans les mêmes conditions que précédemment.

Le degré de détoxication a été établi dans tous les cas par comparaison de la radioactivité de la phase chloroformique seule à celle du total: phase chloroformique + phase aqueuse.

L’étude de la dégradation de l’endothion a été réalisée suivant une méthode analogue; toutefois le broyage des plantes a été effectué en milieu alcoolique à 80%.

Nous signalons également que pour ce produit, seul l’insecticide non décomposé passe dans la phase chloroformique.

ÉVOLUTION DES DÉPÔTS SUPERFICIELS (tableau I)

Comme nous l’avons signalé, la dégradation du film insecticide déposé à la surface de la plante dépend essentiellement des conditions climatiques. Cependant la feuille ne saurait être considérée comme un support inert puisqu’elle est le centre d’échanges actifs entre la plante et l’atmosphère. D’autre part la texture de la surface foliaire, tomenteuse ou lisse, varie d’une plante à l’autre; chez une même plante des différences analogues peuvent également être observées entre feuilles et fruits (tomates par exemple). On pouvait donc espérer quelques variations entre les vitesses de dégradations mesurées à la surface des différentes plantes expérimentées.

Or, pour un traitement réalisé dans des conditions extérieures identiques (série A), les différences constatées sont extrêmement réduites; qu’il
ont été partagés en deux cellophane; ceci pour éviter la surface divisée les plantes selon le programme

gérément décoloré e produit.

été effectué selon et Tietz [1];

insecticide et ses
cavage des feuilles et la présence des dépôts successifs, par
mêmes conditions

cas par comparai-
aille à celle du total:

se suivant une mé-
effectué en milieu
l’insecticide non

insecticide déposé
atives climatiques.

un support inerte
ette et l'atmosphère.
le ou lisse, varie
ères analogues
ruits (tomates par
entre les vitesses
ettes expérimentées,
nières extérieures iden-
ément réduites; qu'il

<table>
<thead>
<tr>
<th>ENDOTHION</th>
<th>Plantes traitées</th>
<th>Temps écoulé entre le traitement et la récolte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feuilles</td>
<td>5 j</td>
</tr>
<tr>
<td>Tomates</td>
<td>71</td>
<td>47,6</td>
</tr>
<tr>
<td>Fruits</td>
<td>74,9</td>
<td>60</td>
</tr>
<tr>
<td>Série A</td>
<td>Feuilles</td>
<td>50,2</td>
</tr>
<tr>
<td>Haricots</td>
<td>Fruits</td>
<td>67,5</td>
</tr>
<tr>
<td>Petit pois</td>
<td>Feuilles</td>
<td>77,2</td>
</tr>
<tr>
<td></td>
<td>Fruits</td>
<td>56</td>
</tr>
<tr>
<td>Série B</td>
<td>Feuilles</td>
<td>78</td>
</tr>
<tr>
<td>Tomates</td>
<td>Fruits</td>
<td>78,2</td>
</tr>
<tr>
<td></td>
<td>Feuilles</td>
<td>88,2</td>
</tr>
<tr>
<td>Haricots</td>
<td>Fruits</td>
<td>88,4</td>
</tr>
<tr>
<td>DÉMÉTON-S</td>
<td>Feuilles</td>
<td>87</td>
</tr>
<tr>
<td>Tomates</td>
<td>et fruits</td>
<td>68,2</td>
</tr>
<tr>
<td>Salades</td>
<td>Feuilles</td>
<td>88,3</td>
</tr>
</tbody>
</table>

* Pourcentage de la radioactivité présente dans la phase chloroformique

s'agisse de surfaces foliaires d'espèces différentes ou bien que la comparaison porte sur les feuilles et les fruits d'une même espèce.

Dans nos conditions expérimentales, la nature de la surface traitée (feuille ou fruit) est donc sans influence pratique sur la vitesse de dégradation sauf peut-être chez le pois où l'on note une décomposition accrue à la surface du fruit.

Il y a une seule exception, semble-t-il; la disparition des résidus de déméton-S paraît retardée sur les feuilles de salade; ceci s'explique dans une certaine mesure par la disposition particulière du feuillage de cette plante qui protège l'insecticide de l'action des agents extérieurs.

Si nous comparons maintenant la vitesse de dégradation respectives des deux insecticides expérimentés, on constate que, pour une même plante, la tomate, les dépôts superficiels d'endothion sont un peu moins tenaces que ne le sont les métabolites actifs du déméton-S.

En l'absence de certaines conditions extérieures, plus abondante ou insolation intense, la détoxication des dépôts superficiels d'endothion ou
de démétion-S semble assez lente. Après 15 jours il reste encore 20 à 50% de l’endothion et 50 à 55% du démétion-S ; après trois semaines 30-40% du démétion-S sont encore présent sur le feuillage.

RÉMANENCE DES RÉSIDUS TOXIQUES DANS LE FEUILLAGE DES PLANTES TRAITÉES (tableau II)

La persistance de la toxicité chez les plantes traitées dépend avant tout de la nature chimique du pesticide qui conditionne l’existence et la nature de métabolites toxiques éventuels.
Or, nous avons déjà signalé, le schéma de dégradation de l’endothion est totalement différent de celui du démetion-S et ne comprend que des produits d’hydrolyse toxiques ; ceci se traduit pratiquement par une rémanence moins accentuée. C’est ce que l’on peut constater en comparant les résultats obtenus sur tomates pour ces deux insecticides. 15 jours après le traitement le démetion-S et ses métabolites toxiques constituent encore 66% des résidus phosphorés présents dans la plante tandis que 90% environ de l’endothion a déjà disparu.

**TABLEAU II**

RÉMANENCE De L’ENDOTHION ET DU DÉMETION-S DANS LES PLANTES TRAITÉES

<table>
<thead>
<tr>
<th></th>
<th>Plantes traitées</th>
<th>Temps écoulé avant le traitement et la récolte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 j</td>
</tr>
<tr>
<td><strong>ENDOTHION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feuilles (série A)</td>
<td>Tomates</td>
<td>41,9</td>
</tr>
<tr>
<td></td>
<td>Haricots</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Pois</td>
<td>55</td>
</tr>
<tr>
<td>Feuilles (moyennes)</td>
<td>Tomates</td>
<td>52,6</td>
</tr>
<tr>
<td></td>
<td>Haricots</td>
<td>30,7</td>
</tr>
<tr>
<td>Fruits (moyennes)</td>
<td>Tomates</td>
<td>58,1</td>
</tr>
<tr>
<td></td>
<td>Haricots</td>
<td>34,8</td>
</tr>
<tr>
<td><strong>DÉMETION-S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feuilles</td>
<td>Laine</td>
<td>93,2</td>
</tr>
<tr>
<td></td>
<td>Tomates</td>
<td>89,7</td>
</tr>
<tr>
<td>Fruits</td>
<td>Tomate</td>
<td>94</td>
</tr>
</tbody>
</table>

* Pourcentage de la radioactivité présente dans la phase chloroformique

DE

La nature de la dégradation d’un insecticide comme le sechradan se décomposant dans la plante, il y a une métabolisation analogue peut être 

Appliqué sur les 

Lentement. 21 jours après, la teneur n’atteint pas 15%. 

Pour l’endothion 

CHEZ LA TOMATE ET LE PERSISTANCE DES 

En premier lieu, le sechradan est aussi 

BLÉME était importé 

Il semble y avoir 

endothion et le démétion - 

Chez les plantes 

Voisine dans 

Dans les fruits 

Parable pendant les 

Suite à une détoxication 

Pour le démétion immédiatement qua 

Son maximum dans la 

Proportion de rés. 

_Feuilles traités : Fruits non traités ;

**VITESSE DE LES**

Temps écoulé le traitement et la récolte

<table>
<thead>
<tr>
<th>Feuilles traités</th>
<th>Fruits traités</th>
<th>Fruits non traités</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pourcentage
La nature de la plante traitée peut elle aussi, affecter la vitesse de dégradation d'un insecticide; BENNETT et THOMAS ont démontré que le schradan se décompose plus rapidement chez le haricot que dans le chrysanthème ou le coléus [2]. METCALF et autres ont constaté pour le di-syston une métabolisation accélérée chez la tomate [3]. Une observation analogue peut être faite pour le démétron-S et l'endothion.

Appliqué sur les feuilles de salades, le démétron-S se dégrade très lentement. 21 jours après le traitement le pourcentage de destruction ne dépasse pas 10%; durant le même temps il atteint 53% chez la tomate.

Pour l'endothion, la vitesse de métabolisation est à peu près la même chez la tomate et le petit pois, mais plus élevée chez le haricot.

**PERSISTANCE DES RÉSIDUS TOXIQUES DANS LES FRUITS**

En premier lieu, il convenait d'établir si la disparition des résidus toxiques était aussi rapide dans les fruits que dans les feuilles. Ce problème était important car pour trois des plantes étudiées le fruit constituait la partie consommable de la plante.

Il semble y avoir sur ce point précis une différence notable entre l'endothion et le démétron-S.

Chez les plantes traitées à l'endothion, la rapidité de la dégradation semble voisine dans le feuillage et les fruits traités.

Dans les fruits non traités, l'évolution des résidus toxiques est comparable pendant les premiers jours (tableau III, série C). On assiste ensuite à une détoxication extrêmement rapide.

Pour le démétron-S, la vitesse apparente de détoxication s'accélère immédiatement quand on passe des feuilles aux fruits traités; elle atteint son maximum dans les fruits n'ayant reçu aucun traitement. Après 14 jours la proportion de résidus toxiques est la suivante:

- Feuilles: 68%
- Fruits traités: 14%
- Fruits non traités: 7%.

**TABLEAU III**

**VITESSE DE DÉGRADATION* DES DEUX PESTICIDES DANS LES DIFFÉRENTES PARTIES DU VÉGÉTAL.**

<table>
<thead>
<tr>
<th>Temps écoulé entre le traitement et la récolte</th>
<th>Endothion (série C)</th>
<th>Démétron-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 j</td>
<td>32,9</td>
<td>21,4</td>
</tr>
<tr>
<td>10 j</td>
<td>30,7</td>
<td>23,7</td>
</tr>
<tr>
<td>15 j</td>
<td>27,6</td>
<td>26,3</td>
</tr>
</tbody>
</table>

* Pourcentage de la radioactivité présente dans la phase chloroformique
La feuille et le fruit étant des organes dont le métabolisme est différent, on conçoit fort bien que les vitesses de destruction d'un insecticide y soient des mêmes différentes.

Ce qui semble moins évident est la disparité que l'on constate entre fruits traités et non traités. Que l'insecticide parvienne par voie vasculaire ou à partir de l'épiderme ne change rien à sa vitesse de dégradation.

On est donc conduit à admettre que ce qui parvient au fruit par la voie vasculaire est surtout composé de produits d'hydrolyse voire même de restes phosphorés ou soufrés réutilisés par la plante et drainés vers les centres d'activité métabolique que constituent les fruits en croissance.

Pour le démétion-S ce transport de métabolites non extractibles par le chloroforme doit commencer très tôt, ce qui expliquerait la faible proportion de résidus toxiques retrouvés dès le septième jour et l'accélération du phénomène dans les fruits non traités (voir tableau III).

Au contraire, c'est l'endothion qui constitue l'essentiel des substances soufrées qui parviennent aux fruits durant les cinq premiers jours qui suivent le traitement ; puis l'insecticide est rapidement remplacé par des dérivés toxiques et dénaturés de pouvoir insecticide.

Comparons maintenant, pour chacun des insecticides, leurs vitesses de dégradation à la surface et à l'intérieur de la plante traitée.

Pour le déméthion-S, au niveau du feuillage, c'est le film superficiel qui se dégrade le plus rapidement ; au niveau des fruits, c'est la pollution interne qui disparait la première.

En ce qui concerne l'endothion dans nos conditions expérimentales, la dégradation est toujours plus rapide à l'intérieur de la plante qu'à l'extérieur.

Dans l'une des séries expérimentales nous avons étudié séparément la persistance de l'endothion dans les fruits mûrs ou encore verts.

Au 5e jour la détoxication s'est révélée beaucoup plus grande dans les fruits verts. Ce fait pouvant présenter une certaine importance au point de vue toxicologie, nous avons repris l'étude sur des broyats de fruits réalisés dans divers tampons allant de pH 6 à pH 8. Dans toutes les expériences réalisées le taux de dégradation du pesticide était très voisin pour les deux types de fruits.

**IMPORTANCE RELATIVE DES DÉPÔTS SUPERFICIELS, DE LA CONTAMINATION DIRECTE ET DU TRANSPORT INTERNE DANS LA POLLUTION DES PLANTES TRAITÉES**

Les modalités de la dénaturation du pesticide ne constituent évidemment qu'une donnée dans le problème des résidus. Nous allons maintenant en aborder l'aspect quantitatif. La technique de traitement que nous avons adoptée pour des raisons de sécurité n'est révélée assez imparfaite et il existe une irrégularité évidente entre les dépôts. Les comparaisons qui vont suivre ont donc été établies sur les moyennes de trois traitements.

**Feuillage**

Les dépôts foliaires d'endothion sont très importants, 150 à 270 ppm, soit environ 15 à 27 γ/cm² (à peu près quatre fois la dose normale). Cette saturation a été re

La teneur en nb

Ceci est sans : n'est pas consomm

**Dépôts superficiels**

**Résidus internes**

Même si l'on envisage par des interprétation rend celui-ci dans le traitement.

Les quantités expérimentales sont d'une application n'est toujours sensiblement peut être une consommation une valeur très gr

Un coefficient de p

Laiton chez ces végétaux particuli

**Fruits**

La teneur en r

Il est évident

Afin d'obtenir des résultats expérimentaux un abord de pr

De manière qu'une approche est largement héritée

Ces conclusions dans le colonne 6 de l'apport vasculaire

On constate que les fruits, pourrait xiques, assure 50 D coût que la pollitio
DEUX INSECTICIDES ENDOThÉRAPIQUES

La saturation a été recherchée afin d'accentuer au maximum la pénétration du produit. Dans ces conditions on retrouve dans le feuillage de 60 à 120 ppm de produits marqués.

Ceci est sans intérêt pour les tomates et les haricots dont le feuillage n'est pas consommé, mais on doit en tenir compte dans le cas des pois car le feuillage de cette plante est parfois utilisé (après cueillette en vert et battage mécanique) pour la nourriture du bétail. Le désherbant est amorti après 15 jours les résidus laissés à la surface et dans la plante, la contamination quinze jours après le traitement serait la suivante :

Dépôts superficiels : 
\[
\frac{153.8}{4} = 40 \text{ ppm} \times 33.3 = 13 \text{ ppm}
\]

Résidus internes : 
\[
\frac{121.2}{4} = 30 \text{ ppm} \times 14.2 = 4 \text{ ppm}
\]

Même si l'on envisage une réduction importante de la pollution superficielle par les intempéries, la quantité de pesticide présente dans le tissu foliaire peut être très dangereux pour l'alimentation du bétail deux semaines après le traitement.

Les quantités de désherbants déposées à la surface des deux plantes expérimentées sont assez réduites et correspondent sensiblement à l'apport d'une application normale. Pour la tomate le coefficient de pénétration est toujours sensiblement plus élevé que celui de l'endothion, mais ceci peut être une conséquence du caractère limité de l'application. Il atteint une valeur très grande lorsque le traitement est réalisé sur les salades. Un coefficient de pénétration aussi important joint à une détérioration très lente chez ce végétal rend imprompt l'emploi de cet insecticide dans ces conditions particulières.

Fruits

La teneur en résidus des fruits traités dépend à la fois de la contamination directe à partir de la surface et de l'apport interne, conséquence du caractère tétoxicité des pesticides.

Il est évidemment difficile d'avoir une idée exacte de l'importance relative de ces deux apports.

Afin d'obtenir un ordre de grandeur, nous avons utilisé un artifice expérimental en abritant un fruit sur deux de manière à ce qu'ils soient voisins et de préférence disposés sur la même « grappe ». Dans ces conditions nous avons admis que l'apport interne était comparable pour l'un et l'autre fruit, compte tenu du désherbant de leur masse respective. Ceci n'est d'ailleurs qu'une approximation, car la répartition d'un pesticide dans une plante est largement hétérogène.

Ces conventions étant admises, la comparaison des résultats portés dans les colonnes 8 et 9 du tableau IV nous permet de juger l'importance de l'apport vasculaire dans la contamination interne des fruits traités.

On constate que la totalité des substances marquées, drainées vers les fruits, pourrait, si elle était constituée uniquement de métabolites toxiques, assurer 50 à 70% de leur contamination interne. En fait, on s'aperçoit que la pollution réelle due à l'effet tétoxicité va en s'amoncelant ra-
pidement (tableau IV). Après 15 jours, elle peut être tenue pour négligeable pour toutes les plantes étudiées, qu'il s'agisse de l'endothion ou du déméton.

Il n'en est pas de même pour la contamination externe. Dans les conditions de nos essais, les dépôts superficiels constituent parfois la source de pollution essentielle des fruits traités. Nous avons pu l'établir pour l'endothion. Quelle que soit la plante que nous ayons traitée, cet insecticide persistait toujours plus longtemps à la surface qu'à l'intérieur du fruit; l'importance relative de la contamination externe croît donc avec le temps.

Ainsi chez la tomate elle représente 83% de la pollution totale au 9e jour et 91% après 15 jours.

En ce qui concerne le déméton, l'évolution est encore plus nette; toujours chez la tomate, la contamination externe assure 54% de la contamination totale au quinzième jour et 76% au vingtième jour.

Quel que soit d'ailleurs le pesticide employé, la quantité de résidus retrouvée dans les fruits de tomate paraît réduite, surtout si nous la comparons à celle présente dans les gousses de haricots et de pois (voir tableau IV, colonnes 8 et 9).

Ceci est lié, d'une part, à la faible contamination superficielle, due elle-même à la surface lisse de ce fruit et aussi à la dilution que subit l'apport interne; chez la tomate, les fruits constituaient, dans nos essais, une masse beaucoup plus importante que les feuilles (500 à 600%)

Inversément, la pollution élevée des gousses de pois et de haricots s'explique autant par une lourde contamination superficielle que par un apport interne notable dû à la masse assez réduite que les fruits représentent par rapport aux feuilles (voir tableau IV, colonne 14).

PÉNÉTRATION ET DIFFUSION DU DÉMÉTON-S ET DE L'ENDOTHION DANS LES POUSSES D'ASPERGES

A la suite d'essais réalisés dans le cadre de la lutte contre la mouche de l'asperge (Platynota poecilocera), une différence d'efficacité avait été remarquée en faveur de l'endothion.

En dehors de toute spécificité vis-à-vis de l'insecte lui-même, nous avons tenté de vérifier si l'efficacité de l'endothion ne pouvait être expliquée par une meilleure pénétration à travers l'écorce ou une diffusion plus importante dans la pousse d'asperge.

Dans ce but deux types d'expérimentations ont été réalisés:

1° Essais de « pénétration »: toute la surface de la pousse étant traitée, nous avons évalué:
   a) la pénétration dans la plante,
   b) la diffusion de la zone corticale vers le centre.

2° Essais de « diffusion »: dans ce type d'expérimentation seule la partie médiane de la pousse recevait l'insecticide. Nous avons ensuite suivi le passage de ce composé:
   a) vers le centre,
   b) vers les extrémités apicales et basales.
### Tableau IV

**Importance relative des dépôts superficiels, de la contamination directe et du transport interne dans la pollution des plantes traitées**

<table>
<thead>
<tr>
<th></th>
<th><strong>Feuillage traité</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th><strong>Fruits traités</strong></th>
<th></th>
<th></th>
<th></th>
<th><strong>Fruits non traités</strong></th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td><strong>Endothion</strong> (série A)</td>
<td></td>
<td></td>
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<tr>
<td>Tomates</td>
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</tr>
<tr>
<td>3,1</td>
<td>210 (4)</td>
<td>81</td>
<td>9</td>
<td>18</td>
<td>8,2</td>
<td>2,8</td>
<td>0,9</td>
<td>0,22</td>
<td>9</td>
<td>0,9</td>
<td>0,9</td>
</tr>
<tr>
<td>1,5</td>
<td>186</td>
<td>56</td>
<td>12</td>
<td>15</td>
<td>8,4</td>
<td>0,15</td>
<td>9</td>
<td>0,9</td>
<td>0,9</td>
<td>0,9</td>
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</tr>
<tr>
<td>0,5</td>
<td>112</td>
<td>64</td>
<td>11</td>
<td>16</td>
<td>13,9</td>
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<td>2</td>
<td>0,3</td>
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<tr>
<td>0,1</td>
<td>61</td>
<td>46</td>
<td>9</td>
<td>16</td>
<td>23</td>
<td>4</td>
<td>9</td>
<td>1,4</td>
<td>2,2</td>
<td>2,2</td>
<td></td>
</tr>
<tr>
<td><strong>Déméton</strong></td>
<td></td>
<td></td>
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<tr>
<td>Tomates</td>
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</tr>
<tr>
<td>3,1</td>
<td>475</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>12,7</td>
<td>0,92</td>
<td>2,6</td>
<td>0,1</td>
<td>2,5</td>
<td>0,012</td>
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</tr>
<tr>
<td>1,5</td>
<td>377</td>
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<td>5</td>
<td>5</td>
<td>12,7</td>
<td>0,8</td>
<td>2,4</td>
<td>0,12</td>
<td>2,4</td>
<td>0,017</td>
<td></td>
</tr>
<tr>
<td>0,5</td>
<td>1,5</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>11,2</td>
<td>0,38</td>
<td>0,5</td>
<td>0,12</td>
<td>0,5</td>
<td>0,008</td>
<td></td>
</tr>
<tr>
<td>0,1</td>
<td>1,27</td>
<td>9</td>
<td>11,2</td>
<td>11,2</td>
<td>0,4</td>
<td>0,14</td>
<td>0,1</td>
<td>11,2</td>
<td>0,1</td>
<td>0,01</td>
<td></td>
</tr>
</tbody>
</table>

1. \( I/T \) : contamination interne/contamination totale.
2. \( RT \) : radioactivité totale exprimée en %.
3. \( E.C. \) : extract chloroformique.
4. \( E.C. \) : extract chloroformique.
5. \( E.C. \) : extract chloroformique.
6. Poids des feuilles / Poids des fruits.

Page 205
De plus, pour le déméton seulement, nous avons complété ce travail en étudiant:

a) l'influence de l'addition d'un émulsifiant,
b) l'efficacité comparée de 3 émulsifiants (formule A et B).

L'endothion a toujours été utilisé avec l'adjuvant A.

Les modes opératoires étaient les suivants:
- l'expérimentation a été réalisée en serre,
- les traitements et l'évaluation des résidus ont été effectués selon les méthodes décrites dans l'essai précédent,
- les deux insecticides ont été utilisés à la dose de 1 mg/cc soit 100 g/hl,
- la durée de l'essai était de 48 h.

Résultats obtenus

1. Pénétration dans la plante (voir tableau V)

   a) Influence de l'émulsifiant (déméton seulement)

L'hétérogénéité des résultats de la série « sans émulsifiant » ne permet pas de conclure avec certitude. Il semble toutefois que l'adjuvant utilisé accroît la concentration de l'insecticide dans les plantes. La teneur importante (2,6 ppm) constatée dans un lot d'asperges traitées en l'absence d'adjuvant pourrait s'expliquer ainsi; ce lot étant constitué de pousses de faible section, le rapport surface/volume se trouvait donc augmenté, ce qui a entraîné un accroissement du taux de pénétration apparente.

**TABLEAU V**

<table>
<thead>
<tr>
<th>Pénétration de l'endothion et du déméton-s à travers l'écorce des pousses d'asperges.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Déméton-s</td>
</tr>
<tr>
<td>Émulsifiant B</td>
</tr>
<tr>
<td>Émulsifiant A</td>
</tr>
<tr>
<td>Sans émulsifiant</td>
</tr>
<tr>
<td>Endothion</td>
</tr>
<tr>
<td>Émulsifiant A</td>
</tr>
</tbody>
</table>

(1) Poids moyen de la partie traitée
(2) Concentration en insecticide (radioactivité totale mesurée dans la pousse, exprimée en équivalent d'insecticide)

2. Diffusion vers le

a) Influence du

L'addition de l'endothion est défavorablement l'étaler dans le tableau.

b) Influence de

Le même tableau peut être vers le cent

3. Diffusion vers le

a) Influence du

Les résultats de la diffusion vers la part de la partie basale de l'a
b) Influence de la nature de l'insecticide.

L'endothion semble pénétrer en quantité beaucoup plus importante que ne le fait le déméton. Qu'il s'agisse des essais de «pénétration» ou de «diffusion», les teneurs élevées en insecticide sont toujours en faveur de l'endothion.

### TABLEAU VI

<table>
<thead>
<tr>
<th></th>
<th>Teneur en insecticide</th>
<th>Taux de diffusion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Écorce</td>
<td>Centrale</td>
</tr>
<tr>
<td>DÉMÉTON-S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Émulsifiant B</td>
<td>46,78</td>
<td>5,44</td>
</tr>
<tr>
<td>Émulsifiant A</td>
<td>30,39</td>
<td>3,75</td>
</tr>
<tr>
<td>Sans émulsifiant</td>
<td>34,35</td>
<td>8,59</td>
</tr>
<tr>
<td>ENDOTHION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Émulsifiant A</td>
<td>116,90</td>
<td>36,80</td>
</tr>
</tbody>
</table>

* Radioactivité totale exprimé en équivalent insecticide.

2. Diffusion vers le centre de la pousse d'asperge (voir tableau VI)

a) Influence du mouillant (déméton seulement).

L'addition de l'un ou de l'autre des adjuvants expérimentés influence défavorablement la diffusion vers la zone centrale, comme on peut le constater dans le tableau VI.

b) Influence de la nature de l'insecticide.

Le même tableau met en évidence une diffusion plus marquée de l'endothion vers le centre de la pousse d'asperge.

3. Diffusion vers les extrémités non traitées (voir tableau VII)

a) Influence du mouillant (déméton seulement).

Les résultats sont comparables à ceux que nous avons observé pour la diffusion vers la partie centrale. C'est le déméton exempt d'adjuvant qui migre en quantité la plus importante.

La diffusion est d'ailleurs essentiellement dirigée vers le haut. La partie basale de l'asperge ne reçoit pratiquement rien.
### TABLEAU VII

**DIFFUSION DE L'ENDOTHION ET DU DÉMÉTON-S VERS LES EXTRÉMITÉS NON TRAITÉES.**

<table>
<thead>
<tr>
<th></th>
<th>Teneur en Insecticide</th>
<th>Taux de diffusion ° (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base non traitée (ppm)</td>
<td>Paroi traitée (ppm)</td>
</tr>
<tr>
<td><strong>DÉMÉTON-S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsifiant B</td>
<td>0,02</td>
<td>0,78</td>
</tr>
<tr>
<td>Emulsifiant A</td>
<td>0,06</td>
<td>0,72</td>
</tr>
<tr>
<td>Sans émulsifiant</td>
<td>0,00</td>
<td>0,47</td>
</tr>
<tr>
<td><strong>ENDOTHION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsifiant A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*° Établi sur les teneurs mesurées en valeur absolue.

**b) Influence de la nature de l'insecticide**

Dans la partie haute de la pousse, la teneur en endothion se montre toujours supérieure à la teneur en déméton. Ceci est dû à la concentration plus forte de l'endothion dans la totalité de la plante, car dans le cas présent c'est le déméton qui possède le taux de diffusion le plus élevé. L'endothion, comme le déméton, ne migre pratiquement pas vers la base des pousses.

4. **Dégradation des insecticides dans les différentes parties de la pousse** (voir tableau VIII)

Il convenait de s'assurer que les molécules marquées sillonnant la plante correspondaient effectivement à des produits actifs et non à des métabolites sans intérêt sur le plan insecticide. Ceci nous a amenés à suivre au cours de l'essai précédant les taux de dégradation dans les différentes parties de la pousse d'asperge.

Pour le déméton, le taux de dégradation reste assez faible mais croît malgré tout de l'écorce traitée vers l'intérieur et les extrémités. Dans le cas de l'endothion le taux de destruction de l'insecticide paraît légèrement plus élevé, mais il ne semble pas que la migration du produit s'accompagne d'une dégradation plus accentuée.

En résumé, la nature de l'émulsifiant stabilisateur ne semble pas modérer de façon notable la pénétration ou la diffusion du déméton. L'action la plus nette des deux formules d'adjuvants serait de réduire légèrement la diffusion de l'insecticide.

### CONCLUSION

Nous avons étudié les conditions de diffusion des plantes maraîchères lors de la plantation-S et l'endothion. En dehors de la zone de dépôt superficiel, l'action est souvent faible. La viabilité des feuilles et des fruits est également altérée. Par contre, ce résultat est particulièrement intéressant pour le cas du déméton.
**DEUX INSECTICIDES ENDOThÉRAPIQUES**

**TABLEAU VIII**

DÉGRADATION* DES DEUX INSECTICIDES DANS LES DIFFÉRENTES PARTIES DE L'ASPERGE

<table>
<thead>
<tr>
<th>Taux de diffusion * (%)</th>
<th>Partie traitée</th>
<th>Haut non traité</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Écorce</td>
<td>Centre</td>
</tr>
<tr>
<td><strong>DÉMÉTHON-S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emslifiant B</td>
<td>78,5</td>
<td>49,7</td>
</tr>
<tr>
<td>Emslifiant A</td>
<td>72,5</td>
<td>68</td>
</tr>
<tr>
<td>Sans émslifiant</td>
<td>62,7</td>
<td>67,5</td>
</tr>
<tr>
<td><strong>ENDOTHION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emslifiant A</td>
<td>64</td>
<td>58,5</td>
</tr>
</tbody>
</table>

| **ÉVAL < pénétration>** |                |                |                |
| **DÉMÉTHON-S**          |                |                |                |
| Emslifiant B             | 86,3           | 76,9           |                |
| Emslifiant A             | 80,4           | 63,5           |                |
| Sans émslifiant          | 83,9           | 66,0           |                |

* Dégénération exploitée en pourcentage de la radioactivité présente dans la phase chloroformique.

En dehors de toute spécificité vis-à-vis de l'insecte l'efficacité accrue de l'endothion pourrait donc s'expliquer par une concentration plus élevée dans les pousses d'asperges.

**CONCLUSION**

Nous avons étudié le problème des résidus toxiques laissés dans quelques plantes maraîchères par deux insecticides endothérapiques, le déméthon-S et l'endothion.

En dehors de toute précipitation atmosphérique, l'importance relative des dépôts superficiels et de la contamination interne évolue de façon différente selon l'insecticide, la plante, et pour une même plante selon qu'il s'agit de feuilles ou des fruits.

La voie vasculaire peut assurer une part importante de la contamination des fruits lorsqu'on utilise l'endothion.

Par contre ce type de pollution reste tout à fait secondaire dans le cas du déméthon.
Quinze jours après le traitement l'effet télétrotoxique de ces deux insecticides semble sans influence pratique sur le taux de contamination interne des fruits traités.

Dans les conditions de nos essais, la contamination interne décroît très lentement chez les salades traitées au déméton.

Par contre quel que soit l'insecticide utilisé, la pollution externe et interne des fruits de tomate reste faible.

La persistance de l'endothion dans les plantes traitées est inférieure à celle du déméton.-S.

L'efficacité plus grande de l'endothion dans la lutte contre la mouche de l'asperge (Platyparena poeciloptera) pourrait s'expliquer par une meilleure pénétration dans la poussée d'asperge.

L'absence de diffusion vers l'extrémité basale de la poussée, qui est l'endroit où les larves de 3e âge creusent leurs galeries, serait également en accord avec l'inefficacité des deux insecticides contre les larves ayant atteint ce stade.

REFERENCES


DISCUSSION

J. E. Casida: Did you subject any of your extracts to chromatographic studies?

M. Hascoët: In view of the large number of extractions which this work required it was not possible for us to carry out a chromatographic test in every case. We did, however, carry out this study for endothion, where only the non-degraded insecticide passes into the chloroform phase. Endothion has three degradation products. One of them, a monomethylated product, is now known. The two others we have not yet been able to identify exactly. One of them does not contain any phosphorus; they are probably hydrolysis products but they do not correspond to the products obtained by normal chemical hydrolysis.

Although these were the only degradation products we subjected to a chromatographic test, each insecticide was given a chromatographic test for chemical purity before being used.

L'ÉTABLISSEMENT ET DIFFUSION AU POPULUS L'ÉTABLISSEMENT ET DIFFUSION AU POPULUS
I'ÉTABLISSEMENT DES PROCESSUS D'ABSORPTION ET DIFFUSION DES INSECTICIDES SYSTÉMIQUES AU POPULUS x EURAMEICANA DODE GUINIER «ROBUSTA»

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INSTITUT DE RECHERCHES FORESTIÈRES DE BUCAREST, ROUMANIE

Abstract — Résumé — Anotarion — Resumen

ESTABLISHMENT OF THE PROCESSES OF ABSORPTION AND DIFFUSION OF SYSTEMIC INSECTICIDES IN POPULUS EURAMEICANA DODE GUINIER "ROBUSTA". The organophosphoric insecticides having systemic properties due to their ability to penetrate into the sap flow of plants set on insects which spend part of their lives either in the cambial region between wood and bark, or in the wood itself. Until the introduction of these new insecticides chemical control of xylophagous insects in trees—woods was almost impossible.

The mechanism of absorption, diffusion and concentration of systemic insecticides in species of poplar and willow, which are frequently attacked by xylophagous insects, was studied using labelled Dipexox on the poplar Robusta R20.

The insecticide was labelled in the reactor, using powdered Dipexox (1.5 g) as target, at a flux of \(10^{11} \text{n/cm}^2 \text{s}\) and at a temperature of 36-40°C. Irradiation was carried on until an absolute target activity of \(1 \text{ mc} \) was obtained.

The insecticide was administered, in the form of a solution, by pouring it on to the soil, except in one case in which the insecticide was sprayed on to the leaves.

One series of experiments was conducted in the laboratory using seedlings grown in pots, and another series was performed in a nursery, using one- and two-year-old saplings.

One to two months after administration of the labelled insecticide solutions the trees were subjected to radiochromatic tests.

In the laboratory experiments the insecticide accumulated in considerable quantities in the leaves, and in smaller amount in the wood.

Both in the nursery and in the nursery a greater amount of insecticide was found in the leaves.

In the nursery experiment, however, a considerable increase was observed in the concentration of the insecticide in the leaves and in the wood of the stem, especially at the extremities.

The mean accumulation of insecticide amounted to 3.50 mg/g green matter in the nursery as against 0.26 mg/g in pots.

It was shown that under field conditions, in dry weather, two-year-old robusta saplings take up Dipexox administered in an aqueous solution at the rate of 9.9% of the amount applied to the soil, as compared with 3.3% in laboratory experiments.

Application of solution to the leaves by spraying yields a low insecticide absorption, due both to the difficult process of uptake through the leaves and to the removal of the insecticide by air, dew and mist.

Very little insecticide was found on the ground and the authors therefore concluded that the method of administering systemic insecticide solutions through the soil should be adopted as a priority measure in the campaign against one—species pests.

L'ÉTABLISSEMENT DES PROCESSUS D'ABSORPTION ET DIFFUSION DES INSECTICIDES SYSTÉMIQUES AU POPULUS x EURAMEICANA DODE GUINIER «ROBUSTA» Les insecticides organophosphoriques à propriétés systémiques, par leur faculté de pénétrer dans le coeur du tronc des plantes, ont une action sur les insectes qui menacent leur vie soit dans la zone cambiale entre le bois et l'écorce, soit dans le bois. Jusque la apparition de ces nouveaux insecticides, la lutte chimique contre les insectes xylophages de la tige des arbres était presque impossible.

Pour étudier le mécanisme d'absorption, de diffusion et de localisation des insecticides systémiques en ce qui concerne les peupliers et les saules, expérimentalmen attaqués par les insectes xylophages, on a fait des recherches en utilisant le le Dipexox marqué sur les peupliers Robusta R20.
Le marquage de l'insecticide a été fait dans un réacteur, en utilisant comme cible le Diphterex en poudre (1,5 %), avec un fluo de 0,5 Tr/cm² et à une température de 50 à 60°C. L'irradiation a été effectuée jusqu'à l'obtention d'une activité absolue de la cible de 1 μc.

L'administration de l'insecticide a été faite par verse des solutions dans le sol, en utilisant des résidus de la marquée suite à l'appareil foliée.

Une série d'expériences a été faite en laboratoire, en utilisant des plantes cultivées en sol; une autre série a été faite en pépinière, avec des plants de 1 et 2 ans.

En un laps de temps de 1 à deux mois après l'administration des solutions d'insecticide marquée, les plants ont été nourris à l'analyse radionucléïde. Les résultats obtenus sont relatifs à la quantité de l'insecticide absorbee par les plantes et en plus petites quantités dans le sol.

Tant en laboratoire qu'en pépinière, l'insecticide a été accumulé en grandes quantités dans les feuilles et en plus petites quantités dans le sol. L'insecticide a été accumulé en plus grande proportion dans les feuilles que dans le sol, en ce qui concerne une augmentation importante des accumulations d'insecticide dans les rameaux et dans le sol de la tige, surtout au bout des plants, dans l'expérience en pépinière.

En général, l'accumulation d'insecticide a été de 1,5 mcg substance venue en pépinière, contre 0,24 mcg/g en sol.

Comme résultat que, dans les conditions de terrain, les plants de pêcher robusta de 2 ans, pendant le temps sec, peuvent mobiliser le Diphterex administré en solution aqueuse à raison de 3,7% de la quantité versée dans le sol, contre 1,32% dans les expériences en laboratoire.

L'administration des solutions aux feuilles par pulvérisation a rendu possible l'absorption de l'insecticide, grâce aussi bien au mécanisme difficile d'absorption que l'ajout de l'insecticide par l'eau de pluie, la roide et le broyéter Plante.

On a décelé une grande quantité d'insecticide sur le sol; c'est pourquoi les auteurs considèrent que la méthode d'administration des solutions d'insecticide systémiques dans le sol doit être adoptée en priorité dans la lutte contre les ennemis des arbres ligneux.

DÉTERMINATION DE L'ETAT DES PLANTES EN ŒUVRE DES INSECTICIDES SYSTÉMIQUES ET DU RAPPORT ENTRE CETTE DÉTERMINATION ET LA DÉTERMINATION DE L'INSECTICIDE PAR LA MÉTHODE DES ÉCLAIRAGES.

La marque du insecticide π = 10¹⁴ m c/s², a une cyan alcoolique de 1 000 cm

Salve en un experimento el suelo con soluciones insecticidas.

Parte de los experimentos es semillero y en las condiciones

Un metodo con propiedades

En los experimentos se acumuló en mayor proporción insecticida acumulado en las plantas.

Por regia general, en este experimento no se obtuvo la actividad de los insecticidas en las plantas. 

Si la solución se pone en contacto con el insecticida, se puede obtener un rendimiento de 10%. 

Como se ha observado que hacen contra los insectos que no son insecticidas por acción indirecta.

1. INTRODUCTION

Par leur propre fonction, les insecticides offrent une grande variété de plantes, les plantes détruisent le fonctionnement systémique des insectes vivants, évitant la propagation de dégâts ou des organes de la dégâts d'autres catégories des insecticides.

Les insecticides sont particulièrement utilisés dans des cas de maladies transférées par des plantes.
INSECTIČIDES SYSTÉMIQUES AU PEUPLIER 213

Ce modèle est `Dipexor en produisant une radiation α à l'effet éclairant, mais dans un cas où l'on a cultivé en pot une autre

*Oncocercis maraquai*, les insecticides marqués, les quantités dans les feuilles sont plus importantes que dans les rameaux.

La détermination de la quantité de l'insecticide dans les tiges est faite par une méthode de comptage de radioactivité. Le degré d'absorption de l'insecticide au lavage de l'insecticide est faible (less than 10% of the quantity washed down, which is why the technique is not used in isolation).

La détermination de l'insecticide est faite en un réacteur, contenant le Dipexor en poudre (1,5 g) à un flux de 24 000 n/cm²-s, à une température de 30-40°C. La préparation de l'insecticide a été obtenue par un procédé radioactif de l'insecticide.

Partie de la technique de laboratoire, les plantes cultivées en macettes, et les plantes en semis; et en conditions normales de terrain, le mélange de l'insecticide est obtenu par le mélange des plantes cultivées en laboratoire. 

En les expériences de laboratoire, l'insecticide a été accumulé en grandes quantités dans les plantes et en semis. En conditions normales de terrain, le mélange de l'insecticide est obtenu par le mélange des plantes cultivées en laboratoire. 

De toute façon, nous avons constaté que l'insecticide est absorbé relativement lentement, et que la quantité de l'insecticide absorbe dans les plantes est très faible. Comme on l'a observé dans le cas précédent, les insectes qui atterrissent sur les plantes sont préférés vermer dans le sol, mais les plantes de insecticides de même nature, ainsi que les plantes de insecticides de même nature.

1. INTRODUCTION

Par leur propriété de s'intégrer dans le courant de sève des plantes, les insecticides organophosphoriques à propriétés systémiques immuissent, d'une part, les plantes aux attaques des insectes nuisibles et, d'autre part, détruisent les insectes nuisibles dans les organes divers de la plante. La fonction systémique assure la pensée de l'insecticide dans les tissus vivants, évitant le danger de lavage de la pellicule d'insecticide sur la surface des organes de la plante. Aussi, on attend que la pensée, ainsi que le degré d'utilisation des insecticides, soient plus grands que dans le cas des insecticides qui sont administrées par pulvérisation ou arrosage.

Les insecticides à fonctions systémiques présentent une importance particulière dans la protection des forêts, parce que les espèces ligneuses sont attaquées par des insectes qui se localisent soit dans le bois, soit entre
l'écorce et le bois. La lutte contre ces insectes nuisibles est donc impossible avec des insecticides sans fonction systémique.

Parmi les espèces ligneuses cultivées dans la République Populaire Roumaine, ce sont les peupliers Euraméricains ainsi que les peupliers indigènes qui souffrent le plus des attaques d'insectes se localisant dans les bois. La Saperda populnea L., Paranthreneae tabaniformis Rott., Cryptorrhinchus lapathi L., etc. appartiennent aux insectes nuisibles les plus répandus. Les larves de ces insectes se développent dans le bois de peuplier. En détériorant les exemplaires attaqués elles compromettent les jeunes peuplements.

L'application des insecticides systémiques dans ce domaine est devenue plus qu'une nécessité. Mais, vu la difficultée de suivre la pénétration de l'insecticide dans la plante, les méthodes d'administration n'ont pas été exploitées scientifiquement, ce qui a empêché leur application sur une grande échelle.

La possibilité de marquer les insecticides systémiques avec des isotopes radioactifs ouvre une nouvelle perspective aux recherches dans ce domaine. Vu l'importance de la lutte contre les insectes nuisibles du peuplier et afin d'assurer un état phytosanitaire optimum des cultures de peupliers dans notre pays, on a entrepris une série de recherches pour étudier en détail l'efficacité des insecticides systémiques par rapport à la méthode d'administration aux insectes nuisibles, aux espèces et aux clones de peupliers et aux facteurs écologiques.

Les premières recherches qui font l'objet du présent mémoire ont comme but l'éclaircissement des processus d'absorption et diffusion du diptére marqué dans les plantes de Populus x euramericana Dode Guinier, cv. « robusta ».

2. MÉTHODES DE RECHERCHES

2.1. La conception de base des recherches entreprises par le Laboratoire d'isotopes radioactifs de notre institut, a été déterminée par le but précis des recherches en espèce, et par l'analyse des méthodes de travail utilisées jusqu'à présent.

Le degré de pénétration des insecticides systémiques dans le flux de la sève des espèces ligneuses a été établi d'après la mortalité des insectes qui attaquent les espèces respectives. Comme méthode d'administration de ces insecticides, on a eu recours soit à la pulvérisation fine sur les feuilles et les branches, soit à l'introduction de l'insecticide dans le sol. Parfois on a essayé le bandage de la tige avec de la sève imbibée d'une solution d'insecticide.

Dans les premières expériences effectuées dans notre pays en vue de déterminer l'efficacité des insecticides systémiques, on choisit comme méthode d'administration la pulvérisation d'insecticide sur les feuilles, considérant qu'une proportion importante s'intègre dans le courant descendant de la sève.

JEPPSON a recours à une méthode moins pratique mais plus efficace. Il a appliqué des bandages de sève imbibée avec metasystox d'une concen-
Les insecticides systémiques au peuplier

Le peuplier est donc imposant dans l'industrie forestière. L'intérêt de l'utilisation des insecticides systémiques au peuplier est de taille, en particulier dans les régions où les peupliers sont le plus répandus. En effet, les pesticides systémiques permettent de traiter les jeunes peupliers de manière régulière et efficace.

Le peuplier a été largement utilisé en agriculture, en particulier pour la lutte contre les maladies et les ravageurs. En effet, les insectes systémiques ont permis de lutter efficacement contre les ravageurs des peupliers, en particulier les larves de la mélèze et de la mélèze attaquées par le Taeniophis laricivorus Krat [1].

Les méthodes ont permis la détermination de l'efficacité de l'insecticide basé sur les observations faites sur la mortalité des insectes et les différences de croissance enregistrées par les exemples des variétés traitées avec des insecticides systémiques et celles non traitées (contrôle).

Les progrès réalisés dans le domaine du marquage des molécules de divers composés chimiques, ainsi que la présence du phosphore, du soufre et du carbone dans la molécule des insecticides systémiques, ont permis aisément le marquage des insecticides organophosphoriques.


En général, les recherches dans ce domaine ont pour objet les cycles métaboliques des insecticides systémiques dans les plantes et dans l'organisme des insectes, poursuivis à l'aide des composés marqués aux isotopes radioactifs et mis en évidence par chromatographie radiochimique [2, 3, 4, 5].

2.2. Les recherches entreprises par notre institut ont le but d'éclaircir tout d'abord la quantité d'insecticides systémiques qui s'accumule dans les plantes de Populus x euramericanus, cv. «robusta». On a utilisé du diterex marqué au réacteur nucléaire de l'Institut de physique atomique de l'Académie de la République Populaire Roumaine.

On a obtenu du diterex radioactif par marquage du phosphore, avec une activité spécifique initiale de 1 mc/g. On a effectué les expériences, soit seulement avec l'insecticide marqué, soit avec l'insecticide marqué auquel on a ajouté l'insecticide stable, par rapport aux concentrations établies. On a travaillé d'habitude avec des concentrations de 1-2% dans une solution aqueuse.

On a administré les solutions de diterex dans la première dilution (10 µc/ml) dans le sol à la racine des plants de peuplier, on a poussé une partie dans de vases de végétation, et une autre partie dans la pépinière. Pour la mobilisation de l'insecticide par les plantes, on a assuré une percolation intense du sol avec de l'eau.

On a observé le degré d'absorption de l'insecticide à l'aide de l'intensimètre «Luci» portable.

Au moment où les mesures radiométriques indiquaient une activité maximale à la cime des plantes, on a enlevé celle-ci pour préparer des échantillons afin de procéder à des mesures exactes à l'aide du dispositif de comptage BSP, avec compteur G-M, type MST-17, à la fenêtre mica de 5 mg/cm².

L'activité des échantillons prélevés en diverses parties de la plante a été déterminée à l'aide de l'étudiant calcinés. Les résultats des mesures permettent de déterminer avec une précision satisfaisante la diffusion du diterex dans diverses parties de la plante, la localisation et la quantité de l'insecticide absorbé par rapport à la quantité administrée.
3. RÉSULTATS DES RECHERCHES

Les recherches ont été effectuées en deux étapes, à savoir, une étape au laboratoire et une étape sur le terrain. Les résultats obtenus diffèrent évidemment surtout en ce qui concerne la quantité de diptérex absorbé du sol par les plants de peuplier.

3.1. Les expériences de laboratoire ont été effectuées avec des plants âgés d'une année, plantés dans de vases de végétation Mitscherlich avec une capacité de 20 l. Dans les vases on a utilisé un sol alluvionnaire à texture sablonneuse, riche en humus.

Les solutions de diptérex marqué ont été administrées à la racine des plants, en concentration de 1,5%. L'activité spécifique des solutions a été $R_o = 5340$ cpm/ml. L'activité totale sur la plante a été de $5,34 \times 10^8$ cpm.

On a vérifié périodiquement l'accroissement de l'activité dans les feuilles, et 30 jours après l'administration de la solution de diptérex marqué on a prélevé des échantillons de bois et de feuilles soumis ultérieurement aux mesures radiométriques, à l'état calciné.

Les déterminations faites montrent que le diptérex marqué a été absorbé par les plants et qu'il est distribué différemment dans le bois et dans les feuilles, sur les secteurs de 20 cm mesurés sur la tige.

L'activité totale (cpm) est plus grande dans les secteurs dans lesquels la masse ligneuse ou la masse de feuilles est plus grande et en général décroît vers la tête des plants (tableau 1).

En échange, l'activité spécifique exprimée en cpm/g (cendre), montre une accumulation plus intense de l'insecticide dans les racines et dans la moitié supérieure de la tige, surtout aux 3/5 de la hauteur. Dans les feuilles l'activité spécifique en cpm/g (cendre) est distinctement plus grande que dans le bois et accumule une quantité plus grande d'insecticide aussi aux 3/5 de la hauteur de la plante et vers le bout de la plante. On remarque

**TABLEAU I**

<table>
<thead>
<tr>
<th>Partie de la plante</th>
<th>La hauteur à partir du sol (cm)</th>
<th>0-20</th>
<th>20-40</th>
<th>40-60</th>
<th>60-80</th>
<th>80-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feuilles</td>
<td>-</td>
<td>723</td>
<td>1519</td>
<td>854</td>
<td>852</td>
<td>-</td>
</tr>
<tr>
<td>Bois</td>
<td>254</td>
<td>184</td>
<td>274</td>
<td>127</td>
<td>57</td>
<td>-</td>
</tr>
<tr>
<td>Boutures</td>
<td>-</td>
<td>1990</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Racines</td>
<td>-</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

La variation de l'activité racine des plants de peuplier

A une tendance de pola avec la présence de l' (fig. 1).

Dans le bois de l et dans les feuilles accumulation d'insecticide que dans le bois. Il en considération les parce que le contenu entre 8-9%.

Il résulte des par les plants de P la mobilisation de l'est due, d'une part, septicide dans l'eau du laboratoire, qui plantes.

De la dernière le bois est de 0,172 verte.

3.2. Les expéditions sorption et la diffusion de la même manière que ces processus ;

L'expérience a travaillé est semblable

La solution actifactivité $R_{o1} = 22 300$ nister $R_{o2} = 566$ cpm exemplaire de peupl deur que dans le cas
une tendance de polarité du dipéterex marqué vers les extrémités de la plante, avec la présence d’un troisième maximum aux 3/5 de la hauteur des plantes (fig. 1).

Dans le bois de la tige, 1° activité varie entre 1500-3800 cpm/g (cendre) et dans les feuilles entre 2400-7600 cpm/g (cendre). Par conséquent, l’accumulation d’insecticide dans les feuilles s’avère être deux fois plus grande que dans le bois. Les différences sont encore plus grandes si l’on prend en considération les valeurs de l’activité rapportée à la substance sèche, parce que le contenu en cendre du bois varie entre 3-4°, et celui des feuilles entre 8-9°.

Il résulte des expériences de laboratoire que l’absorption du dipéterex par les plantes de P. robusta représente 1,34° de la quantité administrée; la mobilisation de l’insecticide en proportion réduite dans ces expériences est due, d’une part, à la percolation intense du sol et à la présence de l’insecticide dans l’eau d’arrosage et, d’autre part, aux conditions écologiques du laboratoire, qui ne permettent pas une activité physiologique intense des plantes.

De la dernière analyse il ressort que l’accumulation d’insecticide dans le bois est de 0,172 mg/g., et dans les feuilles de 0,373 mg/g. substance verte.

3.2. Les expériences sur le terrain montrent qu’en général, l’absorption et la diffusion du dipéterex dans les plantes de P. robusta se produisent de la même manière que dans l’expérience de laboratoire, à la différence que ces processus sont beaucoup plus intensifs. L’expérience s’est déroulée avec la même hypothèse et la méthode de travail est semblable à celle utilisée dans les expériences de laboratoire. La solution active de dipéterex dans la première dilution (d1) a eu une activité R01 = 22 200 cpm/ml. A chaque exemplaire de peuplier on a administré R6 = 888 cpm/ml. L’activité totale de la solution administrée à un exemplaire de peuplier a été de 6,66·10° cpm, donc du même ordre de grandeur que dans le cas de l’expérience de laboratoire.
Au diptérex marqué on a ajouté aussi le diptérex stable de telle manière que la concentration de la solution d'inchécidex fut de 1%. L'activité du diptérex était de 6,6 \cdot 10^{4} \text{ cpm/g (diphtérex)}.

On a observé le progrès de l'absorption de l'insecticide et lors d'une accumulation d'activité facilement measurable on a prélevé les échantillons pour les mesures radiométriques.

L'activité de la substance, à l'état calcifié, montre le même phénomène de polarité dans l'accumulation du diptérex marqué que dans le cas de l'expérience de laboratoire. Dans la tige l'activité maximum apparaît aux 3/5 de la hauteur et est de 11 340 \text{ cpm/g (cendre)}, dans les rameaux aux 2/5 et au sommet avec des valeurs de 28 400 et de 23 100 \text{ cpm/g (cendre)}, respectivement, dans le pétiloe des feuilles aux 4/5 et 1/5 avec des valeurs de 13 200 et de 7 400 \text{ cpm/g (cendre)}, respectivement, et dans les feuilles aux 2/5 et au bout avec des valeurs de 11 250 et 7 870 \text{ cpm/g (cendre)}, respectivement (tableau II).

**TABLEAU II**

LA VARIATION DE L'ACTIVITÉ EN \text{ cpm/g (CENDRE)}

DANS LES DIVERSES PARTIES DES PLANTS DE PEUPLIER TRAITÉS AU DIPTELEX MARQUÉ

(Expérience de terrain)

<table>
<thead>
<tr>
<th>La hauteur ( \text{à partir du sol (cm)} )</th>
<th>Parties de la plante</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bois</td>
</tr>
<tr>
<td>0-50</td>
<td>14 000</td>
</tr>
<tr>
<td>50-100</td>
<td>25 000</td>
</tr>
<tr>
<td>100-200</td>
<td>34 000</td>
</tr>
<tr>
<td>200-300</td>
<td>40 000</td>
</tr>
</tbody>
</table>

En comparaison avec les mêmes valeurs trouvées dans l'expérience de laboratoire, les plans de peupliers élevés dans des conditions de terrain en pleine saison de végétation mobilisent l'insecticide dans une proportion 3-4 fois plus grande sur l'unité de masse.

Contrairement à l'expérience précédente, on constate une augmentation de la radioactivité dans le bois de la tige et les rameaux qui dépasse les valeurs enregistrées dans les feuilles, phénomène inverse à celui enregistré auprès des plans en pleine croissance dans des conditions de laboratoire. Il en résulte que les insecticides systématiques diffusent aussi dans de divers organismes des plantes, selon la même loi que la sève élaborée et en concordance parfaite avec les composés de biosynthèse. Dans la première phase d'accroissement, au début de la saison de végétation, le diptérex s'accumule plus fortement dans les feuilles, et dans la seconde partie de la saison de végétation dans la pratique.

L'accumulation de deux ans se progresse par rapport au feuillage.

En unité de masse, la quantité de diptérex a augmenté approximativement dans le bois et les rameaux de l'appareil végétatif administré dans la lutte contre ce cas, il est utile qui fait relevé d'ailleurs en pérearileges.

On a observé que les peupliers avec 34 et les larves dans le bois ont été détruites, que pour le laboratoire, on a observé les plantes auxquelles on a procédé au levant de ces cas. On a relevé donc ceci. Il résulte donc tice systématiques en conditions météorologiques par la vigueur des conditions de la région.

4. CONCLUSIONS

4.1. L'utilisation et de la circulation systématiques dans le bois.

4.2. Les plans de peuplier administrés dans le temps des insectes divers et insectes nuisibles.

4.3. Dans les conditions de croissance, l'absorption de l'insecticide administré est absente.

4.4. Dans la protection de l'insecticide, dis que dans la sécurité du bois augmente d'utilisation.
de telle manière que l'activité du insecticide et lors d'une même phrase, les échantillons dans le cas de l'ex- même phénomène apparaît aux 3/5 péril de l'insecticide dans les feuilles de 0,9 g, donc, au total, approximativement 2 g des 10 g administrées à une plante.

L'absorption d'environ 20% du dipterex par plants de P. robusta au mois d'août de l'année 1962 montre que l'utilisation des insecticides systémiques administrés dans le sol peut donner des résultats remarquables dans la lutte contre les insectes. Nous considérons cependant que, dans ce cas, il est utile d'augmenter la concentration des solutions jusqu'à 5%, fait relevé d'ailleurs aussi par JEPPSON [1] dans un autre genre d'expériences.

On a observé à la suite de l'infestation artificielle intense des plants de peuplier avec Saperda populnea, les plants ont été faiblement attaqués et les larves dans le bois sont mortes. La quantité d'insecticide administrée dans le bois a été de 1,81 mg/g et dans les feuilles de 2,18 mg/g de substance verte, presque plus grande donc que dans le cas de l'expérience de laboratoire. On a observé également une faible absorption du dipterex chez les plants auxquels on a administré l'insecticide par voie extra-radiculaire, grâce au long processus d'absorption par les feuilles ainsi qu'au lavage de celles-ci par les eaux de pluies.

Il résulte donc que les processus d'absorption et diffusion des insecticides systémiques sont déterminés dans une mesure appréciable par les conditions météorologiques, par l'intensité des processus physiologiques et par la vigueur des cultures de peupliers.

4. CONCLUSIONS

4.1. L'utilisation des tracers radioactifs permet l'étude de l'absorption et de la circulation des insecticides organo-phosphoriques aux fonctions systémiques dans le système sol-plante.

4.2. Les plants de P. robusta absorbent, dans les limites convenables, le dipterex administré dans le sol, et la localisation de l'insecticide dans les organes divers peut assurer une immunité de la plante envers les divers insectes nuisibles.

4.3. Dans les conditions de laboratoire et dans la première phase de croissance, l'absorption de l'insecticide est plus faible, tandis que dans les conditions de terrain en cultures intensives, environ 20% de la quantité administrée sont absorbées.

4.4. Dans la première partie de la saison de végétation, l'accumulation de l'insecticide est plus intense dans les feuilles que dans le bois, tandis que dans la seconde partie de la saison, l'accumulation de celui-ci dans le bois augmente d'une manière considérable (dans une plus grande proportion).
4.5. On remarque un phénomène distinct de polarité dans la diffusion de l'insecticide dans les plantes. Toutefois, il apparaît une zone d'accumulation intense de l'insecticide dans la partie inférieure de la couronne des plantes, tant dans les feuilles que dans le bois, plus prononcée dans le tiers moyen de la tige des plantes.

4.6. Les aspects concernant la stabilité du dipterex et des autres insecticides systémiques constituent l'étape nouvelle des recherches entreprises, et l'établissement de concentrations optima à administrer dans le sol est un problème que l'on est en train d'étudier. Bien que les dates obtenues jusqu'à présent tendent à montrer que certains insecticides systémiques se décomposent après 19-24 jours [2], nous considérons que ce problème doit être encore étudié, surtout dans l'hypothèse de l'administration des solutions de l'insecticide dans le sol. Le phénomène doit être relié surtout au pH de la solution du sol et de la phase liquide contenue dans les plantes.

4.7. Les préoccupations concernant l'utilisation sur une large échelle des insecticides systémiques dans la sylviculture pratiques sont en pleine développement, et nous considérons que la protection des forêts offre le plus important domaine d'application de ces substances.

RÉFÉRENCES


DISCUSSION

J. E. CASIDA: Dipterex is known to be a fairly good systemic in certain trees. It is also somewhat unstable biologically under these circumstances - the period of stability can be estimated at 2 to 10 days, on the basis of bioassays not restricted by localization. I am wondering if this might have some effect on the interpretation of your results. In other words, do you know for sure that you are analysing the active insecticide? Was any attempt made to ascertain that the radioactivity measured was due to Dipterex per se, and not to non-insecticide metabolites?

C. H. SCHMIDT: Yes, I also wanted to ask this question.

I. CATRINA: As regards the period of stability of Dipterex, the research carried out in my country has indicated a rather longer period, i.e. about 30 days. Research work on the problem is still going on. You are certainly quite right, Dr. Casida, in saying that Dipterex decomposes, especially when it is applied topically to plants. The main cause of this phenomenon is the ultra-violet ray effect. In the case of injection of Dipterex into the soil, however, this particular phenomenon is eliminated.
There is no doubt that the radioactivity we measured was due to the Dipterex itself. The biological test used showed it to be Dipterex and not non-insecticide metabolites. The absorption rate was too rapid, the activity appeared too soon for it to have been otherwise. The dead larvae in the wood and the mortality among larvae fed on the leaves suffice to show the presence of Dipterex itself.

G. F. BURNETT (Chairman): It seems to me you are using very large amounts of Dipterex, 10 g to a very small tree. Your 20% utilization is high, and I assume in practice you would use much smaller quantities. Have you considered that in such a case the relative distribution of Dipterex in wood and leaves may be different and fall below the effective concentration in the wood? Are you pursuing any studies along this line to see whether, say 1 g of Dipterex you will get the same type of distribution throughout the tree?

I. CATRINA: The 10-g dose which we used for a two-year-old sapling of Populus robusta was not too large because the insecticide was administered into the soil. We have not studied dosage from the practical point of view. That is for the future.

T. SAITO: Do you do purification of chemical assays of used radioactive Dipterex?

I. CATRINA: After labelling we do no purification or chemical assays of Dipterex because we receive the radioactive insecticide from a radiochemical laboratory as pure labelled Dipterex, and its purity is guaranteed. I might add that paper-chromatographic studies made at the Institute of Atomic Physics of the Romanian Academy of Sciences aim at establishing the quality of labelled insecticides as we use them in our experiments.
RADIOTRACER APPROACH

status one of the major group approaches with radiotracer et methylcarbamates with methyl of these compounds presumal Reaction of carboxyl- or met allow a critical examination reactivation or decarbamyl
examined by administering nervous tissue, or by stilling of cholinesterase inhibition enzymes during analysis. So metabolism of carbamate is seen along with its potential hydrolysis and then further de
decarboxylation mechanism in carbamates by the microsomes is rapidly metabolised in mac
the metabolites appear in the of the N-methyl derivative. carbamyl-3-methylpyrazolyl-
oxidative attack forms N-met these deconjugation reactions this group of insecticides, and into plants is probably also oxl mechanism have not yet been

EMPLOI DES RADIOISOTOPÉS DES Carbamates. Les méthyl-
indicateurs permettent d'étudier des dimethylcarbamates ou un
14C les groupes méthyle et car
bien de l'inhibition de
de la cholinésectase ou d'autre méthyle permettrait de faire u
ont la réactivation ou décar
cholinésectase peut être due
de l'acétylcoline marquée des pour déterminer in vitro le dé
inhibiteur minimum des inhibite
problème dans l'étude des métabol
états de ο-naphtyle méth
thèse selon laquelle le méthabol
products d'hydrolyse, n'a pa
et probablement aussi chez les
carbamates par les microsomes
RADIOTRACER APPROACHES TO CARBAMATE INSECTICIDE TOXICOLOGY

J. E. CASIDA
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Abstract — Résumé — Anotación — Resumen

RADIOTRACER APPROACHES TO CARBAMATE INSECTICIDE TOXICOLOGY. Methylcarbamates constitute one of the major groups of insecticides. Many unsolved problems in their toxicology may be readily approached with radiotracer studies. Dimethylcarbamates have been prepared with carbonyl-C14-labeling and methylcarbamates with methyl-, carboxyl- and ring-labeling utilizing carbon-14. The pharmacological action of these compounds presumably results from acetylcholinesterase inhibition and may involve carboxylation. Reaction of carboxyl- or methyl-labelled carbamates with purified cholinesterase or other enzymes would allow a critical examination of this carboxylation reaction and the ease of spontaneous and induced reactivation or decarboxylation. The physiological significance of cholinesterase inhibition might be examined by administering acetyl-C14 and analysis for radiolabelled acetylcholine accumulation in nervous tissue, or by utilizing acetyl-C14-choline as the substrate for in vitro determination of the degree of cholinesterase inhibition in tissues of poisoned animals with minimal dilution of the inhibitors and enzymes during analysis. Some progress has been made with radiolabelled materials in investigating the metabolism of carbamate insecticides. Sevin (1-naphthyl methylcarbamate) has been most extensively studied along with its potential hydrolysis products. The assumption that the metabolism of Sevin involves an initial hydrolysis and then further decomposition of the fragments was not supported by carbon-14 studies. The major detoxification mechanism in mammals, and probably also in insects, results from initial oxidative attack on the carbamate by the microsomes in the presence of reduced nicotinamide-adenine dinucleotide phosphate. Sevin is rapidly metabolized in mammals, but the fate of certain of the fragments has not been resolved. Some of the metabolites appear in the milk of lactating animals. One step in the metabolism appears to be formation of the N-methyl derivative. Preliminary studies on the metabolism of radiolabelled Dimethan (3-dimethylcarbamyl-3-methylpyrrolidino-(2)-dimethylcarbamate) and a related compound in cockroaches also indicate that oxidative attack forms N-methyl N-methyl derivatives. Much remains to be done on the relationship of these detoxification reactions to the resistance mechanism, the action of synergists, the selective toxicity in this group of insecticides, and the role and significance of residues. Metabolism of Sevin following injection into plants is probably also oxidative rather than hydrolytic, but the nature of the products and the enzymatic mechanism have not yet been established.

EMPLOI DES RADIONUCLIDES POUR L'ÉTUDE DE LA TOXICOLOGIE DES INSECTICIDES À BASE DE CARBAMATES. Les méthylcarbamates constituent l'un des principaux groupes d'insecticides. Les radionuclides permettent d'étudier facilement de nombreux problèmes que pose leur toxicologie. On a préparé des dimethylcarbamates en marquant le groupe carbonylé par 14C, et des méthylcarbamates en marquant par 14C les groupes méthyle et carbonylé et le noyau. L'action pharmacologique de ces composés réside vraisemblablement dans l'inhibition de l'acétylcholinestérase et peut impliquer une carboxylation. La réaction, avec la cholinesterase ou d'autres enzymes purifiées, des carbamates dont on a marqué le groupe carbonylé et méthylé permettrait de faire une étude critique de cette carboxylation et de la faciliter avec laquelle on procéderait à sa réactivation ou décarboxylation, spontanée ou induite. L'importance physiologique de l'inhibition de la cholinestérase peut être étudiée en administrant de l'acétylcholine marquée par 14C et en analysant l'acétylcholine marquée dans le tissu nerveux, ou bien en utilisant de l'acétyl-14C-choline comme substrat pour déterminer in vitro le degré d'inhibition de la cholinestérase dans les tissus d'animaux traités, avec une dilution minimum des inhibiteurs et des enzymes pendant l'analyse. Les produits marqués ont permis certains progrès dans l'étude du métabolisme des insecticides à base de carbamates. On a étudié de manière très approfondie le Sevin (1-naphthyl méthylcarbamate), en même temps que ses produits d'hydrolyse dérivés. L'hypothèse selon laquelle le métabolisme du Sevin implique une hydrolyse initiale, puis une nouvelle décomposition des produits d'hydrolyse, n'a pas été confirmée par les études faites avec le carbamate. Chez les mammifères et probablement aussi chez les insectes, la détoxication résulte principalement d'une oxydation initiale du carbamate par les microsomes en présence de phosphate de nicotinamide adénine dinucléotide réduit. Le
metabolización del Sevin es rápida con las marimbitas, pero no en la de otras especies. Los métodos habituales de investigación no permiten determinar el valor exacto del metabolismo de esta sustancia en los insectos. La metodología utilizada puede ser insuficiente para evaluar el efecto de los metabolitos en los insectos. Es necesario realizar estudios adicionales para determinar la toxicidad de los metabolitos en diferentes especies de insectos.

INTRODUCTION

Insecticidal ear during the past decade 3-methylpyrazolyl-(1-
Dimetilan (2-dimethyl compound VII of Fig Sevin (1-naphtyl m-
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1. RADIOSYNTHETE

The insecticidal methylcarbamates of radioactivity are methylcarbamates:

1. Via the carbamy

COC14+2(C}
INTRODUCTION

Insecticidal carbamic acid esters have been extensively investigated during the past decade. Two dimethylcarbamates, Isolan (1-isopropyl-3-3-methylpyrazolyl-5)-dimethylcarbamate, compound VIII of Fig. 1) and Dimetilan (2-dimethylcarbamyl-3-methylpyrazolyl-5) dimethylcarbamate, compound VII of Fig. 1), are in limited use, and one monomethylcarbamate, Sevin (1-naphthyl methylcarbamate, compound I of Fig. 1), is extensively utilized for pest control. Several other methylcarbamates have been thoroughly field-tested for insecticidal activity, and their residual persistence and toxicity are now being evaluated. Recent reviews have considered the development, mode of action and present status of these carbamates [1-6].

investigations of certain aspects of the action of the carbamate insecticides, particularly those dealing with their detoxification mechanisms, are greatly facilitated by radiotracer approaches. Although few such studies have been reported, many are now in progress. An attempt will be made to review the information obtained to date from such studies, and to indicate where tagged molecules might further assist in clarifying unresolved or incompletely elucidated facets of the mode of action of the carbamate insecticides. 

1. RADIOSYNTHESIS

The insecticidal carbamates of current interest are all methyl- or dimethylcarbamates of substituted phenols or heterocyclic enols. Two routes of radiosynthesis are obvious for the dimethylcarbamates and three for the methylcarbamates:

1. Via the carbamyl chloride

\[
\text{COC}_2\text{H}_5 + 2 (\text{CH}_3)(\text{R})\text{NH} \rightarrow (\text{CH}_3)(\text{R})\text{NC}(\text{O})\text{Cl} + (\text{CH}_3)(\text{R})\text{NH} \cdot \text{HCl}
\]

\[
\text{ROH or RONa} \downarrow
\]

\[
(\text{CH}_3)(\text{R})\text{NC}(\text{O})\text{OR} + \text{HCl or NaCl}
\]
2. Via the chloroformate

\[
\text{COC}_2\text{H}_4\text{OH} + \text{HCl} \rightarrow \text{ClCOC}_2\text{H}_4\text{OH} \quad 2(\text{CH}_3(\text{R})\text{NH}) \rightarrow \text{(CH}_3(\text{R})\text{NC})_2\text{OH} + (\text{CH}_3(\text{R})\text{NH})\text{HCl}
\]

2. Via the isocyanate

\[
\text{CH}_3\text{NCO} + \text{ROH} \rightarrow (\text{CH}_3(\text{R})\text{NC})_2\text{OH}
\]

The first route has been used by Dr. D. P. Ryskiewich (Geigy Chemical Corporation, Ardsley, New York) to prepare carbonyl-\(^{14}\text{C}\)-dimethylcarbamyl chloride in almost quantitative yields. A total of 3 mM of phosgene-\(^{14}\text{C}\) in 3.6 ml dry benzene was transferred to a 35-ml reaction flask equipped with a condenser, drying tube and magnetic pellet in an ice bath. A total of 0.6 mM of dimethylamine in 6.6 ml dry benzene was added with stirring in 1-ml portions at 10-min intervals. The resulting slurry was then stirred at room temperature for one hour. The benzene solution of carbonyl-\(^{14}\text{C}\)-dimethylcarbamyl chloride was separated from the insoluble dimethylamine hydrochloride by filtration and used directly by Dr. Ryskiewich in the preparation of the dimethylcarbamates, Dimetal (VII) and Isolan (VIII). By substituting anhydrous methylamine for the dimethylamine, it should be possible to prepare carbonyl-\(^{14}\text{C}\)-methylcarbamyl chloride under similar reaction conditions. This reaction could also be used to prepare the methyl-\(^{14}\text{C}\)-or methyl-\(^3\text{H}\)-labelled methylcarbamyl chlorides by providing a means of regenerating the amines from the chlorohydrocarbons for completion of the reaction of methyl-\(^{14}\text{C}\)-amine or methyl-\(^3\text{H}\)-amine with phosgene. Reaction conditions suitable for the final coupling to form the carbamate ester have been reported [9-15].

The second route has been used for radiolabelling carbamates of pharmacological interest but not for the methylcarbamate insecticides. For example, in the preparation of 1-ethyl- "cytochrome synthase" carbamate-carbonyl-\(^{14}\text{C}\), the reactions were carried out in collidine at -10°C without isolation of the intermediate chloroformate [16].

Sevin (1) has been prepared by the reaction of 1-naphthol-1-\(^{14}\text{C}\) with methyl isocyanate [17, 18]. Detailed conditions for this reaction conducted in a solution of 3% pyridine in dry benzene at 80°C for 5 h have been reported (17). The product after recrystallization from hot p-xylene was recovered in 82% yield and characterized by melting point and isotopic dilution analysis. Methyl isocyanate-\(^{14}\text{C}\) has also been prepared and reacted to yield labelled methylcarbamate insecticides [19] using the following reaction sequence and conditions adapted from reported procedures for non-labelled materials [20, 21]:

\[
\text{CH}_3\text{C}(\text{O})\text{Cl} + \text{NaN_3} \quad \text{140°C, 2 h} \quad \text{CH}_3\text{NCO} + \text{NaCl} + \text{N}_2
\]

\[
\text{100°C, 2 h, ROH, (C}_6\text{H}_5\text{CH}_3\text{N} \quad \text{CH}_3\text{NHC}(\text{O})\text{OR}
\]

The carbon-\(^{14}\text{C}\)-labelled methylcarbamate and its \(^3\text{H}\)-isotope can be used to determine the position of these insecticide tingle sites of labelling. The results are presented in Table 1. The product reached the p-\(^3\text{H}\) carbamate-\(^{14}\text{C}\) and methyl-\(^{3}\text{H}\) and tritium labelling synthesis of certain in the rings, or in available routes.

2. METABOLISM

The first report dealt with a compound naphthol-1-\(^{14}\text{C}\) in the modification phase by a study in the 35% mobile phase, *Wit domestica* L. Sevin sesame [2-(3,4-methoxyl)] interfere with metabolism, but it was metabolized and is a substance that appears there was a much g
Acetyl chloride, labelled with carbon-14 in either the methyl or carbonyl position, and sodium azide were reacted to yield methyl isocyanate-C\textsuperscript{14}, which was then reacted with the appropriate phenol. A two-compartment reaction tube with a break-seal was utilized. Yields on a 0.5-mM scale were routinely 40-70%. The methylcarbamates were isolated by chromatography on Florisil and crystallization. Their purity and structure were confirmed by infra-red spectra, co-chromatography on Florisil columns and silica gel thin-layer chromatograms, and by their melting points and mixed melting points. Acetyl-1-C\textsuperscript{14} chloride or bromide yielded carbonyl-C\textsuperscript{14}-methylcarbamates, and acetyl-2-C\textsuperscript{14} chloride or bromide yielded the methyl-C\textsuperscript{14}-carbamates. This method has been modified (Dr. Roy Everett, Chemagro Corporation, Kansas City, Missouri) by introducing the acetyl chloride into the reaction tube with a vacuum manifold rather than as a benzene solution to minimize water pick-up, and by completing the first reaction at the higher temperature before introducing the substituted phenol in the case of thermally unstable phenols. Further useful modifications of this procedure include preparation of the methyl isocyanate-C\textsuperscript{14} followed by introduction of the substituted phenol into the other compartment of the reaction tube without triethylamine, and reacting the phenol and isocyanate for either 2 h at 100°C or several days at room temperature [22]. Selenium-carbonyl-C\textsuperscript{14}, as prepared via methyl isocyanate-C\textsuperscript{14}, is available from Volk Radiochemical Co. (Skokie, Illinois). This break-seal tube and reaction sequence should also be appropriate for preparing methyl-H\textsuperscript{3}-carbamates from acetyl-H\textsuperscript{5}-chloride or acetic-H\textsuperscript{5}-anhydride.

The carbon-14-labelled carbamate insecticides currently under investigation are indicated in Fig. 1. The available evidence on the metabolism of these insecticides has emphasized the importance of working with multiple sites of labelling. The toxicological studies discussed later have already reached the point where it would be advantageous to utilize carbonyl-C\textsuperscript{14} and methyl-H\textsuperscript{3} labels on the same methylcarbamate, or carbonyl-C\textsuperscript{14} and tritium labelling of the enolic moiety within the same molecule. The synthesis of certain of these insecticides with carbon-14 at selected sites in the ring or in a ring substituent should also be readily possible with available routes.

2. METABOLISM OF METHYLCARBAMATES

The first reported study utilizing radiolabelled carbamate insecticides dealt with a comparison of the metabolism of naphthyl-1-C\textsuperscript{14}-Sevin and 1-naphthol-1-C\textsuperscript{14} in three insect species [18]. The metabolites were separated by a paper chromatographic system, with glutaronitrile as the stationary phase and isopropyl ether saturated with glutaronitrile as the ascending mobile phase. With a normal susceptible (S) strain of house-fly, Musca domestica L., Sevin penetrated rapidly either alone or with the synergist, sesamex [2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxoanthracene], but sesamex interfered with the process or processes by which the absorbed Sevin was metabolized and excreted. Sevin was rapidly changed to a very polar substance that appeared both in the tissues and the excreta. With sesamex there was a much greater amount of unchanged Sevin in the body and much
less polar metabolite also investigated was 25 times more resistant in susceptibility and in rates of metabolism. The polar metabolite max decreased the unchanged Sevin in with the S flies. S the house-fly, only fasciatus Dal. (.), a Germanica L.). Sevin products in these in differed greatly in isolated to the toxicity synergist, and that trolled the overall of the ester bond by limiting step in the

A recent invest is not as simple as systems then degrade oxidative attack by microsomes, in the dinucleotide phosphate mechanism; not follow oxidation.

Sevin was met different carbamate and two further met. These conclusions labelled with carbon were used in separate with column chromatography and on thin-layer chromatograms. The use of suitable conditions: modification of the from the methyl-OH formalddehyde-OH as action with 4 hydroxphenylthiourea after these labelled derivatives and co-chromatography labelled metabolites layer chromatogram after reaction of the techniques revealed.
less polar metabolite in the excreta. A resistant (R) house-fly strain was also investigated with and without synergist. Although the R strain was 25 times more resistant to Sevin than the S strain, the strain differences in susceptibility and the effect of the sesamex could not be accounted for on the basis of changes in absorption. However, a quantitative difference in rates of metabolism favoured survival of the R strain, since more of the polar metabolite was excreted and less Sevin remained in the body. Sesamex decreased the excretion of metabolites and increased the retention of unchanged Sevin in the bodies of the R flies in exactly the same manner as with the S flies. Sevin metabolism yielded at least three metabolites in the house-fly, only a very polar metabolite in the milkweed bug (Oncopeltus fasciatus Dall.), and six metabolites in the German cockroach (Blattella germanica L.). Sevin and 1-naphthol formed the same series of more polar products in these insects. The authors concluded that the metabolic pattern differed greatly in the three insects, that the rate of metabolism was related to the toxicity to a particular insect species and to the action of the synergist, and that the rate of formation of one key product probably controlled the overall metabolism. They proposed that the rate of hydrolysis of the ester bond by a carbamate esterase enzyme was the first and rate-limiting step in the metabolism of Sevin.

A recent investigation [23] has established that the metabolism of Sevin is not as simple as a carboxyl esterase mediating hydrolysis and other systems then degrading the hydrolysed fragments. It appears instead that oxidative attack by enzymes in liver microsomes, and possibly also insect microsomes, in the presence of the co-factor, reduced nicotinamide-adenine dinucleotide phosphate (NADPH), may be the initial and critical detoxification mechanism and that hydrolysis of the carboxyl ester may or may not follow oxidation.

Sevin was metabolized by rat-liver microsomes to yield at least five different carboxyl metabolites with the C-O-C (3)-N-C skeleton intact, and two further metabolites that lacked the carbonyl and methyl group [23]. These conclusions resulted from studies where three samples of Sevin labelled with carbon-14 in the methyl, carbonyl and 1-naphthyl positions were used in separate but identical experiments. Metabolites were separated with column chromatography on Florisil using mixtures of hexane and ether, and on thin-layer chromatographic plates with silica gel, using hexane–ether mixtures. The carbonyl-C14-labelled derivatives were utilized to ascertain suitable conditions for hydrolysis of the carboxyl metabolites. Possible modification of the N-methyl group was then studied with metabolites from the methyl-C14-labelled sample by hydrolysis and determination of formaldehyde-C14 as 3,3-methylene-C14-bis (4-hydroxycoumarin) after reaction with 4 hydroxycoumarin [24], and of methyl-C14-amine as methyl-C14-phenylthiourea after reaction with phenylisothiocyanate. The structure of these labelled derivatives was verified by both isotopic dilution analysis and co-chromatography on silica-gel thin-layer chromatograms. Ring-labelled metabolites were subjected to hydrolysis and separated on thin-layer chromatograms as the phenolic derivatives or as the methylcarbamates after reaction of the hydrolysis products with methyl isocyanate. These techniques revealed that modifications of both the ring and methyl group
were involved within this series of metabolites. Characterization studies on these Sevin metabolites are in progress.

House-flies and American cockroaches (Periplaneta americana L.) yielded the same series of metabolites as those formed by the rat-liver microsomes, based on their chromatographic characteristics and experiments with the different sites of radiolabelling. Thus insects also formed at least five carbamate metabolites from Sevin [23]. The use of multiple-labelling of the insecticide allowed a more complete evaluation of the metabolism leading to a different interpretation of the metabolic pathway in insects as compared with a study [18] where only Sevin-naphthyl-C\(^{14}\) was investigated.

When Sevin has been fed to or sprayed on lactating animals, no significant residues of Sevin or metabolites responding to the \(p\)-nitrobenzenediazonium fluoroborate chromogenic reagent after hydrolysis have been detected in the milk [25–28]. The complexity of the metabolism of Sevin by liver microsomes prompted the treatment of a lactating goat with carbonyl-C\(^{14}\)-Sevin, using a single oral dose of 1.3 mg/kg [23]. A method for the analysis of milk for Sevin and metabolites was developed by fortification of goat milk with the radiolabelled metabolites formed by rat-liver microsomes. The method involved extraction with acetone and chloroform and chromatography of the organosolubles on a Florisil column. Sevin reached a peak level of 0.004 ppm in the milk after 2 h; a metabolite which appears to be a methylcarbamate with the ring modified, probably by hydroxylation, reached a peak level of 0.075 ppm at 2 h; and a metabolite not formed by rat-liver microsomes reached a peak level of 0.027 ppm at 16 h. The level of total Sevin-C\(^{14}\)-equivalents appearing as metabolites in milk reached a peak of 0.93 ppm at 8 h and diminished progressively thereafter to below 0.003 ppm by 60 h. The majority of these radiolabelled metabolites were not extractable into organic solvents and may represent conjugates of certain hydroxylation products of Sevin. An analysis of the urine of this goat, which contained about half of the administered radioactivity, revealed the presence of the same metabolites. These results indicate that analysis for free and conjugated 1-naphthol, as for detection of possible occupational or other exposure by urine analysis [28–30] or of possible milk contamination following ingestion of feed containing Sevin residues or direct application of Sevin to animals, may detect only a portion of the metabolites eliminated by these routes.

Sevin does not yield persistent tissue residues in animals exposed to this insecticide, according to analyses of tissue extracts using \(p\)-nitrobenzenediazonium fluoroborate before and after hydrolysis [31–33]. In order to examine the mechanism of this rapid dissipation of Sevin residues further, the following six compounds (specific activity 1 mc/mg) were administered intraperitoneally to male rats at 7.5 \(\mu\)M/kg: Sevin labelled with carbon-14 in the carbonyl, methyl and 1-naphthyl positions; 1-naphthol-1-C\(^{14}\); methyl-C\(^{14}\)-amine hydrochloride; and sodium carbonate-C\(^{14}\) [22]. Sodium carbonate yielded rapid (60% within one hour) and almost complete elimination of the radioactivity in the expired air, whereas with Sevin-carbonyl-C\(^{14}\) only about one-quarter of the radioactivity was eliminated by this route and the time for half-elimination of that which was expired was about 4 h. With methyl-C\(^{14}\)-amine, about half the radioactivity was eliminated as expired carbon-14 dioxide, whereas with the administered which was expired by methyl-C\(^{14}\). No naphthyl-C\(^{14}\) or with elimination of radioadministered dose, C\(^{14}\) was largely eliminated in the tissues after 4 days the rate of expired amounts was negligible since-\(^{14}\), but it amine. The total S activity was most pe in the corpuscles b. The localization of quite different with to carbonyl- and mo in the tissue was v with the other labels bolites lacking the b admistration of carb products may have r Sevin compared with localization of meta (about 60%) appears the identity of these.

The fate of Sevin Many studies have b plants, based on col the insecticide durin and cotton plants inji it to metabolites wh aqueous solution [23] animals were not ce water-soluble metab although this seems plants, even with the 50% of the carbon-1- Experiments in \(\alpha\)-isopropoxyphenyl the carbonyl posit roaches yielded two which were not read principal organosolvent methylcarbamate b behaviour of \(\alpha\)-isoprop that of Sevin.
dioxide, whereas with Sevin-methyl-C\textsuperscript{14} this value was only about one-eighth of the administered radioactivity, the times for half-elimination of that which was expired being about 1.5 h for methyl-C\textsuperscript{14}-amine and 8 h for Sevin-methyl-C\textsuperscript{14}. No radioactivity appeared as carbon-14 dioxide with Sevin-naphthyl-C\textsuperscript{14} or with 1-naphthol-1-C\textsuperscript{14}. With all six labelled compounds the elimination of radioactivity in the faeces accounted for less than 1% of the administered dose. The radioactivity from Sevin-naphthyl-C\textsuperscript{14} and 1-naphthol-C\textsuperscript{14} was largely eliminated in the urine, leaving very little residual carbon-14 in the tissues after 48 h. The radioactivity in the tissues at 48 h, by which time the rate of elimination of carbon-14 from the rats with all six compounds was negligible, was higher with Sevin-carbonyl-C\textsuperscript{14} than with sodium carbonate-C\textsuperscript{14}, but was lower with Sevin-methyl-C\textsuperscript{14} than with methyl-C\textsuperscript{14}-amine. The total Sevin-C\textsuperscript{14}-equivalents for ten rat tissues ranged from about 0.1 to 0.5 \( \mu \)M/kg after administration of Sevin-carbonyl-C\textsuperscript{14}, but ranged to 1.7 \( \mu \)M/kg after administration of Sevin-methyl-C\textsuperscript{14}. The radioactivity was most persistent with both carbonyl- and methyl-labelled Sevin in the corpuscles, brain, heart, lung, and particularly the liver and kidney. The localisation of the carbon-14 and specific activity of the tissues were quite different with labelled sodium carbonate and methylamine compared to carbonyl- and methyl-labelled Sevin. Since the radioactive persisting in the tissues was very low for that from the Sevin-naphthyl-C\textsuperscript{14} compared with the other labels, the residual radioactivity was largely due to metabolites lacking the bicyclic ring. Tissue residue differences between administration of carbonyl- and methyl-labelled Sevin and their hydrolysis products may have resulted from variation in distribution of the fat-soluble Sevin compared with its water-soluble hydrolysis products and subsequent localization of metabolites at different sites. Much of the radioactivity (about 60%) appeared in the urine as carbamate metabolites of Sevin, but the identity of these has not been established.

The fate of Sevin after application to plants is incompletely understood. Many studies have been reported on the persistence of this insecticide on plants, based on colorimetric analysis for 1-naphthyl after hydrolysis of the insecticide during the analytical procedure (see, e.g. [34–38]). Bean and cotton plants injected through the stem with Sevin-C\textsuperscript{14} slowly converted it to metabolites which could not be extracted into organic solvents from aqueous solution [23]. Five of the carbamate metabolites as detected with animals were not evident in the plants. It is not known whether the major water-soluble metabolite from the plants is the same as that from animals, although this seems unlikely. Little loss of carbon-14 occurred from the plants, even with the carbonyl-C\textsuperscript{14} material and after 28 days, when over 90% of the carbon-14 from Sevin was present as metabolites.

Experiments similar to those conducted with Sevin were also made with \( \gamma \)-isopropoxyphenyl methylcarbamate (compound II of Fig. 1) C\textsuperscript{14}-labelled in the carbonyl position [23]. The microsomes of rat liver and the cockroaches yielded two organosoluble metabolites and one or more metabolites which were not readily extracted from water into organic solvents. The principal organosoluble metabolite appeared to be \( \gamma \)-isopropoxyphenyl N-methylolcarbamate based on isolation, degradation and synthesis. The behaviour of \( \gamma \)-isopropoxyphenyl methylcarbamate in plants was similar to that of Sevin.
Only limited information is available on the biological activity of these metabolites of the methylcarbamates. The organosoluble metabolites from the liver microsomes of 1-naphthyl and o-isopropoxyphenyl methylcarbamates were less than one-eighth as potent as their methylcarbamate precursors when assayed in vitro for anticholinesterase activity with fly-head homogenates [23].

3. METABOLISM OF N,N-DIMETHYL CARBAMATES

The metabolism of Dimetilan-5-carbonyl-$C^{14}$ (VII) was investigated in adult American and German cockroaches [39]. The half-life of the injected compound in the German cockroach was less than one hour, with the loss occurring from elimination as carbon-14 dioxide and formation of at least three organosoluble $C^{14}$-metabolites, none of which was 3-methylpyrazolyl-(5)-dimethylcarbamate. The metabolites in the American cockroach were examined in more detail using both thin-layer chromatography on silica gel with an ethyl acetate-ethanol (98:2) developing solvent, and column partition chromatography with silica gel developed with hexane-methylene chloride mixtures. Chromatography on the silica-gel column of an extract prepared from roaches 4 h after injection of 1.0 mg Dimetilan-$C^{14}$ per roach resulted in recovery of about half of the original carbon-14 injected. About one-fourth of the injected dose was recovered as Dimetilan, and one-fourth as four metabolites which were partially resolved on the column and more completely so on the thin-layer chromatograms. Two of these metabolites yielded formaldehyde on acid degradation. The biological activity of these metabolites was less than that of the Dimetilan precursor on the basis of toxicity to house-flies and of anticholinesterase activity as assayed in vitro with house-fly head acetylcholinesterase and human plasma butyrylcholinesterase. Infra-red spectra on the two major metabolites, those which yielded formaldehyde on acid degradation, were consistent with the presence of both carbamyl groups on the molecule, but with an alcoholic function which might result from the oxidation of a methyl to a methyol group. On the analogy of the metabolism of similar non-labelled compounds by rat-liver microsomes and insects [40, 41] it would appear likely that the formaldehyde-yielding metabolites from Dimetilan were N-methylol derivatives.

Mammals treated with Pyramat [2-propyl-4-methylpyrimidinyl-(6)-dimethylcarbamate] excreted three metabolites in the urine. One was identified as 2-propyl-4-methyl-6-hydroxypyrimidine and the others were presumed from limited evidence to be 2-propyl-4-methyl-5,6-dihydroxypyrimidine and a carbamate intermediate which might decompose to yield this latter compound [42]. Metabolism of this dimethylcarbamate insecticide thus appeared to involve ring hydroxylation, but methyl hydroxylation and some conjugation may also have occurred as acid hydrolysis increased the recovery of certain of the enolic products.

4. OTHER RADIO TRACER APPROACHES

The availability of methods of preparing $C^{14}$-labelled carbanes with specific activities of 1 to 5 mc/mM should facilitate, and perhaps even stimulate, further relatively new gastrointestinal

The insecticide lated at least in part, types of experiments with compounds [18]. The function of different acquired to the car or as a side-resist strains to detoxify the pounds and other compounds is pro toxification of the inhabiting the micro N-methyl groups of evidence supporting 8, 18, 43, 44]. The knowledge of the properties of insects will for such studies are Detoxification to mammals of evidence is not yet in rat oral LD50 forms of the compounds besides fall in the range large and differ which is more toxic and Dimetilan (VII). Toxicity changes in different routes of administration of compound in the host structure to rates e it may be possible in mammals and less case with Sevin, a weak action. This selection of advanced, such as further the detoxification of mammals. The selective toxicity will.

Radiotagged on the distribution of animals. Consideration of the origin since metabolites formed. The carb tracer or colorim
stimulate, further critical investigations on the mode of action of this relatively new group of insecticides.

The insecticidal activity of the methylcarbamates is known to be related at least in part to their rate of metabolism by insects based on many types of experimental evidence, including a single study with radiolabelled compounds [18]. Species specificity in insecticidal activity may be a function of differences in rate of metabolism of the insecticides. Resistance acquired to the carbamates in strains pressured with agents of this type, or as side-resistance where the insects were selected with non-carbamate compounds, appears to be related to the greater ability of the resistant strain to detoxify the carbamate. The action of methylenedioxyphenyl compounds and others that serve to synergize the insecticidal activity of the carbamates is probably associated with decreasing the efficiency of detoxification of the toxicants. Certain of these synergists may act by inhibiting the microsome-NADPH₂ system involved in hydroxylation of the N-methyl groups of the enolic portion of the molecule. The experimental evidence supporting these relationships has been recently reviewed [1, 2, 3, 18, 43, 44]. The ultimate testing of these hypotheses requires detailed knowledge of the rates of the various detoxification reactions in the appropriate insects. Radiotracer and chromatographic techniques adequate for such studies are now available.

Detoxification rates may also be related to certain aspects of the toxicity to mammals of the methylcarbamates, although adequate experimental evidence is not yet available on this point. Insecticidal carbamates vary in rat oral LD₅₀ from less than 1 to more than 1000 mg/kg, although most of the compounds being considered for development as commercial insecticides fall in the range 10–500 mg/kg. The route of administration may have a large and differential effect on the toxicity, as illustrated by Isolan (VIII), which is more toxic to mammals following dermal than oral administration, and Dimetilan (VII), where the dermal is much less than the oral toxicity. Toxicity changes with different substituents on the molecule and with different routes of administration may be related in part to the stability of the compound in the body. With sufficient information on the relationship of structure to rates of metabolism of the carbamates in different organisms, it may be possible to design a molecule that would be readily detoxified by mammals and less readily decomposed by insects. This appears to be the case with Sevin, which has a considerable level of selectivity in its toxic action. This selectivity might be further increased by the use of suitable adjuvants, such as the methylenedioxyphenyl and other synergists, to reduce further the detoxification rate by insects without affecting the rate by mammals. The fundamental information leading to such approaches to selective toxicity will probably come in large part from radiotracer studies.

Radiotagged compounds have been extremely valuable in determining the distribution of pesticides among tissue and organ systems in plants and animals. Considerable fractionation will be necessary to define the localization of the original radiolabelled carbamate in insects and mammals, since metabolites of somewhat similar solubility properties are rapidly formed. The carbamates which have been investigated, by either radiotracer or colorimetric techniques, are not stored for prolonged periods
in any particular depts. Many ionized carbamates are almost inactive as insecticides, despite very high anticholinesterase activity, presumably because of their inability to penetrate into the insect nervous system at a sufficient rate to achieve the critical levels necessary for blocking impulse conduction. Data on the level of intact carbamate or its anticholinesterase metabolites in the insect nervous system might be advantageously compared with the degree of cholinesterase inhibition occurring within a portion of the nervous system, such as the thoracic ganglia or entire nerve cord. The correlation of the actual level of carbamate in the nervous system with the symptoms and degree of inhibition of acetylcholinesterase and other carbamate-sensitive esterases, such as aliesterases, would contribute to an evaluation of the mode of insecticidal action of these materials. Further studies are needed on the penetration rate into insects as affected by formulation and other adjuvants, and the rate of absorption and distribution following ingestion, particularly since considerable variation occurs in insecticidal activity depending on whether the compounds are applied topically, injected or ingested. Penetration and distribution studies in plants utilizing methylcarbamates-C¹⁴ might be more readily approached than similar studies in animals, since the compounds studied so far are more stable in plants than animals and the plant metabolites vary greatly from the original compound in solubility characteristics.

Studies on the residual persistence of the carbamate insecticides have been made primarily by determination of the enolic or amine portion of the molecule as liberated by hydrolysis during analysis. The validity of any such method depends on the specificity in accounting for all toxic agents, with minimum interference from non-toxic metabolites. It is known that metabolism may modify both the amine and enolic portions of the molecule, and that certain of the metabolites are biologically active. Metabolism involves both oxidation and hydrolysis, with or without conjugation of certain of the hydroxylated derivatives. This same situation also occurs with other pharmaceutically active but non-insecticidal carbamates [45]. Ethyl carbamate (urethane) as studied with a carboxyl-C¹⁴-label and probably also methyl carbamate are rapidly hydrolysed in mammals [46-48]. Ethyl chloracetate [ethyl 2, 2, 2-trichloro-1-hydroxyethylcarbamate] is partially hydrolysed and partially conjugated [49]. Mephentoxin carbamate (3-n-tolyloxy-1, 2-propanediol 1-carbamate) is quite stable in mammals, and its metabolism includes conjugation of a portion of the dose without hydrolysis of the carbamate ester group [50, 51]. In vivo hydrolysis of methocarbamol [3-(o-methoxyphenox)-1, 2-propanediol 1-carbamate] is negligible, according to a carbonyl-C¹⁴ study, and the excreted metabolites include the original compound and its glucuronide and four further metabolites, two of which are glucuronides [52]. Meprobamate (2-methyl-2-n-propyl-1, 3-propanediol diisocarbamate) labelled with both tritium (Wilsbach technique) and carbon-14 has been investigated and its metabolism found to involve almost no hydrolysis but rather predominantly hydroxylation of the 2-methyl position with or without glucuronic acid formation before excretion [53, 54]. Metabolism of both 1-ethynylecyclohexyl carbamate-carbonyl-C¹⁴ and 1-ethylcyclohexyl carbamate-carbonyl-C¹⁴ involves ring hydroxylation. Hydrolytic cleavage was a minor catabolic route and excretion occurred as both ring-hydroxylated 1-ethynylecyclohexyl carbamate and its glucuronide [16]. The nature of the changes
almost inactive as a carboxylic acid, presumably because it is a system at a sufficient distance from the acetylcholinesterase site. Experiments on a portion of the brain or the nerve cord. The metabolism system with the brain or the nerve cord. The brain is also shown to be active in other carbamates, probably due to an evaluation of nerve acetylcholinesterase activity. They differ, however, in the nature of the reaction with the cholinesterase, since the organophosphate inhibition by phosphorylation is only very slowly reversible, whereas the more readily reversible of the carbamate inhibitors may lead to enzyme-inhibitor dissociation during tissue preparation and assay. The reaction with the carbamate and the esterase is complex, the rates of formation of the enzyme-inhibitor complex, carboxylation of the enzyme and hydrolysis of the carbamylated enzyme being affected by concentration of inhibitor, enzyme and substrate. The sequence of reactions at the esteratic site on the enzyme surface appears to be the same for the substrate and the carbamate and phosphate inhibitors, with the marked differences with these compounds resulting from variations in rate of different components of the reaction. Radiolabelled carba-mates are now available with adequately high specific activity for use in studies on the nature of the reaction of the enzyme and inhibitor, an approach allowing possible verification and extension of the kinetic studies which have led to the information now available. Highly purified cholinesterase, from mammalian or insect sources, might be reacted with the radiolabelled carbamates to ascertain whether, for example, a radiolabel on the enolic moiety would be released and the radiolabelled methyl and carboxyl groups would be bound to the protein. The stability of the C_{14}-methylcarbamyl group at the esteratic site of the esterase and the nature of the peptide chain involved at the site of carboxylation might be investigated on similar lines to those adopted for F_{12}-phosphorylated esterases, using J_{12}-organophosphate inhibitors, with the exception that suitable conditions would have to be utilized to prevent spontaneous hydrolysis of the carbamyl group from the esterase or peptide during the studies (for reviews and recent articles relating to this problem see [1, 55-66]). Certain esterases other than cholinesterase have also been observed to hydrolyze some insecticidal carbamates, such as esterases of rabbit plasma, liver and kidney, which catalyse the hydrolysis of dimethylcarbaryl fluoride and the albumin fraction of mammalian plasma which increases the hydrolysis rate of certain methylcarbamates, including Sevin [67-69]. These esterases presumably differ from those that are more sensitive to inhibition by carbamates in that the carbamyl esterases are more rapidly hydrolysed, an assumption susceptible to direct test with radiolabelled carboxylate substrates. A recent proposal [10] to use acetyl-C_{14}-choline as the substrate in studying the cholinesterase inhibition from carbamates is particularly attractive as a means of minimizing enzyme and inhibitor dilution during assay and utilizing much lower substrate concentrations during analysis than has been possible with other procedures. This should result in
higher observed levels of inhibition. The degree of inhibition for tissue from carbamate-poisoned animals might then more closely approximate, although certainly not reproduce, the in vivo situation (for considerations related to this point see [1, 71-73]).

The labelled-pool technique, using phosphorus-32 and carbon-14 biochemicals, has proved very valuable in considering the mode of action of such insecticides as DDT, dieldrin and others (for review see [74]). No such studies have been reported as yet with animals poisoned with carbamate insecticides. The procedure for examining the rate of acetate-14C incorporation into C14-acetylcholine would be of particular interest, especially if applied to the insect nervous system.

ACKNOWLEDGEMENTS

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M. HASCOËT: What solvent system did you use for thin-layer chromatography?

J. E. CASIDA: We have a mixture of about half-a-dozen different solvent materials which works very well. Hexane-ether mixtures of varying proportion give good results, the proportion depending on the type of metabolites being used. We usually run these as two-dimensional thin-layer chromatograms in order to determine the stability of the metabolites on the thin layer and we run them with each radio-label.

M. HASCOËT: What is the capacity of the Florisil column that you use? How many milligrams per gram do you need to obtain a good resolution?

J. E. CASIDA: The capacity is about 50-150 mg. For further information I would refer you to my reference [19]. Actually you cannot obtain as good a resolution by this means as with thin-layer chromatography — this is the experience of many people who have tried both. You can probably run at about 5 mg/g but this is a top level. Optimun resolution would be obtained with about 1 mg/g.

M. HASCOËT: Is there no degradation of Sevin on the column?

J. E. CASIDA: This has been very carefully checked in a variety of experiments. There is no degradation on the column, but there is degradation for some of the metabolites if left for prolonged periods on the silica-gel thin-layer chromatogram.

M. HASCOËT: At what temperature is the silica gel activated?

J. E. CASIDA: Our silica gel is obtained in a large drum and we take it from the drum and add water. We do not activate it. With each new batch of silica gel we re-ascertain the optimal conditions for separating the material. These conditions, like the purity of the solvent, appear to play a large part in determining the quality of resolution obtained.

C. E. SEKERIS: With thin-layer chromatography I think you are able to see how many hydroxyl groups you have on your ring, if you choose the right elution system.

J. E. CASIDA: Yes. We have been using it in this manner to clarify the modifications occurring on the naphthol ring.

H. HUQUE: Insects are said to develop resistance very quickly in the case of Sevin. What are the factors responsible for this?

J. E. CASIDA: My references [1], [4] and [5] provide information on this point. The rate of development of resistance depends on the slope of the dosage-mortality curve, the type and level of field application made and the previous history of exposure of the insects in question to other insecticides. House-flies quickly develop resistance to Sevin, but the Mexican bean beetle does not. Synergists may reduce the rate of developing re-
CARBAMATE INSECTICIDE TOXICOLOGY

The rate of metabolism of Sevin appears to be at least partially correlated with resistance in selected strains and this presumably results primarily from in vivo hydrolysis, as discussed. The techniques are now available for the critical studies on resistance which are needed.

H.I. Petersen: A problem of very great interest in this connection is the toxicity of the metabolites. Can you say anything about this?

J.E. Casida: The experiments that we ran were limited because we had a limited supply of metabolites. However, with Dimetilan it is very easy because we can put 1 mg into each cockroach and recover about 350 μg as pure metabolite by passing through one column — it will then crystallize so that we get hundreds of milligrams of these metabolites. None of them are as potent as Sevin in inhibiting cholinesterase from its source in the house-fly brain. The anti-cholinesterase activity of the materials is, of course, no indication of their toxicity, and until we can make enough of these materials to study their toxicity independently the question remains unresolved. I think it is an important question because they appear in quite a number of foodstuffs produced by currently recommended procedures.

C.C. Hassett (Chairman): Dr. Casida, would you care to comment on problems of cross-resistance with the carbamate insecticides?

J.E. Casida: I believe Dr. Meltzer investigated this rather closely. I think it would be more appropriate for him to answer.

J. Meltzer: We have had a lot of experience in the selection of strains of house-flies using phenyl-dimethyl-carbamate. Within a certain strain of house-flies, this compound induced a remarkable cross-resistance, in particular to chlorinated hydrocarbon insecticides, whereas the resistance to the carbamate was only slight. Work with other strains of house-flies showed that it was possible to obtain resistance to the phenyl-dimethyl-carbamate, but the maximum resistance did not exceed a factor of 10.

I should like to add, however, that our work with selections of resistant strains showed that we should be very careful in generalizing results. If we obtain a certain result by our selections, we can only say that the results apply to the particular strain used, and under the particular circumstances. Under other conditions, we might obtain contradictory results. I would, therefore, like to warn against generalizing the results obtained in this selection work.

J.E. Casida: I would like to make one final comment on this question of resistance, drawing attention in this connection to references [1], [4] and [5] to my paper. From the studies that have been made so far it appears that metabolism in insects is not by hydrolysis but by oxidation. There is considerable evidence in the literature that resistance is related to the rate of metabolism, so that one could deduce that the resistance is due to an increased ability to carry out this oxidation. There is no proof of this, however.
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PROBLEMS OF APPLICATION AND ACTION OF THIODAN STUDIED WITH S\textsuperscript{55} -LABELED INSECTICIDE

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Abstract — Résumé — Resumen

PROBLEMS OF APPLICATION AND ACTION OF THIODAN STUDIED WITH S\textsuperscript{55} -LABELED INSECTICIDE. Thiodan (8,7,8,10-hexachloro - 1,5,5a,6,8,9a - hexahydro-6-0-methano - 8,4,8 - hexahydroxathiepin - 3 - oxide) is an insecticide developed by Farbwirke Hoechst AG. The technical product consists of two isomers with different melting points and different velocities of insecticidal effect. Using highly purified X- and S-isomers together with technical product, all labelled with S\textsuperscript{55} of the same specific activity, the authors studied problems of the application and mode of action of the isomers. Since the insecticide can be experimentally applied in the vapour phase, we tested the effect of different temperatures and air humidities on sublimation on the soil penetration through the cuticles. As intoxication began, a reactive increase in respiration, followed by a remarkable elevation of the insect's body temperature, was found. It was shown with the labelled isomers that this raising of body temperature after some time causes surface removal of the sublimated insecticide substance by way of sublimation into the air. This mechanism influences the complex mechanism of penetration, intoxication and decomposition in connection with temperature and relative humidity of the air.

Further experiments are concerned with the penetration and distribution of the labelled insecticide in the insect organism.

MODES D'APPLICATION ET D'ACTION DU THIODAN ÉTUÉS PAR MARCAGE DE L'INSECTICIDE PAR S\textsuperscript{55}. Le Thiodan (8,7,8,10-hexachloro - 1,5,5a,6,8,9a - hexahydro-6-0-methano - 8,4,8 - hexahydroxathiepin - 3 - oxide) est un insecticide mis au point par la Farbwirke Hoechst AG. Le produit fabriqué comprend deux isomères ayant des points de fusion différents et dont l'effet insecticide ne se manifeste pas avec la même rapidité. En utilisant simultanément les isomères alpha et beta à l'état de résidu et le produit fini, sous trois marquages par S\textsuperscript{55} de même activité spécifique, on a étudié les problèmes relatifs au mode d'application et au mode d'action des isomères. Comme l'insecticide peut être appliqué expérimentalement en phase gazeuse, on a vérifié l'effet de diverses températures et de divers degrés d'humidité de l'air sur la sublimation à la surface de la cuticule et sur la pénétration à travers celle-ci. On a constaté que le début d'intoxication provoquait une accélération du rythme de la respiration, suivie d'une élévation sensible de la température du corps des insectes. Comme on a pu le constater en utilisant des insecticides marqués, cette augmentation de la température du corps provoquée, après un certain délai, la dispersion de l'insecticide se produit à la surface, qui s'évapore de nouveau dans l'air. Ce phénomène est influencé par le mécanisme complexe de la pénétration, de l'intoxication et de la décomposition en fonction de la température et de l'humidité relative de l'air.

D'autres expériences ont porté sur la pénétration et la distribution de l'insecticide marqué dans l'organisme de l'insecte.

ПРОБЛЕМЫ ПРИМЕНИМОСТИ И РЕЖИМЫ СПОСОБА ДЕЙСТВИЯ ТИОДАНА ПРИ РАССМОТРЕНИИ S\textsuperscript{55} ИНСЕКТИЦИДОВ. Тиодан (8, 7, 8, 10-гексахлоро-1, 5, 5a, 6, 8, 9a-гексахидро-6-0-метилно-8, 4, 8-гексахидрохетицин-3-оксид) — инсектицид, разработанный фирмой "Фарбверк Хёхст АГ". Этот технический продукт состоит из двух изомеров с различными температурами плавления и с различной скоростью инсектицидного эффекта. Используя кислотообразование альфа- и бета-изомеров и технический продукт, окисленный S\textsuperscript{55} с одинаковой активностью, авторы исследовали вопросы поглощения и проникновения инсектицида через оболочку. Как только началось воздействие инсектицида, температура тела насекомого начинала резко возрастать вследствие испарения инсектицида в воздух. Это влияло на механизм проникновения и уничтожения инсектицида в организме насекомого. Другие эксперименты были связаны с проникновением и распределением инсектицида в организме насекомого.
INTRODUCTION

Thiodan\(^6\) is an insecticide developed by Farbwerke Hoechst AG, Federal Republic of Germany [1-2]. The commercial product (5, 7, 8, 9, 10, 10'-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepins-3-oxide) consists of two isomers with distinctly different melting points and different rates of insecticidal action [3]. The configuration of the stereoisomers was studied by RIEHLE, SCHNEIDER et al. [5]; in this paper they are designated as the \(\alpha\)-isomer (\(F = 103-110^\circ C\)) and the \(\beta\)-isomer (\(F = 206-210^\circ C\)). As shown in Fig. 1, the Thiodan molecule was labelled with radiosulphur (S\(^{35}\)); the

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Filter papers of 1 ml of a Thiodan-ac glass lid after evaporation insecticide pai the weevils from all temperature of 30\(^\circ\)C, lected from the dish means of a sprayer, moved from the super chloroform for remo to BEAMENT [7], it five fractions were c ration of the solvent,
its specific site of action either by penetration through the cuticle or by other paths. In this case, tracer methods are an excellent way of studying the problems of pick-up and penetration. This paper gives a brief account of a large field of experiments using tracer techniques as a part of complex investigations into the mode of action of Thiodan [3, 4, 6].

PICK-UP OF THIODAN BY THE SURFACE OF THE INTEGUMENT AND THE INFLUENCE OF RELATIVE AIR HUMIDITY

The first part of the work to be described attempts to answer the question: Do the varying relative humidities of the air influence the pick-up of Thiodan and its two isomers? Among the different application methods for testing insecticides, the use of the vapour phase is advantageous in our case, because the test insects are exposed to the pure insecticide in molecular form. The main experiments have been done with the granary weevil Calandra granaria L., on account of its relatively large body surface and small amount of body fat. The insects were kept in closed hygrostatic dishes with an inset made of copper gauze on which they were able to run about (Fig. 2).

The bottoms of the dishes were filled with super-saturated inorganic salt solutions providing the different relative humidities (K₉ SO₄ = 98-100% r. h., NaCl = 74% r. h., Ca(NO₃)₂ = 54% r. h., ZnCl₂ = 17% r. h.).

Filter papers of 7-cm diameter had been previously impregnated with 1 ml of a Thiodan-acetone solution (600 µg Thiodan) and were fixed under the glass lid after evaporation of the solvent. This method permitted the molecular insecticide particles to come into contact with the entire surface of the weevils from all directions. The exposure was done in a constant test temperature of 30°C. After 72 h 50 granary weevils in each case were collected from the dishes and washed five times with 2 ml cold methanol by means of a sprayer. By this means all the insecticide picked up was removed from the superficial layers. We preferred methanol to ether and chloroform for removing Thiodan from outside the insect, since, according to BEAMENT [7], it is a poor solvent for the wax of the insect cuticle. The five fractions were collected in small aluminium planchettes. After evaporation of the solvent, they were measured for 10 min by means of a Frieske
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and Hoepnner electronic scaler in conjunction with an end-window counting-
tube having a window thickness of 1.2 - 1.3 mg/cm².

![Graph](image)

**Fig. 3**
Amount of Thiodan-2H isomers picked up by 50 granary weevils
exposed (72 h; 20°C) to the vapour phase of the insecticide under different air humidities.

The first point of interest in Fig. 3 is that different amounts of Thiodan and its α- and β-isomers were picked up by the superficial layers of the integument. This had been anticipated, because the difference in the quantity picked up depends on physical properties, i.e. the different melting points of the α- and β-isomer, as well as on the composition of the commercial product (α:β = 3 : 1) [8].

Further, it is apparent – and this is the main point of interest – that the pick-up of Thiodan on the epicuticle is considerably influenced by the relative humidity. These effects are likewise due to physico-chemical properties of the superficial layers. As the wax layers of the insect epicuticle consist of mixtures of paraffins, alcohols and fatty acids, i.e. a mixture of lipoids of varying reactivity [9], it has a certain solubility for Thiodan, which is a lipophilic insecticide. If a Thiodan molecule comes into contact with the epicuticle the surface and the particle will be subjected to attractive forces varying in intensity from primary valence bonds through hydrogen bonds to long-range intermolecular or van der Waals forces. The highest rate of solubility is shown in the middle range of relative humidities, at which the wax layer is not covered with a thin film of water and the transpiration rate is relatively low. In this case, we assume that the dissolving forces of the epicuticle are fully effective. At high humidities, a thin film of water covers the epicuticle, which reduces the solubility for the insecticide, while at very low humidities the rate of transpiration and loss of water through the cuticle act against the deposition and pick-up of the Thiodan molecules [6].

THE PICK-UP ANI
THE PICK-UP AND PENETRATION OF THIODAN AND ITS ISOMERS

In the second series of experiments we tried to clarify the problem of the relationship between pick-up and penetration through the integument and the symptomatology of the insecticidal action.

These studies were done with the same experimental arrangement as described above, but now the granary weevils (50 specimens) were exposed directly to deposits of the pure isomers or commercial product on filter paper impregnated with insecticide to 0.63µg/cm² for varying periods at 25°C and 54% r.h. After different periods the symptoms induced by the insecticide were evaluated. For the purposes of the following presentation the symptoms will be designated by numerals: 1 = latency phase; 2 = incipient excitation; 3 = strong excitation; 4 = convulsions; 5 = paralysis and death.

The weevils were collected and first of all washed five times with 2 ml cold methanol. This removed the Thiodan collected on the surface for determination. This fraction is termed the "outside" fraction. After this, the test insects were homogenized in a Potter-Elvehjem homogenizer with 5 times 2 ml acetone. This fraction is termed the "inside" fraction. Since the Thiodan was labelled with S²⁵, combustion and ash measurement was not a convenient procedure; it was therefore necessary to establish a standard curve for the absorption of β-radiation by the body substances which are also extracted. The amount of fat extracted from 50 granary weevils absorbs at least 31% of the β-radiation.

As Fig. 4 shows, different amounts of Thiodan and its isomers had been picked up by the insects from deposits of equal density during equal periods. If the pick-up had been no more than superficial contamination, it might...
have been expected that the amounts of the three substances recovered in the "outside" fraction would have been equal. Since this is clearly not the case, however, it demonstrates that there must be differential solubility for the three substances in the epicuticle, depending on their different physico-chemical properties, in particular their stereoreconfiguration [6]. These facts are confirmed if one also compares the times at which the visible symptoms evoked by the poison appear: For α-Thiodan, the stage of irreversible injury (symptoms 2 and 3) is reached after 24 h and for commercial Thiodan after 36 h, whereas for the β-isomer this stage is not reached for 48 h. It must be emphasized that all three substances are absolutely toxic for insects [3, 6]; the better initial toxicity of the α-isomer is merely a function of its physico-chemical properties. By comparing the symptoms induced by Thiodan intoxication and the amount of "outside" pick-up, it may be seen that up to the end of the incipient excitation phase (symptom 2) and beginning of the strong excitation (symptom 3) insecticide is picked up steadily and the curve has a distinct peak. During the strong excitation phase, however, no more insecticide is picked up; in fact the detectable activity of the outside falls. At a later stage (symptom 4) pick-up again rises. ARMSTRONG et al. [10], working with the isomers of benzene hexachloride, obtained curves of nearly the same type, but were unable to explain this effect. By means of an apparatus which enables us to measure the body temperature in the body cavities of insects [3, 6], we found that the temperature rises from incipient excitation until the end of the strong excitation (symptoms 2 and 3). Parallel to the rise in body temperature, the oxygen consumption curve also has its peak at the same intoxication stages [6].

Another experiment to confirm the fall of the outside curves after intoxication stages 2 and 3 was made by subjecting life weevils and freshly killed weevils under identical experimental conditions to radioactive Thiodan vapour. It was found that the pick-up of labelled Thiodan showed a linear increase in the dead weevils, whereas the live weevils demonstrated the typical fall of activity during excitation stage 3 [6]. These experiments represent an absolute proof that the fall of the "outside" curve is due to a physiological reaction on the part of the insects.

If one examines the shape of the "inside" curves it appears that the α-isomer penetrates the insect more rapidly than the commercial product and the β-isomer. After two hours' exposure, the α-isomer and commercial product already appear in substantial amounts "inside" the insects. Thus it is confirmed that the structural difference between the isomers and the quantitative composition of commercial Thiodan have a considerable effect on the ability to penetrate the insect cuticle [6]. Allowing for the different amounts of "outside" pick-up, the "inside" curves show nearly the same shape. There is a visible increase from the latency phase 1 through the incipient excitation stage 2, whereas during the strong excitation stage 3 the rise of the curves is very small. As we have stated before, all three substances possess absolute toxicity; a total amount of 0.4 μg of Thiodan or its isomers must be present "inside" the insects to cause irreversible injury in 50 granary weevils.

It has been shown that the postulated and sufficient to weevils 100% of the site of action.


B. DARIS: Did humectates after the W, KLOFT: Yes elsewhere.
B. DARIS: I am 98-100%. Did you W. KLOFT: Yes on the insecticide of the experiments periods at different adapted to particular attention has been D. A. CROSSLEY these insects? W. KLOFT: Would we have at different vessels and were in First, the rate of the respiratory quotation until death. C.T. LEWIS: insecticide from the nation, or any views been picked up by the. Do you agree that a insecticide that had...
It has been shown, by working with the labelled insecticide, that Thiadan possesses the postulated properties of an active insecticide; it has an absolute and sufficient toxicity for the insect (0.008 μg per insect will kill granary weevils in 100% of the cases) and the ability to gain access to its specific site of action.

REFERENCES


DISCUSSION

B. DARIS: Did you count the number of dead weevils in different relative humidities after the period of 72 h?
W. KLOFT: Yes, naturally we did that, but the results will be published elsewhere.

B. DARIS: I am especially interested in the high relative humidity of 98-100%. Did you find live weevils at these high relative humidities?
W. KLOFT: Yes, even at 98-100% r. h. some weevils survive, depending on the insecticide concentration and on the exposure periods. The purpose of the experiments was in fact to find the best concentrations and exposure periods at different relative humidities, in order to establish control methods adapted to particular climatic conditions. We think that so far insufficient attention has been paid to this.

D. A. CROSSLEY: What method do you use to measure respiration for these insects?
W. KLOFT: We measured respiration using Warburg manometric techniques at different temperatures. The insects were put directly into the vessels and were intoxicated—this is important, I feel—within the vessel. First, the rate of oxygen consumption was measured for two hours, and then the respiratory quotient was measured throughout the whole course of intoxication until death.

C. T. LEWIS: I was very interested in the figures for the uptake of insecticide from the vapour phase. I wonder whether you have any information, or any views, on the possibility that sublimed insecticide might have been picked up by the weevils from the gauze on which they were walking. Do you agree that a significant fraction of the uptake might have come from insecticide that had condensed on the gauze surface?
W. Kloft: Yes, we cannot totally exclude this factor. Only at the beginning of the exposure was the uptake exclusively from the vapour phase, and I am sure that after about two hours we had a supplementary intoxication of the type you mention.

F. T. Phillips: Your results on the pick-up of Thiodan vapour by insect epicuticle under different relative humidities are interesting. Did you use other surfaces for comparison, for example a glass surface (as a standard surface) and a glass surface covered with a layer of insect wax? I realize that it probably would not be possible to obtain the same wax structure on a glass surface as on insect bodies, but this might be overcome by using excised cuticle from the insects.

W. Kloft: We have not done any experiments exposing glass surfaces under different relative humidities, since we are of the opinion that the differences observed are mainly due to physiological processes, for the reasons explained in greater detail in the paper. In particular, the loss of "outside" activity after a rise in internal body temperature shows that physiological processes are involved, and this was proved by the simultaneous exposure of dead weevils. We were also able to show this sublimation effect using a glass model heated inside by an electric resistance.

E. Horber: Is there no interaction to be expected between the insecticide vapour and the ions of the solutions in the bottom of the Zwölfer hygrostatic dishes?

W. Kloft: No, because Thiodan is practically water-insoluble. In addition, the filter with the insecticide is fixed under the lid and the vapour phase comes from above, while the aqueous salt solution is underneath.
THE USE OF RADIOISOTOPES TO STUDY THE ABSORPTION, DISTRIBUTION AND ELIMINATION OF VARIOUS INSECTICIDES IN ANIMALS. When insecticides are used against farm-animal parasites it is important to ensure that no harm is done to the health of the animal or the consumer. Radioisotopes provide a means of studying the behaviour of labelled insecticides in animal organisms and of obtaining extremely accurate data on residues of insecticides and insecticide decomposition products in meat and milk.

The present study was carried out on the rats in which DDT-C\textsuperscript{14}, polychlorinephene-C\textsuperscript{13}, and chloropropane-\textsuperscript{14} were absorbed through the skin, accumulated in the organ and tissues, and eliminated from the organism of farm and laboratory animals.


donnement des radioisotopes pour l'étude des processus d'absorption, de distribution et d'excrétion de certains insecticides dans l'organisme vivant. L'emploi des insecticides dans la lutte contre les parasites des animaux de ferme ne doit pas compromettre la santé des animaux ni celle des personnes utilisant les produits animaux. Les radioisotopes permettent de suivre le comportement d'incidents marqués qui a été introduits dans l'organisme animal, et de déterminer par analogies, avec une grande précision, la teneur du lait et de la viande en insecticides et produits de désintégration.

Le laboratoire fournit des données sur la vitesse d'absorption à travers l'épiderme, l'accumulation dans les organes et tissus et l'excrétion, par l'organisme d'animaux de laboratoire et d'animaux de ferme, de DDT-C\textsuperscript{14}, de polychlorine de phtalène-C\textsuperscript{13} et de chloropropane-\textsuperscript{14}.  

Применение радиоизотопов в изучении процессов всасывания, распределения и выведения из живого организма некоторых инсектицидов. Применение инсектицидов для борьбы с паразитами оказывает существенное влияние на здоровье животных, человека, потребителя и окружающую среду. Использование радиоизотопных инсектицидов позволяет определить их распределение и выведение из организма и оценить их токсичность, определять метаболизм инсектицидов и продуктов их распада в животных и человеке.

В работе приведены данные о скорости всасывания через кожу инсектицидов, их накоплении в органах и тканих и выведении из организма животных и человека инсектицидов ДДТ-\textsuperscript{14}, полихлоринафтен-\textsuperscript{13} и хлоропропан-\textsuperscript{14}.

El uso de los radioisótopos en el estudio de los procesos de absorción, distribución y eliminación de ciertos insecticidas en el organismo animal. El empleo de los insecticidas en la lucha contra los parásitos del ganado no debe perjudicar a la salud de los animales y de las personas que utilizan los productos de los insecticidas. Los radioisótopos permiten seguir en el organismo animal el comportamiento de un insecticida marcado y determinar con gran precisión el contenido de residuos de insecticidas y de productos de su desintegración en la leche y en el carne.

En la memoria se presentan datos sobre la velocidad de absorción a través de la piel, la acumulación en los órganos y tejidos y la eliminación del DDT-\textsuperscript{14}, del polícloro-\textsuperscript{13} y del clorofosfuro-\textsuperscript{14} por el organismo de animales de laboratorio y por el del ganado.

Всесоюзный научно-исследовательский институт ветеринарной санитарии применяет радиоактивные изотопы для изучения процессов
восстановления, распределения и выделения из организма инсектицидов, используемых для борьбы с эктопаразитами сельскохозяйственных животных, а также при кожноводовой инвазии.

ИЗУЧЕНИЕ ВСАБЫВАНИЯ, ДЕПОНИРОВАНИЯ И ВЫДЕЛЕНИЯ ИЗ ОРГАНИЗМА ЖИВОТНЫХ ПРЕПАРАТА ДДТ, МЕЧЕННОГО С14.

Широкое использование препаратов ДДТ (дихлордифенилтрихлорэтан) в ветеринарии как средства борьбы с эктопаразитами сельскохозяйственных животных и разорвавшиеся должны по поводу его в организме сельскохозяйственных животных заставили провести специальное исследование по изучению вопросов всасывания, депонирования и выделения из организма животных этого препарата при нанесении его на кожу в виде масляного раствора в обычно применяемых дозах.

Меченный С14 препарат ДДТ в количестве 8,6 г растворялся в 200 мл вазелинового масла при подогревании. На одну обработку лактирующей коровой брели 100 мл раствора, в котором содержалось 4,3 г ДДТ с общей активностью 19,35 мккюри, что составляло 62 мккюри на 1 кг веса животного или 14 мг ДДТ на 1 кг живого веса.

Раствор втирался щеткой в область спины коровы от переднего края лопатки до корма хвоста. Активность препарата ДДТ, меченного С14, в образцах крови, молока и мочи животных определяли радиометрически на установке Б-2 с торцовым счетчиком Т-25-РФЛ. Полученные результаты переводились в весовые количества ДДТ.

В соответствии с ветеринарным законодательством корова обрабатывалась 4,3%-ным масляным раствором ДДТ дважды, с промежутком в 20 дней.

После первой обработки корова уже через 2 часа в крови обнаруживалось наличие ДДТ. Через сутки оно достигает 0,37 мг/л. Максимальное содержание ДДТ в крови - 0,43 мг/л обнаруживается к 6-му или 7-му дню, после чего резко уменьшается и к 10-го дня не обнаруживается.

После второй обработки (через 20 дней) ДДТ появляется в крови вновь и достигает максимальной величины на 13-й день (0,1 мг/л). Через 19 дней ДДТ в крови не обнаруживается.

Наблюдались 2 случаи после первого нанесения на кожу 4,3%-ного масляного раствора ДДТ в пробах молока можно обнаружить С14.

На следующий день в утреннем удое содержалось 0,48, а в вечернем - 2,78 мг/л ДДТ.

Максимальное содержание С14 - ДДТ выделяется в молоке через 36 часов и соответствует 8,44 мг/л. Через 24 часа содержание ДДТ в молоке уменьшается, однако до 10 - 12 суток содержание его превышает 1 мг/л. На 20-й день после первой обработки в молоке еще содержалось до 0,46 мг/л ДДТ.

После второй обработки (на 21 день) количество С14 - ДДТ в молоке возросло до 0,67 мг/л. Максимальное содержание ДДТ в молоке, после второй обработки, обнаружено на 4 сутки - 7,69 мг/л,

после чего идет по обработку в молоке.

Опыт за вре 590 мг ДДТ, что составляет 1149 мг, или 13% от общего количества ДДТ, которое было получено с молока в течение 72 часов.

Так, в ходе картина содержания ДДТ обнаружено около 10% количества его в организме в 24-часовом виде.

ИЗУЧЕНИЕ ПРОВОДНЯ ОРГАНИЗМА ЖИВОТНЫХ

В опытах на депонирование и применение С15 в с.

В опытах с мечёнов С15 на кожу (150 мг ПХП) на эпителий через 1 ч и после 24 час их о моче в течение 72 часов в течение 144.

После задавали в органах и тканях в печени и почках.

В опыт на жёлчных мочев. мечёнов 225 мккюри С15 обнаружено было в течение 72 часов в течение 144.

В моче все.

Пошел четыре шизофрения С15 не об.

по 16.

Учитывая, что деления активности измерения проводили как правило, С15 и С14 сдали на аналиты в организм и вид
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после чего идет постепенное уменьшение. Однако, до 130 дней после обработки в молоке можно обнаружить С14 - ДДТ.

Всего за время проведения опыта с молоком выделось около 590 мг ДДТ, что составляет 7% от количества нанесенного на кожу.

За опытный период (130 дней) с мочой выделилось около 1149 мг, или 13,3%, а с калом - 1383 мг, или 15,7% от нанесенного количества ДДТ.

В органах и тканях подопытной коровы, забитой через 5 месяцев с момента первой обработки, обнаружено содержание ДДТ преимущественно в желудке.

Так, в хряже слизистой, жире подкожной клетчатки и жировой перикард содержание ДДТ составляло 1,2 - 1,47 мг/кг.

Таким образом, за 5 месяцев после обработки коровы ДДТ выделяется около половины нанесенного препарата, причем наибольшее количество его выделяется в первые 4 суток.

Оставшийся в организме ДДТ депонируется во всех органах с преимущественно в желудке.

ИЗУЧЕНИЕ ПРОНИДОЧСКИХ ЧЕРЕЗ КОЖУ, ДЕПОНИРОВАНИЯ И ВЫДЕЛЕНИЯ ИЗ ОРГАНИЗМА ЖИВОТНЫХ ПОЛИХЛОРИНЕНЕ, МЕЧЕННОГО С13На

В опытах на коровках и дойной корове изучалось всасывание, депонирование и выделение из организма препарата полихлорена, меченного С13На применяемого в стране для защиты животных от гнуса.

В опытах с нанесением 3%-ной эмульсии полихлорена, меченного С13На на кожу подопытных коровок из расчета 0,75 мкг (150 мг ПХП) на 1 кг живого веса, показало, что С13На появляется в крови через 1 час и обнаруживается в ней до 24 часов. В моче и кале С13На полихлорена появляется через 3 часа и выделяется с мочой в течение 96 часов, а с калом - 144 часов.

После забоя подопытных коровок определили содержание С13На в органах и тканях. Наибольшее количество его зарегистрировано в печени и почках.

В опытах на дойной корове с нанесением на кожу 3%-ной водной эмульсии меченного полихлорена в количестве 1,5 мг с содержанием 225 мкг С13На было установлено, что препарат ПХП быстро обнаруживается в крови и в небольших количествах регистрируется в течение 72 часов. В моче и кале С13На выделяется соответственно в течение 144 - 166 часов.

В молоке С13На обнаруживается на 48 - 72 часа.

После четырехкратной обработки коровек эмульсией полихлорена С13На не обнаруживается в крови спустя 120 часов, в моче и кале - после 168 часов.

Учитывая, что полициклен не растворяется в воде при определении активности животных подопытных коровок и коров, измерение проводилось в водных и спиртовых вытяжках, при этом, как правило, С13На регистрировалось только в водном экстракте. Отсюда было сделано предположение о быстром расщеплении молекулы ПХП в организме и выделении его в виде продуктов распада.
Для подтверждения этого были проведены хроматографический анализ ПХП и продуктов его распада, содержащихся в органах и тканях и выделяющихся с мочой, мочой и калом. Хроматография проводилась на бумаге Ленинградской фабрики марки "М" по видоизмененной методике Киснера, Джона, Миллера и Келдера. В качестве подвижного растворителя применялся гексан и этилацетат.

Прохождение хроматограмм производилось опрессованием бумажных полос 0,01%-ным водным раствором флюоресцена и последующим выдерживанием хроматограмм в парах брома.

При этом флюоресценс, реагируя с перами брома, дает яркую окраску (образование флюоресценс). В месте расположения полиаминов флюоресценс сохраняет свою нормальную окраску, давая жёлтую пятну.

Данные хроматографического анализа подтверждают, что полиамин в организме животного быстро расходуется, так как в одном случае не было выделено пяти, KF которых соответствовали бы целевой молекуле полиаминов.

ИЗУЧЕНИЕ МЕТАБОЛИЗМА ХЛОРФОСА В ЖИВОТНОМ ОРГАНИЗМЕ

Хлорфос как фосфорорганический инсектицид в настоящее время широко применяется для обработки сельскохозяйственных животных против энтомофагов и в различных свиноводства. В нашей лаборатории был опробован препарат метилового хлорфоса, меченный P32, с удельной активностью 250 мккрей.

При наружной обработке лабораторных животных, в том числе крупного рогатого скота было установлено, что хлорфос в первые минуты после нанесения на кожу обнаруживается в крови, через 15 минут достигает максимальной величины, затем постепенно снижается и к 6-7-му дню не обнаруживается.

Исследование проб мочи конюх коровы показало, что через 30 минут в моче появляются следовые количества P32 хлорфоса, через 6 часов активность образов достигает максимальной величины, а затем уменьшается. На 6 - 7-й день радиоактивность фосфор хлорфоса в моче обнаружить не удалось.

Были получены данные о том, что в коже достигает максимальной величины через 6 часов после нанесения на кожу и длится на высоком уровне до 8-10 часов. После 6 часов содержание P32 хлорфоса в моче оживает и на 3-ий день становится в 10 раз меньше максимальной величины.

Однако при 18-24 часах после обработки активность 1 мк мочи достигает 900 рел/мин. Были получены данные о том, что в коже зарегистрированы через 2 часа после наружной обработки коровы меченным хлорфосом.

Максимальное количество P32 в коже обнаруживается через 18 часов, после чего уменьшается и к 18 дням не обнаруживается.

Возможно, что радиоактивным методом определения P32 можно провести более точный и надежный анализ поглощения хлорфоса.
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к продуктов его распада проводилась на бумаге марки "Б" Ленинградской фабрики. В качестве подложного растворителя использовался смесь, состоящая из 2,5 частей 0,2%-ного раствора соляной кислоты и 7,5 частей 95%-ного этанола.

Проявлением пятен итальянская свинка в течение 2-5 минут. Соединения, содержащие фториды эфиров проникались в виде булавообразных пятен. Неорганические фосфаты обнаруживались в виде желтых пятен. В качестве свидетелей использовались препараты в растворах соляной кислоты, метилформиловой кислоты и ДМФА, а также заготовленные в виде порошков, соответствующие различным фазам в кислотах и воде.

Кроматография моции и экстрактов печени подопытных животных, забитых в различные сроки после наружной обработки хлорофосом, показала отсутствие патен, а также заготовленные в виде порошков, соответствующие различным фазам в кислотах и воде.

В моции и экстрактах печени крыс, которым хлорофос вводился регулярно, были обнаружены соединения, а также заготовленные в виде порошков, соответствующие различным фазам в кислотах и воде.

Изучение кинетики активности крови, моции и внутренних органов подопытных животных подтвердило динамику вызываемых и нивелирующих организма препаратом хлорофосом.

DISCUSSION

R. von BORSTEL: What other insecticides are you planning to use in your future research?

G.V. FILATOY: Phosphoric organic compounds, mainly of the Trole and Rogor types, and also carboxamides of the Sevin type.

J. HALLENSTEIN: Were any specific assay techniques used for determining the original compound and its breakdown products in tissues, organs and excreta?

G.V. FILATOY: For this purpose we used the chromatographic method, and also inhibition of the choline esterase of standard serum.
STUDIES ON THE SKI
insects, while it is highly toxic
distribution of toxicants in the
toxicants.
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definite relationships were
echollinesterase to toxicants as
existed among these insects.
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Electron microscope of
chewing insects were epidermis
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The author came to the
difference of their metabolism
sensitivity of insect cholinester
persons in insect bodies. The
which acts as a barrier against
the selective toxicity of Schiz

ÉTUDE DE LA TOXICITÉ
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distribution des substances tox
leurs métabolisme.
On a observé des différen
dans le métabolisme du Schrad
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la toxicité. Par contre, on a obser
l’action de Schradan-
que ceux des in
L’observation au microscope
des insectes broyeurs
acides sont entourés d’une in
L’auteur est parvenu à la
pas aux différences de métab.
sensibilité de la cholinesterase
Schradan dans l’organisme des
et la nature de la gaine des re
stimuler les principaux facteurs d

ИЗУЧЕНИЕ ОБСЯЗЫВАЮЩИХ
метод для определения
некоторых вредных
веществ в
насекомых и о их
STUDIES ON THE SELECTIVE TOXICITY OF SCHRADAN

TETSUO SAITO
LABORATORY OF APPLIED ENTOMOLOGY, FACULTY OF AGRICULTURE,
NAGOYA UNIVERSITY, ANJO, AICHI, JAPAN

Abstract — Résumé — Аннотация — Resumen

STUDIES ON THE SELECTIVE TOXICITY OF SCHRADAN. Schradan is of low toxicity to chewing insects, while it is highly toxic to sucking insects. The selective toxicity of Schradan may be associated with distribution of toxicants in insect bodies, susceptibilities of insect cholinesterases to toxicants and metabolism of toxicants.

The absorption, excretion and metabolic rates of $^{32}$P-Schradan in these insects varied considerably and no definite relationships were found between these factors and toxicities, nor found between susceptibility of cholinesterases to toxicants and toxicities; but quantitative differences in $^{32}$P-Schradan distribution patterns existed among these insects. Much more $^{32}$P-Schradan accumulated in the central nervous system of sucking insects than in those of chewing insects. Isolated nervous tissues of sucking insects absorbed much more $^{32}$P-Schradan than those of chewing insects.

Electron microscope observations on the central nervous system disclosed that the thoracic ganglia of chewing insects were enclosed in a thick and robust sheath, while those of sucking insects were surrounded by simple, thin double membranes.

The author came to the conclusion that the selective toxicity of Schradan to insects was not due to the difference of their metabolizing activities of Schradan as regards activation and detoxication, nor to the sensitivity of insect cholinesterases to activated Schradan, but to the differences of Schradan distribution patterns in insect bodies. Thus the distribution of toxicants in insects and the characters of the nerve sheath, which acts as a barrier against the penetration of toxicants, may be the most important factors responsible for the selective toxicity of Schradan.

ÉTUDE DE LA TOXICITÉ SÉLECTIVE DU SCHRADAN. Le Schradan est peu toxique pour les insectes buveurs, alors qu'il l'est extrêmement pour les insectes suceurs. Sa toxicité sélective pourrait dépendre de la distribution des substances toxiques dans l'organisme de l'insecte, de leur action sur la cholinestérase et de leur métabolisme.

On a observé des différences considérables entre ces insectes, dans les taux d'absorption et d'excrétion et dans la métabolisation où Schradan marqué par $^{32}$P, mais on n'a pas pu établir de rapport bien défini entre ces facteurs et la toxicité, pas plus qu'entre la sensibilité de la cholinestérase aux substances toxiques et leur toxicité. Par contre, on a relevé des différences quantitatives dans la distribution du Schradan- $^{32}$P. L'accumulation de Schradan- $^{32}$P a été beaucoup plus forte dans le système nerveux central des insectes suceurs que dans celui des insectes buveurs. De même, les tissus nerveux isolés des poils ont absorbé beaucoup plus de Schradan- $^{32}$P que ceux des insectes buveurs.

L'observation au microscope electronique des gaines dans les canaux nerveux a révélé que les ganglions thoraciques des insectes buveurs sont recouverts d'une gaine épaisse et robuste, tandis que ceux des insectes suceurs sont recouverts d'une membrane simple ou d'une membrane mince et double.

L'auteur est parvenu à la conclusion que la toxicité sélective du Schradan pour les insectes ne tient pas aux différences de métabolisation du Schradan au cours des phasès d'activation et de détoxication, ni à la sensibilité de la cholinestérase au Schradan marqué, mais qu'elle est due aux différences de distribution du Schradan dans l'organisme des insectes. La distribution des substances toxiques dans l'organisme des insectes et la nature de la gaine des nerfs, qui s'oppose à la pénétration des substances toxiques, pourraient donc constituer les principaux facteurs de la toxicité sélective du Schradan.

ИССЛЕДОВАНИЕ СЕЛЕКТИВНОЙ ТОКСИЧНОСТИ СХРАДАНА. Представляя мало токсичен для насекомых, но очень токсичен для сосущих насекомых. Селективность токсичности вредна может быть связана с различиями в токсических веществ в тех насекомых, которые представляют хтонистеразы насекомых и токсичные веществ, метаболизмы последних.
TOXICITY

Rice is not
American co.
House-fly
Green rice
Black rice
Rice bug

Schradan is conv.
esterase in the centra
Consequently the sel
follow the following three main
(1) Differences
(2) Differences:
and
(3) Differences:
detoxification:

ABSORB
BY VARIOUS

INTRODUCTION

Generally, a given insecticide is not always equally effective against all kinds of insects. Systemic insecticides, which are absorbed by a plant and translocated to other parts of the plant, so that insecticidal activity is transferred to untreated parts, are effective against sucking insects, but not so effective against chewing insects.

Schradan, which is one of the systemic insecticides, was found to be of low toxicity for the larva of the rice stem-borer, Chilo suppressalis Walker, the adult of the American cockroach, Periplaneta americana L., and the adult of the house-fly, Musca domestica vicina Macq., while it was highly toxic for the adult of the rice bug, Lepidocoris variicornis Fabricius, the black rice bug, Scutinophara lurida Burmeister, and the green rice leafhopper, Nephotettix bipunctatus intimatep Uhler (Table 1).
### Table I

<table>
<thead>
<tr>
<th>Insect</th>
<th>LD50 (µg/g)</th>
<th>Regression coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice stem-borer</td>
<td>&gt;36,222</td>
<td>--</td>
</tr>
<tr>
<td>American cockroach</td>
<td>2,170</td>
<td>0.201</td>
</tr>
<tr>
<td>Horse-fly</td>
<td>1,662</td>
<td>0.242</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>160</td>
<td>0.149</td>
</tr>
<tr>
<td>Black rice bug</td>
<td>92</td>
<td>0.394</td>
</tr>
<tr>
<td>Rice bug</td>
<td>23</td>
<td>-</td>
</tr>
</tbody>
</table>

Schradan is converted, by enzymatic oxidation in the insect body, into oxidized Schradan, a strong anticholinesterase. The inhibition of cholinesterase in the central nervous system may be the principal action of Schradan. Consequently the selective toxicity of Schradan may be associated with the following three main factors:

1. Differences in the distribution of toxicants in insect bodies;
2. Differences in the susceptibility of insect cholinesterase to toxicants; and
3. Differences in the metabolism of toxicants as regards activation and detoxification.

### Table II

<table>
<thead>
<tr>
<th>Insect</th>
<th>Absorption/excretion (%)</th>
<th>After 1 h</th>
<th>After 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Outer</td>
<td>Internal</td>
</tr>
<tr>
<td>Rice stem-borer</td>
<td>24.5</td>
<td>3.7</td>
<td>68.8</td>
</tr>
<tr>
<td>American cockroach</td>
<td>31.3</td>
<td>17.7</td>
<td>41.2</td>
</tr>
<tr>
<td>Horse-fly</td>
<td>11.5</td>
<td>1.6</td>
<td>83.7</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>26.6</td>
<td>3.3</td>
<td>76.1</td>
</tr>
<tr>
<td>Black rice bug</td>
<td>17.8</td>
<td>18.7</td>
<td>68.5</td>
</tr>
<tr>
<td>Rice bug</td>
<td>19.8</td>
<td>3.1</td>
<td>77.3</td>
</tr>
</tbody>
</table>
RESULTS

(a) Distribution of Schradan in various insect species

No quantitative differences concerning the absorption and excretion capacities of Schradan were observed as between the susceptible and non-susceptible insects (Table II). There were, however, quantitative differences in the Schradan distribution patterns. Much more Schradan accumulated in the central nervous systems of susceptible insects than in those of non-susceptible insects (Tables III, IV).

(b) Cholinesterase inhibition of Schradan in various insect species

Schradan itself is a very weak anticholinesterase, but oxidized Schradan is a powerful anticholinesterase, oxidation enhancing the activity by as much as 1000-10 000 times. The susceptibility of the cholinesterases of the various insects to Schradan and to oxidized Schradan varied considerably, and no definite relationships were found between enzyme inhibition and toxicity (Table V).

(c) Metabolism of Schradan in various insect species

Schradan was activated and detoxicated by the species studied. The metabolic rates of Schradan in those species varied considerably and no definite relationships were found between metabolism and toxicity (Table VI).

(d) Penetration of Schradan and oxidized Schradan to isolated nervous tissues in various insect species

Isolated nervous tissues of susceptible insects absorbed much more Schradan than those of non-susceptible insects (Table VII), and desheathed nervous tissues of cockroach absorbed much more Schradan and oxidized Schradan than untreated tissues (Table VIII).

(e) Electron-microscope observation of the ganglionic sheaths of various insect species

Electron-microscope studies on the central nervous sheath showed that the thoracic ganglia of non-susceptible insects were enclosed in a thick and robust sheath, while those of susceptible insects were surrounded by single or thin double membranes.

DISCUSSION

Several hypotheses have been put forward to explain the physiological selectivity of Schradan. CASIDA et al. have shown that the susceptibility of insects to Schradan depends upon the sensitivity of the particular insect cholinesterase for the activated Schradan and that the cholinesterase from the non-susceptible insects required 6-24 times as much activated Schradan.
TABLE III

DISTRIBUTION OF SCHRADAN IN THE TISSUES OF VARIOUS INSECTS TREATED WITH 500 µg/g P32-SCHRADAN BY TOPICAL APPLICATION

(Percentages)

<table>
<thead>
<tr>
<th>Insect</th>
<th>Nerve cord</th>
<th>Thoracic ganglion</th>
<th>Gut</th>
<th>Fat</th>
<th>Cora</th>
<th>Reproductive system</th>
<th>Remainder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice room-borer</td>
<td>0.903</td>
<td>-</td>
<td>0.202</td>
<td>0.490</td>
<td>-</td>
<td>-</td>
<td>0.462</td>
</tr>
<tr>
<td>American cockroach</td>
<td>0.151</td>
<td>-</td>
<td>3.146</td>
<td>1.113</td>
<td>1.971</td>
<td>0.147</td>
<td>10.623</td>
</tr>
<tr>
<td>Horse-fly</td>
<td>-</td>
<td>0.583</td>
<td>0.403</td>
<td>0.090</td>
<td>0.105</td>
<td>0.138</td>
<td>2.132</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>-</td>
<td>0.583</td>
<td>0.293</td>
<td>-</td>
<td>0.293</td>
<td>-</td>
<td>16.017</td>
</tr>
<tr>
<td>Black rice bug</td>
<td>-</td>
<td>0.500</td>
<td>1.400</td>
<td>0.500</td>
<td>0.900</td>
<td>0.300</td>
<td>22.550</td>
</tr>
<tr>
<td>Rice bug</td>
<td>-</td>
<td>0.500</td>
<td>2.850</td>
<td>1.500</td>
<td>2.550</td>
<td>0.875</td>
<td>25.119</td>
</tr>
</tbody>
</table>

SELECTIVE TOXICITY OF SCHRADAN
TABLE IV

DISTRIBUTION OF SCHRADAN IN THE TISSUES OF VARIOUS INSECTS TREATED WITH 500 µg/g P92-SCHRADAN BY TOPICAL APPLICATION

(Amounts, µg/mg)

<table>
<thead>
<tr>
<th>Insect</th>
<th>Nerve cord</th>
<th>Thoracic ganglion</th>
<th>Gut</th>
<th>Fat</th>
<th>Cora</th>
<th>Reproductive system</th>
<th>Remainder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice stem-borer</td>
<td>0.018</td>
<td>-</td>
<td>0.011</td>
<td>0.008</td>
<td>-</td>
<td>-</td>
<td>0.008</td>
</tr>
<tr>
<td>American cockroach</td>
<td>0.088</td>
<td>-</td>
<td>0.138</td>
<td>0.038</td>
<td>0.056</td>
<td>0.044</td>
<td>0.073</td>
</tr>
<tr>
<td>Horse-fly</td>
<td>-</td>
<td>0.025</td>
<td>0.007</td>
<td>0.007</td>
<td>0.017</td>
<td>0.008</td>
<td>0.007</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>-</td>
<td>0.100</td>
<td>0.018</td>
<td>0.028</td>
<td>-</td>
<td>-</td>
<td>0.024</td>
</tr>
<tr>
<td>Black rice bug</td>
<td>-</td>
<td>1.800</td>
<td>0.048</td>
<td>0.085</td>
<td>0.183</td>
<td>0.142</td>
<td>0.121</td>
</tr>
<tr>
<td>Rice bug</td>
<td>-</td>
<td>0.500</td>
<td>0.097</td>
<td>0.100</td>
<td>0.360</td>
<td>0.350</td>
<td>0.095</td>
</tr>
</tbody>
</table>

CONCLUSION

The author considers that the effects observed in the various insects were due to the mode of action of Schradan, which is non-susceptible to the cholesterol esterase. The results show that the insecticide has a synergistic effect on the cholinesterase and that the cholinesterase is not inhibited by Schradan, as indicated by the results of the experiments. The nature of the decrease in the cholinesterase activity is not clear, but it is thought to be associated with the presence of Schradan in the tissues of the insects. The data also show that the mode of action of Schradan is not due to inhibition by the insecticide, as is the case with other insecticides.
for inhibition as that of the susceptible insects [1, 2]. O'BRIEN and SPENCER (3, 4, 5), on the other hand, considered that the physiological selectivity of Schradan was not due to the sensitivity of the particular insect cholinesterase to the Schradan metabolite, but that susceptible insects converted Schradan to the cholinergic oxidation product slowly within the nerve tissue, while in the non-susceptible species the conversion rate in the fat body of the insect was so great that little or no unconverted Schradan reached the nerve tissue and the oxidation product of Schradan was too unstable to penetrate the lipid nerve sheath. They proposed the hypothesis that non-susceptible insects have a barrier which prevents the penetration of the converted Schradan to the site of action. TSUYUKI et al. suggested (6) that the active anti-cholinesterase agent was isomerized to a much more stable component, the methyl ether. They considered that the selective toxicity of Schradan might be associated with this isomerization.

CONCLUSION

The author came to the conclusion that the selective toxicity of Schradan to insects was not due to differences in their metabolism of Schradan as regards activation and detoxication, nor to the sensitivity of insect cholinesterases to activated Schradan, but to the differences of Schradan distribution patterns in insect bodies. Thus the distribution of toxicants in insects and the nature of the nerve sheath which acts as a barrier against the penetration of toxicants may be the most important factors responsible for the selective toxicity of Schradan.
### Table VI

**Per-Cent Metabolism of P32-Schradan**

Indicated by slice of tissue in various insect species

(30°C, 3 h)

<table>
<thead>
<tr>
<th>Insect</th>
<th>Schradan</th>
<th>Oxidized Schradan</th>
<th>Hydroxates</th>
<th>Protein Incorporated</th>
<th>Corrected Hydroxates</th>
<th>Corrected Oxidized Schradan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice stem-borer</td>
<td>94.8</td>
<td>1.2</td>
<td>3.8</td>
<td>0.2</td>
<td>3.6</td>
<td>1.5</td>
</tr>
<tr>
<td>American cockroach</td>
<td>90.2</td>
<td>1.0</td>
<td>7.9</td>
<td>0.9</td>
<td>7.0</td>
<td>2.8</td>
</tr>
<tr>
<td>House-fly</td>
<td>93.4</td>
<td>1.7</td>
<td>4.8</td>
<td>0.4</td>
<td>4.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>92.6</td>
<td>1.2</td>
<td>5.6</td>
<td>0.6</td>
<td>5.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Rice bug</td>
<td>97.0</td>
<td>0.8</td>
<td>1.8</td>
<td>0.2</td>
<td>1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>
### Table VII

**Absorption of $P^{32}$-Schradan by Isolated Nerve Tissues of Various Insects Immersed in Phosphate-Buffer Ringer Solution of 0.1% Schradan (pH 7.1, 30°C, after 60 min)**

<table>
<thead>
<tr>
<th>Nerve tissue</th>
<th>Tissue weight (mg)</th>
<th>Absorbed Schradan (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice stem-borer</td>
<td>0.16</td>
<td>0.313</td>
</tr>
<tr>
<td>American cockroach</td>
<td>11.20</td>
<td>0.528</td>
</tr>
<tr>
<td>House-fly</td>
<td>0.16</td>
<td>0.506</td>
</tr>
<tr>
<td>Green rice leafhopper</td>
<td>0.06</td>
<td>0.583</td>
</tr>
<tr>
<td>Black rice bug</td>
<td>0.10</td>
<td>0.770</td>
</tr>
<tr>
<td>Rice bug</td>
<td>0.14</td>
<td>0.621</td>
</tr>
<tr>
<td>Desheathed American cockroach</td>
<td>9.90</td>
<td>0.612</td>
</tr>
<tr>
<td>Insecticides</td>
<td>Concentration (%)</td>
<td>Exposure time (hr)</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Schradan</td>
<td>0.0114</td>
<td>40</td>
</tr>
<tr>
<td>Schradan in 50% ethanol</td>
<td>0.0114</td>
<td>40</td>
</tr>
<tr>
<td>Schradan in 95% ethanol</td>
<td>0.0114</td>
<td>40</td>
</tr>
</tbody>
</table>

S. FUZEAU-FECTicide, and if so or non-susceptible, T. SAITO: The susceptible or resistant species which had been available for J, E. CASIDA: T. SAITO: T. permanganate oxic chromatography, I fraction was then a chromatography, anticholine: K van ASPER E. that resistant insects so quickly that very the nerve sheath co in toxicity in different differences in distribution.
T. SAITO: I think that the different enzymatic and C.D. HASSET problem of the insecticide is a relatively Roeder and colleagues that the insect nor same way as the n.
J, E. CASIDA: in saying that O'Bri barrier in the reservoir, it is easy for more or less in the pound acts on an in the more one come to a single factor, of the nerve sheath distributing factor.
SELECTIVE TOXICITY OF SCHRADAN

REFERENCES


DISCUSSION

S. FUZEAU-BRAESCH: Do you possess strains resistant to this insecticide, and if so can you compare insects of the same species, susceptible or non-susceptible, instead of comparing different species?

T. SAITO: The study reported compares species which were naturally susceptible or resistant to Schradan. No strains of any of the susceptible species which had acquired resistance through treatment with this compound were available for study.

J.E. CASIDA: How did you prepare oxidized Schradan?

T. SAITO: The oxidized D-32-Schradan was prepared by potassium permanganate oxidation from D-14C-Schradan and separated by silica-gel chromatography, by the method described in ref. [6] to the paper. One fraction was then analysed by means of infra-red spectra, paper chromatography, anticholinesterase activity and chloroform-water partition coefficient.

K. van ASPEREN: I have an idea that O'Brien and Spencer suggested that resistant insects may oxidize the Schradan outside the nervous system so quickly that very little penetration of the new more polar substance through the nerve sheath could occur. If that is true, their explanation of differences in toxicity in different insects is more or less similar to yours, i.e. a difference in distribution. However, my memory may mislead me.

T. SAITO: I had some correspondence with O'Brien on this problem, I think that the different results may be due to the different methods. O'Brien used enzymatic analysis, but I am analysing radiometrically in situ.

C.C. HASSETT (Chairman): I might make a general comment on the problem of the insect nerve sheath in relation to Dr. Saito's work. This sheath is a relatively impermeable barrier to drugs and it was not until Roeder and colleagues devised the technique of removal that it was found that the insect nervous system responded to various agents in much the same way as the mammalian system.

J.E. CASIDA: Perhaps I could also add a comment. Dr. Saito is right in saying that O'Brien and Spencer postulated that the nerve sheath was a barrier in the resistant species more than in the susceptible species. However, it is easy for the author of a paper to throw out several hypotheses more or less in passing; as you know, the more one works with how a compound acts on an insect in relation to resistance, be it natural or acquired, the more one comes to the conclusion that the effects cannot be pinned down to a single factor. I think the evidence Dr. Saito has presented for the role of the nerve sheath shows better than anything else that this is a major contributing factor.
D. F. HEATH: Your results undoubtedly indicate that differences in distribution are important, but taking the results as a whole, one would conclude that the house-fly should be the most susceptible species, since house-fly anticholinesterase was found to be inhibited at very much lower concentrations than the anticholinesterase of any other species, and this would quite outweigh the other effects you observed.

This does not necessarily mean that your conclusions are wrong. The concentration of inhibitor in the nervous system as a whole can only be a very crude indicator of the concentration at the synapses. Desheathing a locust ganglion preparation increases the sensitivity of the preparation to eserine and acetylcholine, but the ganglion is still much less sensitive than mammalian ganglia. This and other evidence suggests that the synapses are protected by additional membranes. Consequently, the concentration of inhibitor in the synaptic region cannot be assumed to be similar to the concentration in a whole nerve. It would be very useful to determine inhibitor concentrations in the synaptic regions, but I have no idea of how this could be done.
that differences in
le one would con-
ies, since house-
uch lower concen-
, and this would
are wrong. The
ple can only be a
De-sheathing a
preparation to
less sensitive than
the synapses are
concentration of
similar to the con-
determine inhibitor
of how this could

IV.

INSECT METABOLISM: TRACER APPLICATIONS
STUDIES ON THE UTILITY OF CHOLESTEROL IN THE GROWTH AND DEVELOPMENT OF THE HOUSE FLY, *MUSCA DOMESTICA* L.

The utilization of cholesterol in adult flies is an important aspect of their diet. In the House Fly, *Musca domestica* L., cholesterol is used in the mobilization of growth and development. The study of cholesterol utilization helps in understanding the nutritional requirements of the fly, which can have implications in pest management. The quantification of cholesterol in the fly's diet is crucial for its proper growth and development. This research also has significance in the broader context of entomology, where understanding the dietary habits of insects is essential for controlling pest populations.
STUDIES ON THE UTILIZATION, METABOLISM AND FUNCTION OF STEROLS IN THE HOUSE-FLY, MUSCA DOMESTICA

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Abstract — Résumé — Anotaciones — Resumen

STUDIES ON THE UTILIZATION, METABOLISM AND FUNCTION OF STEROLS IN THE HOUSE-FLY, MUSCA DOMESTICA. Insects generally have been found to require a dietary source of sterol for normal larval growth and metamorphosis. Our work has pointed to two additional physiological roles for sterols in the house-fly, Musca domestica L.: (1) A dietary source of sterol is essential for sustained viable egg production in the female fly; on a sterol-deficient diet eggs are produced but hatch and viability are low. (2) Cholesterol is also involved in the mobilization and utilization of nutrient reserves associated with the initiation of ovarian maturation in the female fly.

The quantitative sterol requirements for the above physiological processes and the metabolic conversions that occur during growth, metamorphosis and reproduction have been studied in this insect, using C\(^4\) - and H\(^2\)-labeled sterols in conjunction with a variety of analytical tools, including reverse isotope dilution, gas-liquid chromatography and spectroscopy, and employing aseptic rearing techniques and semi-defined larval and adult diets.

Both C\(^4\)-cholesterol and H\(^2\)-\(\beta\)-sitosterol have been used as a source of sterol in either the larval or the adult diet of the house-fly, and the pattern of utilization and metabolism was found to be almost identical for these two sterols. However, there was no detectable conversion of \(\beta\)-sitosterol to cholesterol.

Sub-minimal quantities of cholesterol have also been used in the larval diet in combination with "sparing sterol" such as cholestrol, which will suffilce in part but not entirely the sterol requirement of this insect. The utilization and fate of the "spearing sterol" has been investigated using C\(^4\)-cholesterol, and the metabolism of the minimum quantity of essential cholesterol is currently under study using high-specific activity C\(^4\)-cholesterol.

Other species of insects, including the German cockroach (Blattella germanica), have been examined in relation to the patterns of utilization and the metabolic pathways for sterols found in the house-fly.

UTILISATION, MÉTABOLISME ET FONCTIONS DES STÉROLS CHEZ LA MOUCHE DOMESTIQUE (MUSCA DOMESTICA). On a déterminé que, de façon générale, les insectes ont besoin de stérols dans leur régime alimentaire pour que leurs larves se développent et se métamorphosent normalement. Les travaux de l'année ont fait apparaître deux autres fonctions physiologiques des stérols chez la mouche domestique (Musca domestica L.): 1. Il est indispensable que le régime alimentaire de la femelle contienne des stérols pour que celle-ci puisse régulièrement des œufs viables; s'il y a carence de stérols, la femelle continue à pondre mais son faible pourcentage des œufs parvient à éclore et sont viables; 2. le cholestérol joue également un rôle dans l'utilisation des réserves nutritives dont s'accompagne le début du développement des œufs.

On a étudié chez cet insecte les quantités de stérols qui sont nécessaires pour que les processus physiologiques ci-dessus se déroulent, ainsi que les transformations métaboliques qui se produisent au cours du développement, de la métamorphose et de la reproduction. Cette étude a été faite à l'aide de stérols marqués par C\(^4\) et H\(^2\), en appliquant diverses méthodes analytiques: dilution isotope inversée, chromatographie à phase gazeuse et phase liquide, spectroscopie; on a recours à des méthodes d'élevage en milieu aseptique et on a étudié les régimes alimentaires semi-synthétiques pour les larves et les adultes.

Le cholestérol marqué par C\(^4\) et le sitostérol-\(\beta\) marqué par H\(^2\) aux été utilisés comme sources de stérols dans le régime alimentaire de la mouche domestique aux stades larvaire et adulte; il s'est révélé que l'utilisation et le métabolisme de ces deux stérols étaient à peu près identiques. Toutefois, on n'a pas observé de transformation du sitostérol-\(\beta\) en cholestérol.
INTRODUCTION

In 1935, HOBS sericata, require a ment. Since this d representing seversterol requirementean dietary or exogen metamorphosis [2]. been considered to h this has been confirster precursors ar of the more primiti of biosynthesis [11,12]. Although chole has been found to su and phytophagous in lieu of cholesterin beetles, Dermestes related C-27 sterol sect, CLARK et al, vided us with a work of sterols in insect grow and mature esterol if the diet will not fulfill the w they have proposed the "staring-sterol quicken of Derm cellular component to serve a metabo sterol metabolites, C+cholesterol by tions, CLAYTON requirements of thi that in insects cho cannot be fulfilled. 1

In addition to L ported to play a ro phytoestrogens appear mori, [16,17] and essential for norm germanica [18]. In to be involved in the for sustained viab by KOBAYASHI et e
INTRODUCTION

In 1985, HOBSO [1] reported that larvae of the blowfly, Phaenicia sericata, require a dietary source of sterol for normal growth and development. Since this discovery, the immature stages of a number of insects, representing several of the major orders, have been examined as to their sterol requirement and every insect studied has been found to require a dietary or exogenous source of sterol for normal larval growth and metamorphosis [2]. This nutritional requirement for sterols has generally been considered to indicate the absence of sterol biosynthesis in insects and this has been confirmed biochemically in several species, using C14-labelled sterol precursors [3-10]. However, there is some indication that certain of the more primitive insects may be capable of some degree of cholesterol biosynthesis [11, 12].

Although cholesterol has generally been used as the dietary sterol and has been found to support growth in every species studied, many omnivorous and phytophagous insects can also use certain C-28 and C-29 phytosterols in lieu of cholesterol. For certain carnivorous insects, such as the hide beetle, Dermestes maculatus, however, only cholesterol and certain closely related C-27 sterols are satisfactory sterol sources [13, 14]. Using this insect, CLARK et al. [14], in a particularly significant study, recently provided us with a working hypothesis for investigating both the fate and function(s) of sterols in insects. These workers have shown that the hide beetle will grow and mature on a larval diet containing sub-minimal quantities of cholesterol if the diet is supplemented by certain "sparing-sterols" which alone will not fulfill the sterol requirement of this insect. Based on their work, they have proposed a dual role for sterols in insects. They postulate that the "sparing-sterols", which will replace up to 97% of the cholesterol requirement of Dermestes, serve in a structural capacity, as cellular or subcellular components. The remaining irreplaceable cholesterol is believed to serve a metabolic role, perhaps as a precursor for physiologically active sterol metabolites. However, in a subsequent study on the metabolism of C14-cholesterol by the cockroach, Blatta orientalis, under "sparing" conditions, CLAYTON et al. [15] found that the minimum essential cholesterol requirements of this insect were not completely metabolized and suggested that in insects cholesterol may also have a specific structural role which cannot be fulfilled by the "sparing-sterols".

In addition to larval growth and metamorphosis, sterols have been reported to play a role in several other physiological processes. Certain phytosterols appear to act as phagostimulants for silkworm larvae, Bombyx mori, [16, 17] and a dietary source of sterol has been reported to be essential for normal reproduction in the German cockroach, Blatta germanica [18]. In the house fly, Musca domestica, we have found sterols to be involved in the initiation of ovarian development [19] and to be necessary for sustained viable egg production [20, 21]. Finally, the recent reports by KOYABASHI et al. [22, 23] that cholesterol has "brain hormone" activity...
in debrained, diapausing silkworm pupae, suggest that in insects sterols may either serve as hormones per se or as precursors to hormones.

From this brief review it is apparent that sterols are either involved in or essential to a number of the important physiological and biochemical processes of insects.

Information on the biochemical and physiological function(s) of sterols and their metabolic fate in insects is not only important for a more complete knowledge of insect physiology and biochemistry, but should also add to our general understanding of the role(s) of neutral sterols in living systems generally.

Both the minute quantities of cholesterol required, especially when used in combination with other sterols, and the small size of many insects, make it difficult to follow the uptake, utilization, metabolism, and transfer of cholesterol from one development stage to another. Radioisotope-labelled sterols, which have been extensively used in this area of research, have not only provided an extremely sensitive and efficient technique for following the above processes, but have permitted certain experiments to be carried out with ease which otherwise would have been extremely difficult, if not impossible.

The purpose of this paper is to briefly report on certain facets of our research on the function(s) and metabolic fate of sterols in the house fly, Musca domestica, and in so doing to illustrate some applications of radioactive-tracer techniques to this area of insect physiology.

MATERIALS AND METHODS

The house flies used in these studies were from a 1948 NAIDM strain. The stock colonies were reared by the CSMA procedure [24] and fed an adult diet of dry defatted milk-sucrose (1:1). Unless otherwise noted, a semi-defined adult diet [21] and an aseptic semi-defined larval diet [28] were employed in these experiments. The "vitamin-free" casein used in preparing these diets was exhaustively extracted to remove sterols. The $^{14}$C- and $^{3}$H-labelled sterols were repurified just before use and their radiochemical purity checked by several analytical methods. All radioassays were made on replicated "weightless" samples using windowless gas-flow proportional counters. Gas-liquid chromatographic analyses of the sterols were made using the systems of VANDENHEUVEL et al. [28].

STEROLS AND REPRODUCTION

In contrast to the numerous studies on the requirement for sterols by immature insects, our knowledge of the role of sterols in the adult insect is extremely limited. MONROE [20, 21], in a study from our laboratory, has shown that flies fed on a sterol-deficient semi-defined diet lived as long and produced approximately the same number of eggs as flies fed on the same diet containing cholesterol. However, the cholesterol-deficiency caused an overall reduction of about 90% in egg hatch or viable egg production. When flies were fed on the semi-defined diet containing cholesterol, egg hatch ranged from 92 to 98% throughout all the egg collections. When cholesterol was omitted from the diet, until by the fourth instar, no eggs were laid at all; the insects simply died. In contrast, when the eggs of flies fed on a sterol-deficient semi-defined diet were transferred to a sterol-supplemented diet, there was a significant increase in egg hatch and viable egg production.

The production of sterol-free eggs suggests that sterols are essential for reproduction in the house fly. However, the specific sterol required for reproduction is unknown.

The above studies suggest that sterols are required for reproduction in the house fly. However, the specific sterol required for reproduction is unknown.

From the above data, it is clear that sterols are essential for reproduction in the house fly. However, the specific sterol required for reproduction is unknown.

An earlier study by VANDENHEUVEL et al. [28] showed that sterols are essential for reproduction in the house fly. However, the specific sterol required for reproduction is unknown.

Eggs from flies containing $0.2\% \text{C}_{14}-\text{cholesterol}$ and $0.1\% \text{C}_{14}-\text{cholesterol}$ were found to contain sterol-like substances. When the crude extracts were analyzed by column chromatography, peaks representing...
was omitted from the diet, egg hatch was initially lower and decreased rapidly until by the fourth egg collection it was only 5%. If at this time these flies were given a diet containing cholesterol, the egg hatch was found to increase progressively until by the ninth collection it was similar to that found for the eggs of flies fed the diet plus cholesterol. In addition, only about 50% of the larvae from the eggs from flies fed on the sterol-deficient diet produced adults as compared with 91% of those from the diet containing cholesterol.

The production of some viable eggs up to about the fourth egg collection suggested a physiological mechanism whereby the female fly may use sterols stored from the larval diet for the early groups of eggs produced. The presence of such a storage mechanism was confirmed in a subsequent study [27].

The above-mentioned finding prompted us to examine the relative contribution of the stored sterols and the sterols from the adult diet to the total sterol content of the eggs. This was accomplished by using either C14-labelled or unlabelled cholesterol in the larval diet and C14-cholesterol in the adult diets and then examining the first groups of eggs produced for both the sterol content and the specific activity of the sterols present. Under our rearing conditions, and using optimal concentrations of cholesterol in both the larval and adult diets, we have found that as much as 75% of the sterol present in the first group of eggs produced originates from the stored reserves of the female fly. However, when flies are reared on larval diets containing combinations of "sparring sterols" and sub-minimal quantities of cholesterol, the adult females, which contain very little, if any, excess cholesterol, then must depend almost wholly on the sterols from the adult diet for egg production [28].

### STEROLS OF HOUSE-FLY EGGS

From the above studies, it is apparent that the sterols present in the house-fly egg are essential for both normal embryonic development and early larval growth. These findings have prompted us to undertake a thorough study on both the amount and nature of the sterols present in the eggs and to examine any qualitative or quantitative changes that may occur in the sterols during these early developmental stages.

An earlier study [29], in which we injected C14-cholesterol into adult female flies reared by the CSMA procedure, provided us with some qualitative information on the egg sterols. The injected C14-cholesterol was efficiently incorporated into the eggs where it was found to be present both in the free and esterified form. A portion of the C14-cholesterol in the egg and adult fly had been converted to its 5,7-diene, 7-dehydro-cholesterol. However, because of the presence in these eggs of phytosterols from the adult flies and unlabelled cholesterol from the adult diet, the total sterol content could not be studied.

Eggs from flies reared aseptically on a semi-defined larval diet containing 0.2% C14-cholesterol and fed on a semi-defined adult diet containing 0.1% C14-cholesterol were used for the quantitative studies. These eggs were found to contain about 0.051 µg-equivalents of radioactive sterol per egg. When the crude total lipid from these eggs was fractionated into lipid classes by column chromatography on silicic acid, two major radioactive peaks, representing sterol esters and free sterols, were detected [30].
these eggs the sterol esters accounted for about 41% of the total C14-sterols present, but in some of our other studies we have found ester fractions as high as 80%. Analysis by ultra-violet (UV) spectroscopy of either the total lipids before fractionation or of the free sterol and sterol ester fractions indicated the presence of an appreciable amount of a 5,7-diene. For confirmation of this finding, the total sterols, as their acetates, were fractionated by chromatography on an alumina column which separates Δ5 and Δ5,7 sterols. Two major radioactive peaks were detected: The major peak (Δ5 fraction), which accounted for about 86% of the radioactive material, was identified as cholesterol by reverse-isotope dilution and purification through the dibromide and by its relative retention time in several gas-liquid chromatographic systems. The minor, more polar, peak (Δ5,7 fraction) contained about 13.8% of the total radioactivity and, when analysed by UV spectroscopy, was found to have a spectrum and molecular extinction coefficient, computed from the micromoles of radioactive sterol present, identical to 7-dehydrocholesterol [28]. Further confirmation of the identity of the compound has been made using gas-liquid chromatography and reverse-isotope dilution.

A summary of our findings on the sterol content of house-fly eggs is presented in Table 1. Although we have not determined the minimum sterol requirement for egg viability, we have found that as little as 0.015 μg total sterol per egg or 25 to 30% of the maximum sterol content we have observed, is sufficient to support normal embryonic development and early larval growth [28].

TABLE 1

<table>
<thead>
<tr>
<th>STEROL CONTENT OF HOUSE-FLY EGGS</th>
<th>Equivalent per egg (μg)</th>
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<tr>
<td>Total C14-sterols</td>
<td>0.061</td>
</tr>
<tr>
<td>Free sterols</td>
<td>0.029</td>
</tr>
<tr>
<td>Sterol esters</td>
<td>0.021</td>
</tr>
<tr>
<td>Polar compounds</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.043</td>
</tr>
<tr>
<td>7-Dehydro-cholesterol</td>
<td>0.007</td>
</tr>
</tbody>
</table>

We have also examined the fatty acid moiety of the sterol esters of the eggs and found these to be composed of greater than 92% mono-unsaturated C-16 and C-18 fatty acids, with C-18:1 accounting for about 76% and C-18:1 another 16% [30]. Cholesteryl oleate has recently been reported to be the major sterol ester in the cockroach, *Eurycotis floridana* [31].

An examination of the sterol content of newly-hatched house-fly larvae has shown that there is little, if any, detectable quantitative change in the sterol content of the eggs and newly-hatched larvae. However, about 80% of the sterol esters embryonic develop fractions appeared: continued using high-speed measurement of both

"SPARING" STUDIES

The house fly, I taking sub-minimal We have found that *Dermestes*, including fly larvae. When ad may replace great still provide for opti of studies in which we techniques for mals we have found that t more of the order of per insect.

An interesting efficient selective u diet by the house fly, of the concentration of this phenomenon in a 1:10 000 ratio to of the cholesterol av

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<table>
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<th>Sterol concen</th>
<th>(Dry wt)</th>
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<tr>
<td>0.2% Cholesterol</td>
<td>+ 0.01% C4-</td>
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We have study C14-cholesterol plus semi-defined larval came about 14.5 μg of C4- free sterol. Analysis tography showed that
of the sterol esters present in the egg are converted to free sterols during embryonic development. Both the cholesterol and 7-dehydro-cholesterol fractions appeared to remain constant [28]. These studies are being continued using high-specific-activity C\(^{14}\)-sterols which permit a more precise measurement of both the changes and overall balance of these sterols.

"SPARING" STUDIES

The house fly, like Dermestes [14], may be reared on a larval diet containing sub-minimal quantities of cholesterol plus a "sparing sterol" [32, 33]. We have found that a number of the sterols which "spare" cholesterol in Dermestes, including cholesterol, will perform this same function in house-fly larvae. When added to an aseptic semi-defined larval diet, these sterols may replace greater than 95% of the dietary cholesterol requirement and still provide for optimal growth and development. However, from a number of studies in which we have used different "sparing sterols" and tested various techniques for making the "essential" cholesterol available to the larvae, we have found that the actual "essential" cholesterol requirement may be more of the order of 0.5% of the total sterol requirement, or less than 0.1\(\mu\)g per insect.

An interesting phenomenon observed in these sparing studies is the efficient selective uptake and/or accumulation of cholesterol from the larval diet by the house fly, even when the cholesterol is present at only a fraction of the concentration of the sparing sterol (Table II). An extreme example of this phenomenon was observed in a study in which we used cholesterol in a 1:1000 ratio to the "sparing sterol" and found the pupae to contain 80% of the cholesterol available in the larval diet [28].

<table>
<thead>
<tr>
<th>Sterol concentration (Dry weight)</th>
<th>C(^{14})-sterols per insect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Available in diet ((\mu)g)</td>
</tr>
<tr>
<td>0.2% Cholesterol</td>
<td></td>
</tr>
<tr>
<td>+ 0.01% C(^{14})-cholesterol</td>
<td>3.06</td>
</tr>
<tr>
<td>+ 0.0005% C(^{14})-cholesterol</td>
<td>0.186</td>
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We have studied the fate of a sparing sterol in house-fly larvae using C\(^{14}\)-cholesterol plus sub-minimal quantities of cholesterol in an aseptic semi-defined larval diet. Pupae reared in this manner were found to contain about 14.5\(\mu\)g of C\(^{14}\)-sterol per insect, nearly all of which was present as free sterol. Analysis by reverse-isotope dilution and/or gas-liquid chromatography showed that there was no conversion of cholestanol to cholesterol.
and that greater than 99% of the C^{14}-sterol present in the insects represented unchanged cholestanol [33]. The house-fly then differs from two species of cockroaches, Blattella germanica and Eurycota floridana, which convert nearly half of the cholestanol to Δ^{7} -cholesterol during its utilization as a "sparking sterol" [34, 35] and which contain a high percentage of both the cholestanol and Δ^{7} -cholesterol in the tissues as sterol esters [15, 36]. It would appear that this conversion of a sterol to a Δ^{7} -sterol is related to the dehydrogenation of Δ^{5} -sterols such as cholesterol to form their corresponding 5,7-diensa. We have found this metabolic pathway in both the adult house-fly [29, 37] and the nymphal German cockroach [38], but it occurs at an extremely low level in house-fly larvae [28].

We are currently using C^{14}-cholesterol to study the fate of the minute "essential" cholesterol requirement of house-fly larvae. Our preliminary findings confirm those of CLAYTON et al. [15] in that greater than 95% of the C^{14}-compounds present in pupae and adults reared under sparing conditions, using C^{14}-cholesterol, represent unchanged cholesterol. However, both balance studies on the different developmental stages and the presence of C^{14}-labelled polar metabolites in the larval medium and in the insects suggest that at least a portion of the "essential" cholesterol requirement of house-fly larvae is metabolized during its use for growth and metamorphosis [28], and it is in this area that we are currently concentrating our effort.

UTILIZATION AND METABOLISM OF PHYTOSTEROLS

The literature suggests that a number of the phytophobicous and omnivorous insects which utilize the C-28 and C-29 phytosterols convert these sterols to cholestane derivatives. Much of the evidence for such conversions involves either the use of natural diets [39, 40] or identifications which rely on bioassay and/or paper chromatography [41, 42, 43]. However, the use of radiotracer-labelled plant sterols in semi-defined diets and the availability of gas-liquid chromatographic systems which separate with ease the various phytosterols and cholesterol now permit a more critical investigation of this problem. Recently CLARK et al. [44], using C^{14}-labelled ergosterol, have definitely established that the German cockroach (Blattella germanica) can convert this C-28 trienol to 22-dehydro-cholesterol by saturation of the Δ^{3} double bond and demethylation of the side chain. In a subsequent study using H^{3}-labelled β-sitosterol and gas-liquid chromatographic analysis, we have found the German cockroach to also available the biochemical mechanism for dealkylating the β-sitosterol side chain to form cholesterol [45].

Since the house-fly is routinely reared on the standard CSMA larval medium, which consists solely of plant material, this insect then must be able to use certain plant sterols to fulfill at least a part of its dietary sterol requirement. Adult flies reared on this medium have been reported to contain a mixture of sterols, and the major sterol(s) present was found to differ from both cholesterol and β-sitosterol in its chemical and physical properties [46, 47]. In a recent study THOMPSON et al. [48] have shown the sterols present in CSMA-reared flies to consist primarily of a mixture of campsterol (74%) and β-sitosterol (21%) and that the campsterol origi-
Sterols in the House-Fly

The presence in these insects of a mixture of phytosterols is in contrast to the reports that larvae of Musca domestica vicina convert β-sitosterol to cholesterol [42, 43]. To resolve these differences, we have used H3-β-sitosterol to study its metabolism in house-fly larvae and adults [37, 49]. When fed in a semi-defined adult diet, the H3-β-sitosterol was efficiently utilized for egg production. Although the major portion of the radioactive compounds in both the adult flies and eggs behaved as free sterols, as much as 30% of the H3-sterol in the eggs was esterified. Column chromatographic analysis of the total sterols indicated the presence of both Δ7 and Δ7-sterol. The Δ7 sterols, which accounted for 85 to 95% of the total H3-labelled compounds, was found to consist of greater than 90% of β-sitosterol when analysed by gas-liquid chromatography and reverse-isotope dilution. The Δ7,7-diene fraction was tentatively identified as 7-dehydro-β-sitosterol using the same techniques. Similar results were obtained with pupae reared asexually on a semi-defined larval diet containing H3-β-sitosterol in which greater than 90% of the sterols present behaved like β-sitosterol. There was no detectable conversion of β-sitosterol to cholesterol in either the larvae, the adult fly or the eggs. This was determined by reverse-isotope dilution and purification through the dibromide and by gas-liquid chromatographic analysis with concurrent trapping of the radioactive compounds. The house-fly then uses β-sitosterol directly as a precursor for 7-dehydro-β-sitosterol, to fulfil at least a part of its sterol requirement, without detectable conversion of this phytosterol to cholesterol.

In the course of these investigations we have observed an interesting physiological difference between adult flies reared on larval diets containing phytosterols and those reared on larval diets containing cholesterol. The adult female house-fly has generally been found to require a complete diet for ovarian maturation and to show little or no ovarian growth on a diet consisting solely of carbohydrates. When flies from our NAIDM strain were reared on the CSMA larval diet, which contains a mixture of phytosterols [48], and were held on an adult diet of sucrose and water, only 1 to 2% of these flies developed mature ovaries. However, when the flies were reared on this larval medium supplemented with cholesterol and held on sucrose, then about half the flies developed mature ovaries [19]. This ovarian maturation occurred in the absence of any mortality, an indication that it was not brought about by the females feeding on dead flies, and eggs from these flies were viable and produced larvae which developed to adults. These results have been confirmed with four other laboratory strains of house flies.

In order to eliminate the possibility that the activity was brought about by micro-organisms, these tests were repeated using a semi-defined larval diet and sterile rearing techniques. The phytosterol β-sitosterol, which is the major sterol present in the CSMA medium [48], was used for comparison. About 50% of the flies reared on the larval diet containing various concentrations of cholesterol and held on an adult diet of sucrose and water were found to contain mature ovaries, as compared with only about 5% of flies reared on the diet containing β-sitosterol. We are currently attempting to determine whether cholesterol, as such, is responsible for this effect or...
whether it serves as a precursor for a sterol metabolite which regulates gonad development.

This involvement of cholesterol in ovarian maturation and yolk deposition points to an interesting role for this sterol in the house-fly, since these physiological processes are known to be under endocrine control in a number of insects [50, 51]. Both this effect, and the minute amount of "essential" cholesterol that is required for either embryonic development or complete metamorphosis, in which there is a 400-500 fold increase in weight, strongly support the current concept that at least a portion of the sterol required by insects serves in a metabolic capacity, perhaps as a hormone or a hormone precursor [14, 22].

(Since the preparation of this paper, the moulting hormone (ecdysones) of insects has been reported to be a sterol [KARLSON, P., HOFFMEISTER, H., HOPPE, W. and HUBER, R., Ann. Chem. Liebig's 602 (1963) 1].

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[22] KOBAYASHI, M., KIRUMA, T. and SATO, K., J. Biochem. 52 (1952) 91.

K. HAGEN: Does any correlation or relationship appear that we can establish or verify in the adult house-fly, wherein the larval period is prolonged? Probable periods of storage, if any, might be taken as an indication of the first egg, or if it is the case, to determine that it is very carefully extruded. However, there may prove to be an active material, stored within an insect to a
STEROIDS IN THE HOUSE-FLY 279

which regulates yolk deposition by the fly, since these control in a number of "essential" amount of "essential" of control or complete weight, strongly sterol required by one a hormone hormones (ecdysone) of (1963) 1).


DISCUSSION

K. HAGEN: Do you have any information on, or has your work shown any correlation or relationship between, choline and sterol requirements? It appears that we can get good egg production and fertility without cholesterol or sterol in the adult diet.

W. E. ROBBINS: No, we do not have such information. When it emerges, the female fly probably has from 10 to 15 µg of sterol. If we hold flies for a prolonged period and collect the eggs, we notice that the sterol content goes down. Probably at least half the sterol can be used for egg production. It might take some time to detect any cholesterol deficiency because the storage mechanism is very good, but the house-fly puts a lot of sterol into the first egg and if she runs out, you very soon get non-viable eggs. However, 13 000 µg for egg hatch and larval viability is not the minimum. You could probably go far below that. When Drosophila was studied, it was at first thought that it did not require sterol at all, but when the casein was very carefully extracted it was found that it did. In some of these Diptera, however, there may actually be enough in the protein source. It is a very active material, you know, and only a tenth of a microgram is required to carry an insect to a five- or six-hundredfold increase in mass. When we
extract it from the casein we do not refer to a sterol-free casein, but to a sterol-deficient casein, because though it contains less than one microgram per gram, it is even then not sterol-free. The sterol is firmly bound in it. The same is true of soya-bean protein. I do not know what you were using as a source of protein, but you may have been giving a little sterol along with it.

W. KLOFT: You mentioned that certain of the more primitive insects may be capable of some degree of cholesterol biosynthesis. Dr. G. H. Schmidt of Würzburg has worked out a qualitative and quantitative chemical analysis (proteins, carbohydrates and total lipoids) for the ant Formica polyctena Foerst. During the metamorphosis, which is divided into 10 stages, beginning with presynps and ending with young imagos, he analysed females, workers and males. He found an increase in sterol content during metamorphosis only in workers, although they did not take up any external food. He had to conclude that in this differentiated social hymenopteran workers may have regained the possibility of biosynthesizing sterols, which appears to have been lost in the course of evolution to higher insects. We have no knowledge of the existence of symbiotic bacteria. They seem to have been lost, but I myself am not yet certain whether this is true. Your special tracer techniques may be able to throw light on this problem.

W. ROBBINS: That is a very interesting observation. However, as you are probably aware, the subject of cholesterol biosynthesis in primitive insects needs considerably more study. The use of radioactive precursors, I agree, should be very helpful in resolving your problem, but care should be taken to avoid contamination due to the biosynthesis of sterols by symbionts or intestinal micro-organisms. Axenic culture methods, antibiotics or the injection of labelled precursors should be employed.

In another series of experiments on the biosynthesis of cholesterol, we took another species of Thysanura and by-passed the intestinal tract by injection. Our preliminary studies showed no incorporation of injected C¹₄-acetate into the sterols. We did, however, obtain good incorporation into the total lipids.

J. HALBERSTADT: Was gas-liquid chromatography (GLC) used as a separation or isolation tool for the reverse isotope dilution analysis?

W. ROBBINS: Most of the gas-liquid chromatographic columns for sterols are low capacity, in the microgram to fraction-of-a-milligram range, because of the low percentages of liquid phase used. Because of this, adsorption chromatography is usually used for separation and isolation. However, we do analyse our radioactive samples by GLC with concurrent trapping of the radioactive compounds.

J. HALBERSTADT: Is there any indication as to what the mechanism might be that cause the efficient selection and/or accumulation of cholesterol as observed in some species?

W. ROBBINS: We have not investigated this phenomenon as yet but plan to do so in the very near future.
TYROSINE METABOLISM IN THE BLOWFLY, CALLIPHORA ERYTHROCEPHALA

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Abstract — Résumé — Anotexum — Resumen

TYROSINE METABOLISM IN THE BLOWFLY, CALLIPHORA ERYTHROCEPHALA. Sclerotization, i.e. the transformation of the soft, white larval cuticle into the hardened, dark puparium, is due to interaction of q-quinones with the cuticle proteins. Using radioactively labelled amino-acids, it has been shown that N-acetyl dopamine is the immediate precursor of the sclerotizing quinones in Calliphora erythrocephala. The intermediate steps in the biosynthesis of N-acetyl dopamine are hydroxylation of tyrosine to dopa, decarboxylation of dopa to dopamine and N-acetylation of dopamine to N-acetyl dopamine. This metabolic pathway of tyrosine is followed only in the final larval stage; early third-instar larvae catalyse tyrosine by transamination to p-hydroxyphenyl pyruvic acid and reduction to p-hydroxyphenylactic and -propionic acid. The metabolic shift from transamination to hydroxylation and decarboxylation is brought about by the hormone of the prothoracic gland, ecdysone, and can be inhibited by ligase or destruction of the ring gland.

Injection of ecdysone into the ligated animals leads within 10-14 h to activation of the dopa decarboxylase, presumably as the result of biosynthesis of enzyme protein. The action of the hormone is not a direct one on the biosynthetic mechanism, but indirect, the first action of the hormone being on the chromosomes (puffing phenomenon). The working hypothesis is that the hormones interacting with the genetic material leads to stimulation of the synthesis of specific (message-) RNA which is transferred to the cytoplasm and produces enzyme protein.

MÉTABOLISME DE LA TYROSINE DANS LA CALLIPHORA ERYTHROCEPHALA. L'induration, c'est-à-dire la transformation de la cuticule larvaire, dure et blanche, en un papier translucide et sombre, est due à l'interaction des orto-quinones avec les protéines de la cuticule. Grâce à l'emploi d'acides aminés marqués au moyen de substances radioactives, on a montré que la N-acétyl dopamine est le précurseur immédiat des quinones produites lors de l'induration dans la Calliphora erythrocephala. Les stades intermédiaires de la biosynthèse de la N-acétyl dopamine sont l'hdroxylation de la tyrosine, qui se transforme en dihydroxyphénylalanine (dopa), la décarboxylation de la dopa en dopamine et la N-acétylation de la dopamine en N-acétyl dopamine. Ce processus métabolique de la tyrosine ne se produit qu'au dernier stade de l'état larvaire. Les stades intermédiaires produisent le catalyseur de la tyrosine par transamination à acide p-hydroxyphényl-pyruvique et réduction à acide p-hydroxyphénylactique et -propionique. Le processus métabolique de la transamination à l'hdroxylation est provoqué par l'hormone de la glande prothoracique, l'ecdysone, et peut être rendu impossible par l'interdiction de l'ablation de la glande annulaire.

L'injection d'ecdysone aux animaux ligérés entraîne un accroissement de la décarboxylase de la dopa, dus vraisemblablement à la biosynthèse de la protéine de l'enzyme. L'effet de l'hormone sur le mécanisme de biosynthèse est indirect, le premier effet de l'hormone étant d'agir sur les chromosomes (épacymation de gonflement). L'hypothèse de travail est que l'hormone qui entre en interaction avec la matière génétique a pour effet de stimuler la synthèse de l'ARN spécifique (messager) qui est transmis au cytoplasme et produise les protéines d'enzymes.

METABOLISMO DE LA TIROСÍN EN LA CALLIPHORA ERYTHROCEPHALA. La sclerotización, es decir la transformación de la cutícula larvaria, dura y blanca, en papel transparente y oscuro, está debido a la interacción de las quinonas orto con las proteínas de la cutícula. Gracias al empleo de aminoácidos marcados con isótopos radiactivos, se ha demostrado que la N-acétyl dopamine es el precursor inmediato de las quinonas producidas durante la sclerotización en la Calliphora erythrocephala. Los pasos intermedios de la biosíntesis de la N-acétyl dopamine son la hidroxilación de la tirosina, que se transforma en dihidroxifenilalanina (dopa), la decarboxilación de la dopa en dopamina y la N-acetilación de la dopamina en N-acetil dopamina. Este proceso metabólico de la tirosina solo se produce en el último estadio del estado larvario; los tres primeros pasos producen el catalizador de la tirosina por transaminación a ácido p-hidroxifenil-piruvico y reducción a ácido p-hidroxifenilactic y -propiónico. El proceso metabólico de la transaminación a la hidroxilación es provocado por la hormona de la glándula prothorácica, la ecdisona, y puede ser hecho imposible por la ligadura o la ablation de la glándula anual.

La inyección de ecdisona en animales ligados provoca, a los 10-14 h, la activación de la decarboxilasa de la dopa, pero de forma indudablemente debido a la biosíntesis de la proteína de la enzima. El efecto de la hormona sobre el mecanismo de biosíntesis es indirecto, el primer efecto de la hormona es de actuar sobre los cromosomas (hifomación de parpadeo). La hipótesis de trabajo es que la hormona que entra en interacción con la materia genética tiene el efecto de estímular la síntesis de ARN específico (mensajero) que es transmitida al citoplasma y produce las proteínas de enzimas.
METABOLISMO DE LA TROSINA EN LA MOSCA CALLIPHORA ERYTHROCEPHALA. La desaminación (transformación de la cutícula blanda y blanca de la larva en la cubierta dura y oscura de la ninfa) se debe a la acción recíproca de o-quinonas con las proteínas de la cutícula. Empleando aminoácidos marcados se ha comprobado que la N-acetil dopamina es el precursor inmediato de las quinonas acetilaminas en la Calliphora erythrocephala. Las etapas intermedias en la biosíntesis de la N-acetil dopamina son la hidroxilación de la trosina que da 6-difenil amino, la descarboxilación de esta que da dopamina, y la N-acetilación de la dopamina que da la N-acetil dopamina. Este proceso metabólico de la trosina se produce solamente en las larvas del último estadio; al principio del tercer estadio las larvas catalizan trosina por transaminación, dando ácido p-hidroxifenilacético, y por reducción, dando los ácidos p-hidroxifenilacético y p-hidroxifenil propiónico. Esta transición metabólica desde la transaminación a la hidroxilación y descarboxilación se debe a la hormona de la glándula proctocólica (ecdysterona) que puede inhibir ligando o desencadenar la glándula similar.

Si se inyecta ecdysterona en los anales ligados se produce al cabo de 10 a 14 h una activación de la dopa decarboxilasa, debida probablemente a la biosíntesis de proteínas continuadas. La acción de la hormona sobre el mecanismo de biosíntesis no es directa sino indirecta, definiendo sobre sus primeros efectos sobre los cromosomas (fenómeno de turgencia). La hipótesis de que se partió es que la hormona que actúa sobre las sustancias genéticas y que sufre la acción de éstas estimula la síntesis de un ARN específico ("vehículo") que se traslada al citoplasma y produce proteínas continuadas.

1. TYROSINE AND SCLEROTIZATION

Interest in tyrosine metabolism in insects arose mainly in connection with sclerotization, i.e., the hardening and darkening of the larval cuticle during pupation [1]. This process is brought about by the interaction of o-quinones with the cuticular proteins [2]. These quinones arise from the respective o-diphenols which are oxidized in the cuticle by the polyphenol-oxidase present there. Many such phenolic substances, particularly acids, were extracted from cuticles of different insects and their structural relationships to tyrosine pointed to this amino-acid as the precursor of the phenols. A more direct indication of the involvement of tyrosine in the sclerotization process was its high rate of incorporation in the cuticle of pupating larvae, whereas other amino-acids, for example leucine, were hardly incorporated at all [3].

2. TYROSINE METABOLISM IN LATE III-INSTAR LARVAE

We injected generally labelled C14-tyrosine into final-instar Calliphora larvae and looked for radioactive metabolites that might be potential sclerotizing agents on paper chromatograms. The main metabolite was identified as N-acetyl dopamine [4]. Its role in sclerotization was established by the high incorporation of this metabolite during III-instar pupation.

Using larval N-acetyl tyrosine to DOPA bound enzyme system such as DPNH or a DOPA to dopamine the epidermis cells system and thus the class of compounds could be used for puparii an o-glucosidase.

3. TYROSINE METABOLISM

The injection of "spectrum" of meta propionic acid) were homogenates also. From the incubation phenylactic acid we phenylpyruvic acid acid. The first revealed a transamination reaction redoxal phosphate of tyrosine in early.
by the high incorporation of injected $^{38}$C14-labelled N-acetyldopamine in the cuticle of pupating larvae and also by following the in vivo concentration of this metabolite during larval development [5]. The concentration is low in early III-instar larvae, rises in the late III-instar larvae and disappears during pupation.

Using larval homogenates as the enzyme source, we studied the biosynthesis of N-acetyldopamine [6]. The first reaction is the hydroxylation of tyrosine to DOPA. This hydroxylation is brought about by a particle-bound enzyme system whose action is potentiated by the presence of $\text{H}^+$-donors, such as DPNH or ascorbic acid. The next step is the decarboxylation of DOPA to dopamine by a pyridoxal-phosphate-dependent decarboxylase in the epidermis cells. Dopamine is further acetylated by a transacetylase system and thus protected from ring closure, which is so common for this class of compounds [7]. Most of the N-acetyldopamine thus formed will be used for puparium formation, while a small part is being stored up as an $\alpha$-glucoside which plays a role later on in the eclosion of the imagos.

3. TYROSINE METABOLISM IN EARLY III-INSTAR LARVAE

The injection of tyrosine into early III-instar larvae showed a different spectrum of metabolites: phenol carboxylic acids (such as $\alpha$-hydroxyphenylpropionic acid) were isolated [6]. The incubation of tyrosine with Calliphora homogenates also gave $\alpha$-hydroxyphenylpropionic acid as a main product. From the incubation mixtures $\alpha$-hydroxyphenylpyruvic acid and $\alpha$-hydroxyphenylactic acid were isolated so that the pathway is tyrosine $\rightarrow$ $\alpha$-hydroxyphenylpyruvic acid $\rightarrow$ $\alpha$-hydroxyphenylactic acid $\rightarrow$ $\alpha$-hydroxyphenylpropionic acid. The first reaction was studied in greater detail and was shown to be a transamination reaction with $\alpha$-ketoglutarate as amino-acceptor and pyridoxal phosphate as coenzyme. In Fig. 1 we have summarized the metabolism of tyrosine in early and late III-instar Calliphora larvae.

![Fig. 1](image-url)

Tyrosine metabolism in early and late III-instar Calliphora larvae (from Karlson and Skeris [5]).

4. CONTROL OF TYROSINE METABOLISM BY ECYDSONE

The metabolic shift from transamination to hydroxylation and decarboxylation coincides with the time of secretion of the hormone ecdysone. Ecdy-
Fig. 2

DOPA decarboxylase activity in relation to time after injection of ecdysone

- $\Delta$ - $\Delta$ - Hatched control larvae
- O - O - injected with 2 Calliphora Units
- $\circ$ - $\circ$ - injected with 4 Calliphora Units

(from KARLSON and SEKERIS [9]).

Fig. 3

Schematic representation of the mode of action of hormones

The primary action of the hormone is the activation of gene loci (DNA) which leads to formation of nuclear (messenger) RNA from nucleic acid precursors. The RNA formed enters the cytoplasm and attaches itself to the ribosomal surface, thus serving as a template for specific protein (enzyme) synthesis.

(from KARLSON [8]).
Fig. 4

Influence of antimetabolites on the pupation rate of Calliphora larvae.

Groups of 60 larvae were injected with 4 different antimetabolites (concentration in brackets) and their pupation rate was followed in comparison with that of untreated larvae.

- Continuous line: untreated larvae
- 
  - 
  - Larvae injected with streptomycin (300 μg)
- 
  - Larvae injected with puromycin (5 μg)
- 
  - Larvae injected with actinomycin (0.02 μg)
- 
  - Larvae injected with mitomycin (0.1 μg)

In Fig. 4(a) the substances were injected during the last day of larval life, while in Fig. 4(b) 1–2 days before pupation.
sone, the product of the prothoracic or ring gland, induces a series of changes which lead to pupation [9]. It is natural to conclude that the shift in the metabolism of tyrosine is related in some way with the secretion of the hormone. Ligation of late III-instar larvae prevents pupation; the cuticle remains white and soft, thus retaining its larval character. The concentration of the sclerotizing agent N-acetyltyrosine in the ligated animals remains low in comparison with the normal larvae, which points to an impaired synthesis. We studied the activity of the enzymes involved in the biosynthesis of N-acetyltyrosine in these animals and found that the activity of the hydroxylase and decarboxylase remained low [9]. The injection of ecdysone leads to activation of the enzymes, as seen in Fig. 2 with reference to decarboxylase. This activation is dose-dependent and cannot be clearly seen until 8-10 h after injection of the hormone, i.e. the effect is a late one.

5. NATURE OF ECDYSONE ACTION

This induction of enzyme activity by ecdysone brings up the question of the nature of the activation: does the hormone activate a pre-existing inactive proenzyme, is it a de novo synthesis of enzyme protein, an action on a coenzyme, etc.? A new approach to this problem has been opened up by the work of CLEVER and KARLSON [10]. Injection of ecdysone into final-instar Chironomus larvae leads within 30 min to modification of the structure of the salivary gland chromosomies. These morphologically modified gene loci, known as "puffs", are active centres of RNA formation. This was shown by autoradiography of salivary gland cells of Chironomus after injecting H2-uridine or H3-cytidine [11]. Recalling the role of nuclear RNA in protein synthesis, we can picture the action of the hormone indirectly on protein synthesis through activation of DNA → messenger RNA synthesis → protein (enzyme) synthesis (Fig. 3) [12].

We have indirect evidence that de novo synthesis of enzyme protein takes place. Injection into Calliphora of substances which inhibit protein formation (such as puromycin or streptomycin) leads to delayed pupation and also to inhibition of decarboxylase induction. No significant inhibition can be seen if the substances are given in the last 10-15 h of larval life, while maximum effectiveness is observed if they are injected 24-48 h before pupation, at the time of hormone secretion when the activation of the biosynthetic chain begins (see Fig. 4). Similar action is exhibited by some DNA and RNA inhibitors (such as actinomycin, mitomycin and 5-F-deoxyuridine), which fits in with the hormone action mechanism proposed.

REFERENCES

DISCUSSION

R. von BORSTEL: The conclusions from the work of Clever and Karslon must be approached with caution, since recent work by H. Kroeger in Zürich indicates that the same "puft" that can be activated by edysone can also be activated by several different narcotics as well as butanol. Even though the edysone activation of the puff occurs at extremely low concentrations of the compound, the effect may still be a secondary one.

C. E. SEKERIS: Ecdysone induces the puffing phenomenon in concentrations of about 0.01 μg, that is with minute amounts of substance, which points to the specific nature of the effect. There is a possibility that edysone does not act directly on the puff but indirectly by combining with a repressor (according to the theory of Jacob et al.). This still remains to be proved.

S. FUZEAU-BRAESCH: Do you think that N-acetylaminophenol is responsible for hardening and pigmentation or just for one of these? Can you give reasons?

C. E. SEKERIS: In Calliphora sclerotization and pigmentation cannot be separated. We believe that N-acetylaminophenol is the sclerotizing agent in Calliphora and that, during the quinone tanning, it also gives the dark colour to the puparium. Of course this is not true of all insects and your paper has a bearing on this problem.
ÉTUDE DE DES INSECTES

LABORATORY L'UNIVERSITÉ

RADIOISOTOPE STUDY which is made up in large p certain questions regarding the following method.

The labelled substance before secretion of the cuticle deposited and, lastly, at the

After a suitable interval Photographic comparison of the formation of pigment, due to

The findings showed tyrosine and tryptophane - as to give direct proof of the ontogeny mechanism in forming pigments.

All cuticular pigments were applied to them with substratum of the mélanosomes specific role in the formation of the role of the tyrosine.

From the standpoint of insects, granular melanin be chromic, derived from trypt..

ÉTUDE DE LA FORMATION raison de la nature de la cuticule pour répondre à certains que

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Les résultats prévus taut carbons-14, du soufre-35 sont l'origine des pigments dans les formations pigmentaires.

Les pigments cuticulaires mélaniques qui témoin été intégré dans la cuticule derme. Le soufre organique laire, contrairement à ce que
ÉTUDE DE LA PIGMENTATION TÉGUMENTAIRE DES INSECTES A L'AIDE DE RADIOÉLÉMENTS

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Abstract — Résumé — Abstrakt — Resumen

RADIOISOTOPE STUDY OF TEGUMENTARY PImentation IN INSECTS. The nature of insect cuticle, which is made up in large part of chitin polypeptides, calls for the use of labelled isotopes to obtain answers to certain questions regarding pigmentation.

The following method, which has the advantage of being quick and easy to apply, has been developed. The labelled substance is injected into the animal at various phases of its skin-shedding cycle: before secretion of the cuticular protein, i.e. when the cuticle is at rest; at the time these proteins are deposited; and, lastly, at the time their sclerotization begins.

After a suitable interval the cuticle is removed, suitably treated, and subjected to x-ray diffraction. Photographic comparison of the results then indicates whether or not the substance chosen has been used for any formation of pigments, due account being taken of prior chemical processes involving the substance.

The findings presented in the paper relate to three labelled substances: two carbon-14 amino-acids — tyrosine and tryptophan — and inorganic sulphate-35 in the form of sodium sulphate. It has thus been possible to give direct proof of the origin of variously-coloured cuticular pigments and to discuss the role of tryptophan and sulphate in forming pigments in insects' cuticles.

All cuticular pigments arising from the metabolism of tyrosine, thus confirming the term "melanic" hitherto applied to them without direct proof. Tryptophan, exceptionally integrated into the cuticle, is the element of the onychochrome red and black pigments in the hypodermis. Inorganic sulphate plays no regular specific role in the formation of cuticular pigments, contrary to what has been suggested by various hypotheses on the role of the sulphhydril group.

From the standpoint of comparative biochemistry, the melanins appear to be purely cuticular among insects, granular melanins being confined to the venenbrates. Dark hypodermic granules in insects are onychochrome, derived from tryptophan, and not melanin.

ÉTUDE DE LA PIGMENTATION TÉGUMENTAIRE DES INSECTES A L'AIDE DE RADIOÉLÉMENTS. En raison de la nature de la cuticule des insectes — constituée en grande partie de chitinopolypeptides — il est nécessaire, pour répondre à certaines questions relatives à la pigmentation, d'utiliser des éléments marqués.

La méthode suivante a été mise au point; elle présente l'avantage de la rapidité et de la facilité. L'élément choisi est injecté à l'animal à différents moments de son cycle de mue; avant la sécrétion des protéines cuticulaires, la cuticule étant alors au repos, au moment du dépôt de ces protéines, à l'instant où commence le processus de sclérotisation de ces protéines.

Après un laps de temps adéquat la cuticule est prélevée, traitée de façon appropriée et autoradiographiée in toto. La comparaison photographique des résultats permet de conclure à l'utilisation ou non de l'élément choisi dans cette formation pigmentaire, compte tenu des processus chimiques antérieurs relatifs à l'élément.

Les résultats présentés concernent trois éléments marqués; deux acides aminés — tyrosine et tryptophane — au carbone-14, du soufre-35 inorganique sous forme de sulfate de sodium. On a pu ainsi démontrer directement l'origine des pigments cuticulaires de différentes couleurs et discuter le rôle du tryptophane et du soufre dans les formations pigmentaires du tégument des insectes.

Les pigments cuticulaires sont tous issus du métabolisme de la tyrosine, confirmant le terme de "mélancieuse" qui leur était jusqu'à présent attribué sans preuve directe. Le tryptophane, exceptionnellement intégré dans la cuticule, constitue le substrat des pigments noirs et rouges onychocromiques de l'hypoderme. Le soufre inorganique est hors de toute spécificité régulée dans la formation des pigments cuticulaires, contrairement à ce que laissaient supposer différentes hypothèses sur le rôle des groupements sulfhydriles.
S. FUZEAU-BRAESCH

El punto de vista de la bioquímica comparada, los melanínios se encuentran entre los insectos, los gametocitos, a menudo en los plegados en el tegumento. En algunos casos, los melanínios, en general, se encuentran en los insectos, de ácaros acaros, derivados de la tripofán y el mithionine.

MÉTODO

La méthode utilisée pour la rapidité et la précision, elle permet de déterminer le type de l'insecte et son métabolisme. En d'autres termes, le dimethylglyoxime est réalisé ici pour les formations pigments.

Un certain nombre d'éléments radioactifs sont utilisés dans les marquages d'insectes.

Les animaux utilisés sont ceux de Geer (gry) gratoria (acrididae).
éléments en réalisant la détection non globalement mais, pour la partie externe, par autoradiographie. Étant donné le cycle de mue fondamental de l'insecte et son métabolisme particulier en rapport avec la formation de la nouvelle cuticule, on doit distinguer des étapes et rechercher les variations dans l'incorporation des substances au cours de ce cycle. Une telle étude est réalisée ici pour le soufre à l'occasion d'une analyse du tégument dont les formations pigmentaires étudiées à l'aide de tyroside et tryptophane radioactifs vont servir de base de comparaison.

MÉTHODE

La méthode utilisée d'autoradiographie in toto présente l'avantage de la rapidité et de la facilité. Si elle ne fournit pas de données quantitatives précises, elle permet d'atteindre par contre un niveau que l'analyse chimique ne peut approcher : celui des différences dans la structure fine, pigmentaire ou morphologique de la cuticule.

L'élément choisi est injecté à l'insecte dans la cavité générale, dissous dans du liquide de Ringer pour insecte, en quantité variant de 10 à 20 µl représentant une activité totale voisine du microcurie. L'animal est nourri au gaz carbonique et l'injection effectuée dans l'abdomen, à travers une membrane intersegmentaire, à l'aide d'une micropipette de verre.

Les injections sont réalisées à des moments variés du cycle de mue : a) avant la sécrétion des protéines cuticulaires, lorsque la cuticule est au repos,

b) au moment du dépôt de ces protéines avant la mue,

c) à l'instant où commence le processus de sclérisation et de pigmentation de ces protéines.

Après un laps de temps adéquat, la cuticule est prélevée, nettoyée à la loupe binoculaire, traitée rapidement de façon appropriée par une solution de potasse bouillante qui élimine toute trace de tissus sous-jacents (hypoderme), puis lavée, séchée, et autoradiographiée sur film spécial «Radio dentaire KODAK». L'autoradiogramme donne l'image des régions radioactives, que l'on peut comparer avec la cuticule elle-même, ou avec une photographie de celle-ci lorsqu'il s'agit d'étudier de près les dessins pigmentaires. Un exemple des résultats obtenus est présenté en figure 1.

Les substances suivantes ont été utilisées :

1. Tyroside, marquée uniformément au 14C (Amersham, Radiochemical Centre).
2. Tryptophane, marqué au 14C, premier atome de la chaîne (Amersham, Radiochemical Centre).
3. Sulfate de sodium marqué au 35S (C.E.A.)
4. dl - méthionine marqué au 35S (C.E.A.)
5. dl - cystine marqué au 35S (C.E.A.)

Les animaux utilisés sont des insectes hétérométaboles : Gryllus bimaculatus de Geer (gryllidae, orthoptères), (substances 1-5), et Locusta migratoria (acrididae) (substance 1).
FORMATION DE LA CUTICULE ET PIGMENTATION

Il est bon de rappeler ici l’essentiel des processus de formation de la cuticule chez les insectes.

Avant la mue et sous l’ancienne cuticule se constitue une couche de protéines sécrétée par les cellules de l’hypoderme sous-jacent; ce sont elles qui deviendront la partie solide de la future cuticule. Molles et incolores avant la mue, aussitôt après le rejet de l’ancienne cuticule ces protéines sont durcies, sclérisées selon un processus complexe de «tannage» par des substances quinoniques, en même temps qu’elles deviennent pigmentées.

Une étude de la cuticule générale de l’ensemble du corps a été effectuée; parallèlement à celle-ci une observation plus détaillée a été réalisée dans une région intéressante à cause du décalage chronologique de sa formation: il s’agit de deux processus latéraux du condyle de l’articulation fémur-postérieur/tibia, petites zones en forme de croissant renforçant l’articulation en rapport avec la fonction saillatrice de la patte, qui durcissent et se pigmentent avant la mue. Enfin, une attention spéciale a également été accordée aux grosses épines du tibia postérieur.
Il faut dès le départ distinguer l'incorporation des substances :

1° dans les protéines cuticulaires mises en place avant la mue (injection: un ou deux jours avant la mue, - prélèvement à l'étuvage ou après la mue, à condition de tenir compte éventuellement de l'activité propre à la période qui suit la mue);

2° après la mue au moment du durcissement et de la pigmentation (injection: à la mue, - prélèvement après durcissement: un jour).

3° dans la cuticule au repos (injection entre les deux mues plus de deux jours après la mue précédente et au moins trois jours avant la mue suivante, prélèvement un ou deux jours après).

Le tableau I schématise les résultats obtenus.

1. Tyrosine et tryptophane dans la cuticule

La tyrosine est incorporée dans les protéines cuticulaires d'une façon uniforme. Par contre, après la mue, elle l'est uniquement dans les zones pigmentées, quelle que soit la couleur du pigments. Les autoradiogrammes montrent une concordance parfaite entre les régions radioactives et les zones pigmentées de la cuticule, tandis que les zones dures sans pigmentation sont dépourvues de radioactivité quelle que soit l'espèce considérée, les animaux ayant vécus groupés ou isolés (fig. 1). La fraction pré-éxuviale de la tyrosine est protéinogène, tandis que la fraction post-éxuviale est pigmentogène.

Les zones non pigmentées de la cuticule durcissent apparentemment autant que les zones pigmentées, bien qu'elles n'intègrent pas de tyrosine après la mue. Le processus chimique de ce durcissement n'est pas établi. On voit donc que la fraction post-éxuviale pigmentogène de la tyrosine ne peut être mise en évidence que par la comparaison des autoradiogrammes pré- et post-éxuviaux et doit passer inaperçue à l'analyse chimique globale. C'est peut-être la raison pour laquelle CARLSON et coll. [2] n'ont pas trouvé de différences très nettes entre les insectes mélanisés, Schistocerca gregaria et ceux d'une souche albino dépigmentés de la même espèce, tandis qu'ici la concordance est toujours parfaite entre tyrosine et mélanine lorsque l'étendue de cette dernière varie, par exemple sous l'influence du groupement.

Dans le processus du condyle, l'incorporation se fait uniquement et fortement avant la mue, attestant bien de l'achèvement précoce inhabituel de cette zone. Les épines du tibia ne présentent pas de caractères distinctifs.

Le tryptophane est lui aussi intégré dans les protéines cuticulaires; il jone, par contre, fort peu de rôle dans les processus qui se déroulent après la mue et n'est pas en rapport évident avec la pigmentation.

Dans la cuticule au repos, aucun des deux acides aminés n'est incorporé.

Par contre le tryptophane est fortement intégré aux granules d'omochrome, pigments hypodermiques sous-jacents, dont il est la substance mère, ce qui permet de distinguer parfaitement ces granules des granules mélaniques issus de la tyrosine tels qu'on les connaît chez les vertébrés, et qui jusqu'à présent n'ont pas été trouvés chez les insectes. Chez ces derniers, les pigments polymérisés issus de la tyrosine semblent bien être
TABLEAU I

RADIOACTIVITÉ DE LA CUTICULE APRÈS INJECTIONS DE DIFFÉRENTES SUBSTANCES RADIOACTIVES EN FONCTION DU CYCLE DE MUE

<table>
<thead>
<tr>
<th>Substances radioactives injectées</th>
<th>Radioactivité pré-éuvulale des protéines cuticulaires (injections avant la mue)</th>
<th>Radioactivité post-éuvulale de la cuticule (injections à la mue)</th>
<th>Radioactivité de la cuticule au repos (injections entre deux mues)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cuticule générale</td>
<td>Procèsus du condyle</td>
<td>Epines tibiales</td>
</tr>
<tr>
<td>Tyrosine (14C)</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Tryptophane (14C)</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulfate de sodium (1H3)</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Méthionine (1S3)</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cystine (14S)</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Détection effectuée par autoradiographie ; nombres de croix (x) = évaluation visuelle approximative des intensités.
1° unique apanage de la cuticule au sein de laquelle ils se trouvent à l'état diffus et non granulaire [1].

2. Analyse comparée du rôle du soufre dans la cuticule

1° Soufre inorganique

Il apparaît que le soufre inorganique sous forme de sulfate de sodium est uniformément incorporé à la cuticule, avant et après la mue. Seuls, les processus du condyle ne sont pas radioactifs avant la mue, tandis que les épines tibiales le sont au contraire très fortement après la mue, et même en période de repos de la cuticule.

2° Soufre organique

La cuticule devient là aussi uniformément radioactive. Il est remarquable cependant que la méthionine provoque une radioactivité extrêmement forte lorsque l'injection est faite avant la mue, beaucoup plus intense que celle que l'on obtient dans les mêmes circonstances avec la cystine. Les deux substances sont - contrairement au soufre inorganique - présentes dans le processus du condyle avant comme après la mue. Les épines tibiales ne présentent pas ici d'activité particulièrement intense.

Il ressort de ces résultats que le soufre est souvent incorporé dans la cuticule, mais avec des modalités variées.

Le fait le plus frappant est la très forte radioactivité des protéines cuticulaires dans le cas d'injections de méthionine qui doit être l'acide aminé souillé essentiel pour cette formation.

Des différences entre soufre inorganique et soufre organique se font jour : au niveau des processus du condyle, seul le soufre organique est incorporé, de façon d'ailleurs importante, tandis que pour les épines tibiales, c'est le soufre inorganique qui paraît le plus utilisé, et cela même en période de repos.

CONCLUSIONS

Il faut tout d'abord souligner l'incorporation de soufre dans la cuticule au repos alors que la tyrosine et le tryptophane ne provoque pratiquement pas de rayonnement. Sauf dans le cas des épines du tibia, cette incorporation reste faible ; on doit néanmoins en tenir compte dans toutes les expériences de ce type. L'interprétation n'en est pas aisé. La cuticule, duree et pigmentée, est théoriquement inerte. En réalité, elle reste en relation avec l'hypoderme vivant sous-jacent qui conserve des prolongements cytoplasmatiques dans les innombrables canalicules de la cuticule - relation qui reste à étudier.

Depuis longtemps la présence de soufre est connue par analyse chimique dans la cuticule et la possibilité de réactions de groupements sulphydriés des protéines cuticulaires avec des quinones est envisagée [3,4]. Cette hypothèse n'est pas incompatible avec les résultats obtenus ici par autoradiographie. Comparés à ceux obtenus avec la tyrosine, ils attestent
que la présence de pigments issus de cet acide aminé n’est pas liée à une richesse différentielle en soufre ni que le soufre soit à l’origine des variations de coloration, la dissolution étant ici la même dans une région pigmentée en noire ou une zone colorée en jaune.

Par contre, l’attention est attirée par le fait que seul le soufre organique est intégré notablement dans les processus du condyle dont on connaît l’accélération du durcissement et de la pigmentation et l’intégration également accélérée de la tyrosine. On peut peut-être évoquer ici les couplages obtenus in vitro entre dérivés quinoniques de la tyrosine et des thioles [5] pourtant donner naissance à des pigments sans que l’état hormonal de l’animal ne permette la libération des enzymes conditionnant l’évolution générale de la cuticule (voir le rôle de l’Ecdyson dans le déclenchement de l’activité décroxylique [5]).

Enfin, le fait que le soufre inorganique est incorporé à des structures organiques complexes telle que la cuticule amène à poser le problème de la conversion chez Gryllus du soufre inorganique en soufre organique, démontré chez quelques autres espèces [7]. Cet insecte possède en outre un segment intestinal à micro-organismes lequel, d’après MARTOJA [8], incorpore le soufre inorganique injecté dans la cavité générale, et peut jouer un rôle ici.

En conclusion, on peut dire que les pigments cuticulaires ont tous leur origine dans la tyrosine incorporée après la mue; le soufre est présent dans la cuticule de façon régulière, mais ne paraît pas jouer de rôle spécifique dans la pigmentation.

Références


Discussion

C. E. Sekeris: The autoradiographic method that you use is very interesting. In Munich, Frau Dr. Schlossberger is using similar techniques to study the incorporation of labelled tyrosine and tryptophane in the wild and albino strains of the grasshopper Schistocerca gregaria.

It would be interesting to use this technique for the incorporation in the cuticle not only of tyrosine but also of its metabolites, acids as well as dopamine in the different developmental stages. This could give some insight into the problems of sclerotization and pigmentation, especially in insects where these two processes are clearly separated.

Concerning your autoradiograms after injection of S-labelled compounds, is it possible that these substances are merely being inactivated and excreted in this way, as cuticulum?

S. FUZEAU-BRAESCH: Yes, that is certainly a possibility.
W. KLOFT: Mme Fuzeau-Braesch's method helps one to understand the mechanism of moulting in hemi-metabolic insects. The incorporation of the labelled substance in the femur condyle is most interesting from this point of view, and I am most grateful to her.
E. HORBER: I should like to mention that with certain Diptera, as Oscinia panthis or Meromyza americana, which develop in graminaceous plants, the phenomenon of sexual dimorphism has been observed in addition to very pronounced seasonal dimorphism. The males showed more melanin than the females, and the hibernating flies more than the generations produced during the summer. Perhaps these species could be considered in the studies as well as the Orthoptera which you have mentioned.
S. FUZEAU-BRAESCH: Thank you very much, that might be useful.
J.R. OGLE: I should like to say something with regard to your observations on the appearance of S$^8$ from methionine in cystine into the cuticle. If, as Dr. Sekeris suggested, this could be caused by excretion into the cuticle, do you think that, in fact, it might be due to the deposition of the non-metabolized d-isomers, rather than a genuine incorporation of the sulfur from the l-isomer? Would it not be preferable to use the l-isomers of these labelled amino-acids?
S. FUZEAU-BRAESCH: Yes, this ought to be done. The substance we had at the time of the experiments was a mixture, not the pure l-isomer.