ing the concentration of neopeptone in the culture medium from 1 to 3 percent, failed to reverse the inhibition; this suggests that no antimetabolic action is involved.

Inhibition was unaffected by interposition of dialysis tubing between tissues and seeded agar. However, there was no apparent diffusion of the inhibitory factor into the agar contiguous to the conjunctiva, for there was normal growth on the rest of the slide.

Lysozyme (muramidase) is present in high concentrations in tears and conjunctiva and is bacteriostatic or bacteriocidal for susceptible organisms (*6*). No lytic or inhibitory action of lysozyme on *C. albicans* could be demonstrated. The fungus was unaffected after exposure for 1 hour at 37°C to lysozyme (1 mg/ml) in .07*M* phosphate buffer at *p*H 6.2. There was no loss of viability after exposure for 28 hours of 2500 *Candida* cells to 50 and 500 μg of lysozyme in the same buffer. This small inoculum was recovered quantitatively by plate counts on Sabouraud's agar, with and without the addition of 100 μg of lysozyme to the medium. Thus, the inhibitory effect of the conjunctiva toward *C. albicans* is not apparently attributable to lysozyme.

In preliminary control experiments human endocervical and endometrial tissues failed to inhibit the growth of *C. albicans* under the aforementioned conditions.

Conjunctiva extracts were prepared by grinding coarsely minced tissue (10 g wet weight) in a mortar and extracting with 50 percent ethanol (250 ml). A turbid extract was obtained. The alcohol was removed by evaporation in a pan held in a 42°C water bath; the aqueous residue was sterile.

The extract was inoculated with *C. albicans* (10^6 cells per milliliter) in the log phase of growth and incubated at 37°C on a rotator (20 rev/min) by the tumble tube technique. Viability tests by the plate-count method were performed before and after 2½ hours' incubation (Table 1).

Three different batches of conjunctival extract had this lethal effect on *C. albicans*. The drop in viability was paralleled by a decrease, indicating cell lysis (Table 2), in the number of cells as judged by counting the cells directly. Neither water nor 50 percent alcohol evaporated to half-volume under test conditions had an inhibitory effect on the growth of *C. albicans*.

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5. Cycloheximide, 20 mg; streptomycin, 1 g; penicillin G, 500,000 units; chloramphenicol, 250 mg; volume made up to 100 ml with sterile distilled water.
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7. Supported by U.S. Public Health Service grant HD-00288 and by the Jewish Philanthropic League of Brooklyn.

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Chlorinated Insecticides: Fate in Aqueous Suspensions Containing Mosquito Larvae

Abstract. *Aldrin, p,p'-DDT, dieldrin, gamma chlordane, heptachlor, heptachlor epoxide, 1-hydroxychlordene, and lindane were bioassayed against larvae of* Anopheles quadrimaculatus *Say; the remnant amounts of insecticide and metabolites in larvae and medium were determined by electron-affinity gas chromatography. The insecticides were surprisingly nonpersistent at concentrations normally used.*

Procedures sensitive enough to trace insecticides and their products in larvae at the concentrations of normal usage have usually depended on labeling the compounds with radioactive elements. With such tracers the codistillation of DDT and its heterogeneous distribution in aqueous suspensions have been demonstrated (*1, 2*); the effect of these phenomena has been correlated with absorption by and toxicity to mosquito larvae (*3*), and the solubility of DDT in water has been determined (*4*).

Table 1. Fate of chlorinated insecticides in standard jar bioassays (250 ml) with 25 fourth-instar larvae of *Anopheles quadrimaculatus* Say after 20 hours at 26.5°C; determined by electron-affinity gas chromatography.

Insecticide	Added		Recovered		
	Per jar (μg)	Concn.* (ppm)	Substance†	From jar (μg)	From larvae (μg)
aldrin	5.98	0.024	aldrin	0.333	0.006
			dieldrin	none	.055
aldrin	67.5	.27	aldrin	9.08	.266
			dieldrin	none	.420
dieldrin	5.95	.024	dieldrin	2.52	.186
dieldrin	64.8	.26	dieldrin	29.0	2.22
heptachlor‡	53.0	.21	heptachlor	2.16	
			hept. epox.	none	
			1-hyd.-chlor.	2.43	
heptachlor	61.0	.24	heptachlor	4.51	0.136
			hept. epox.	none	.372
			1-hyd.-chlor.	2.99	.015
heptachlor	269	1.08	heptachlor	33.3	.855
			hept. epox.	none	1.18
			1-hyd.-chlor.	13.9	0.060
hept. epox.	62.5	0.25	hept. epox.	34.5	1.52
hept. epox.	264	1.06	hept. epox.	164	10.0
1-hyd.-chlor.	264	1.06	1-hyd.-chlor.	255	1.00
p,p'-DDT	1.39	0.0056	*p,p'*-DDT	0.816	0.060
			p,p'-DDE	none	.089
γ-chlordane	51.0	.20	γ-chlordane	14.6	.613
lindane	5.81	.023	lindane	4.02	.025

* By analysis; † hept. epox., heptachlor epoxide; 1-hyd.-chlor., 1-hydroxychlordene; ‡ no larvae present.

Isotopically labeled compounds are expensive and often difficult to synthesize; more important, in the aforementioned work, radio-labeled products could not be identified as part of the analysis, that is to say, the determined product might be the original pesticide or a product thereof, or both. Such identifications have been made by us, using electron-affinity gas chromatography to study the fate of seven chlorinated insecticides and a degradation product of heptachlor in aqueous suspensions containing mosquito larvae (*5*), and in the larvae.

One milliliter of acetone containing an appropriate quantity of insecticide was stirred into 225 ml of distilled water in each of a number of wide-mouth half-liter jars. Twenty-five fourth-instar larvae (*Anopheles quadrimaculatus* Say) in 25 ml of water were added to each open jar, and mortality was noted after 20 hours at 26.5°C. From 12 to 18.5 g of water volatilized during the test. Both the suspension and separated larvae (50 larvae were rinsed with hexane and homogenized for analysis) were extracted with hexane and analyzed by electron-affinity gas chromatography (*6*). The fact that added insecticides could be recovered practically quantitatively from suspensions and larvae at the outset demonstrated the reliability of the procedure; no metabolites or other products were found in freshly made suspensions. Identification of products was based on retention times (Table 1).

Metabolic conversion of aldrin to dieldrin, DDT to DDE (*7*), and heptachlor to its epoxide by larvae of *A. quadrimaculatus* was determined quantitatively. These metabolites must have been formed by the larvae because (i) the metabolites were not present initially, (ii) they could not be found in appreciable quantities in the aqueous medium, and (iii) 50 percent or more of the insecticide in the larvae was present as the metabolite. In contrast with this result, formation of nontoxic 1-hydroxychlordene from heptachlor appeared to be exogenous to the organism, since appreciable amounts of it formed in the absence of larvae.

More than half of the DDT in aqueous suspensions (0.001 to 0.100 ppm) at 25°C was lost in 1 day by codistillation with water (*1, 2*). The low recovery of DDT was therefore expected; but the incomplete recovery of the other insecticides suggests that they too may codistil. This premise is supported by the finding that the concentration of lindane necessary to kill 50 percent of the larvae was greater in open jars (0.032 ppm) than in closed jars (0.012 ppm) which precluded codistillation.

If we assume that the low recovery of insecticide (Table 1) is due to codistillation, our data are consistent with the concept that the less polar compounds codistil with water more readily than polar compounds. Thus aldrin and heptachlor, which are less polar than their corresponding epoxides, codistilled to a greater degree (82 to 94 percent) than their epoxides (27 to 56 percent); the most polar compound, 1-hydroxychlordene, codistilled very little (3 percent).

About three times more dieldrin than aldrin and from three to five times more heptachlor epoxide than heptachlor were found in larvae exposed for 20 hours to approximately equivalent initial concentrations of insecticide (metabolites expressed as original insecticide). However, the conclusion that aldrin and heptachlor have a lesser affinity for the larvae than their epoxides must be considered tentative because certain variables are not controlled in the bioassay. For example, the lower uptake by larvae of aldrin and heptachlor, when compared with their epoxides, may be due in part to the exposure of the larvae over the test period to a lower concentration of aldrin and heptachlor, the lower concentration resulting from the greater loss of these insecticides (probably by codistillation) than of their epoxides.

Our findings illustrate the potential of electron-affinity gas chromatography in determining the fate of chlorinated hydrocarbons at very low concentrations in aqueous suspensions and in minute organisms. Codistillation with water may be an important route for the loss of aldrin, dieldrin, heptachlor, heptachlor epoxide, γ-chlordane, and lindane, and codistillation should be considered in investigations dealing with these insecticides as water contaminants.

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5. W. V. King *et al., U.S. Dept. Agr. Handbook No. 69* (May 1954), p. 6.
6. Using a Jarrell-Ash model 700 instrument equipped with a 1 m by 0.6 cm outside diameter stainless steel column packed with 5 percent wt/wt purified silicone grease (*8*) on 80-100 mesh acid-washed Chromosorb W. Operating parameters were: injection port 200°C, column 180°C, detector 200°C, voltage 22, range 10^{-9} amp, nitrogen flow rate 200 ml/min (exit). Mention of an instrument does not necessarily imply its endorsement by the U.S. Department of Agriculture.
7. DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane; DDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.
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Averaged Brain Activity Following Saccadic Eye Movement

Abstract. *Since a change of stimulus is required to effect a visual response, and since saccadic eye movements change the locus of the retinal image, the hypothesis was developed that there should be a brain response following saccadic eye movement. The hypothesis was tested experimentally by averaging the activities following successive saccadic eye movements. A response was found whose characteristics were dependent on illuminance of the stimulus.*

A change in stimulation is generally recognized as necessary to elicit a response in the visual system. This can be shown by electrical recording; electroretinograms and other forms of evoked visual responses are elicited only by flashes, flickering, or other changes in stimulation. The importance of a change in stimulation is also demonstrated by experiments with stabilized images (*1*). With this procedure, the stimulus viewed by an observer moves exactly in step with movements of the eye so that the retinal image does not move on the retina, and the retinal stimulus is continuously presented to the same set of receptors. Stabilized images gradually fade. Since the change of stimulation ordinarily produced by fine eye movements is no