Opisthosomal homogenates were subjected to electrophoresis on polyacrylamide gel columns for 45 min, and then incubated in the glycerophosphate solutions of Gomori's method. They showed a single moiety of low mobility with alkaline phosphatase activity. The moiety could be seen as a white disk after incubation. This rendered subsequent treatment with cobalt nitrate and ammonium sulphide unnecessary. Incubation of com-parable gel columns using Barka's⁴ method for acid phosphatase showed the presence of phosphatase activity at acid pH. It is worth noting, however, that in neither case did the inhibitors of vertebrate phosphatases, 0.01 molar sodium fluoride and 0.01 molar potassium cyanide, have any effect on these reactions.

Both acid and alkaline phosphatases have long been known to occur in insects⁵⁻⁸, and alkaline phosphatase is particularly common in epithelia secreting silk. It has been suggested that at this latter site the alkaline phosphataso may be involved in the release of silk from an intracellular nucleoprotein complex. It is clear that it may have a similar function in the silk glands of spiders.



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Topically Applied *n*-Decyl Acetate as a Precursor for Metabolic Investigations in Insects

WHEN small insects are injected with labelled metabolites trouble is caused by mechanical damage, excessive bleeding, clogging of the needle, and the serious distortion of metabolism which results from the sudden administration of up to 20 per cent of the insect's weight of water plus 10-60 µmoles of metabolite/g of live weight. One way to overcome these difficulties would be to make a fat-soluble acetate precursor which could be applied directly to the cuticle in the same way as an insecticide. The use of n-decyl 1-14C-acetate prepared by direct esterification of n-decyl alcohol has been investigated. No effect on mortality, pupation or emergence was found to result from topical application of acetone solutions, or even up to 1 µl. of undiluted n-decyl acetate, to larvae of the blowfly Calliphora erythrocephala.

Each member of batches of eight blowfly larvae 3-4 days old was treated with 1 µl. of undiluted and labelled *n*-decyl acetate, and the batches were placed in wire gauze cages. Each cage was suspended in a 15-ml. glass-stoppered tube containing 10 cm² of filter paper saturated with 10 normal potassium hydroxide. The experiment was conducted at 20° C. Every 30 min the cage was transferred quickly to a fresh tube and 3 h after treatment it was dropped into liquid nitrogen. The cage was then washed thoroughly in ethanol at -10° C and re-frozen. The larvae were extracted with perchloric acid¹, and three times with chloroform and methanol (1:1) at room temperature, and the residue was heated for 24 h at 100° C in a sealed tube with 2 ml. of 50 per cent potassium hydroxide. The insoluble residue of chitin was washed with water and dried.

About one fifth of the activity recovered was metabolized and was found in carbon dioxide, fat-soluble, or water-soluble extracts. Small amounts of activity (less



Fig. 1. Production of carbon dioxide labelled with carbon-14 by insects. $\bigcirc --- \bigcirc$. Adult houseflies after injection with labelled acetate (ref. 2); $\bigcirc -- \bigcirc$, larval blowflies after topical application of labelled *n*-decyl acetate.

than 0.1 per cent of the total recovered) were found in the alkaline digest of the residue and similar small amounts in chitin. Chromatography of fat-soluble extracts on thin layers of silicic acid with benzene and ether (60:40)as solvent failed to reveal any intact n-decyl acetate. This thin layer system was the only one out of many that were tested which separated n-decyl acetate from all the detectable insect fat fractions.

The production of labelled carbon dioxide at various times after topical application of the n-decyl acetate is compared with earlier data² from adult houseflies injected with labelled acetate (Fig. 1). There was no sign of a lag period between application of the ester and the evolution of ¹⁴C carbon dioxide and it was concluded that n-decyl acetate is rapidly hydrolysed, and the free acetate produced further metabolized, as soon as it is absorbed by the insect.

These results demonstrate that the use of a fat-soluble precursor permits a water-soluble metabolite to be given to an insect, however small, in a dose smoothly applied over several hours without loss of blood, risk of mechanical damage or unnecessary metabolic disturbance.

I thank Mr. F. P. W. Winteringham, who first suggested that a fat-soluble acetate precursor might be a valuable tool.

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Absorption of Exogenous and Endogenous **Biliary Copper in the Rat**

THE daily intake of copper in man exceeds normal requirements¹, but abnormally high body stores have only been recorded in Wilson's disease^{2,3}. Knowledge of the exact mechanism of the absorption of dietary copper is incomplete⁴ and even less is known about the absorption of endogenous biliary copper or its entero-hepatic circulation^{4,5}. This report concerns the relative intestinal absorption of exogenous copper-64 in two chemical forms (ionic cupric acetate and chelated copper (II-EDTA)) and the absorption of endogenous biliary copper and caeruloplasmin copper.