ty in the nature of inhibition of the two kinase reactions investigated suggests that this phytokinin may inhibit similar phosphorylation reactions involving ATP.

Our results are not proposed as the explanation for all the varied effects of the phytokinins on plant metabolism (cell division and enlargement, germination, protein synthesis, directed transport, delay of senescence, and others). Nevertheless, these experiments suggest that respiratory inhibition induced by N^6-benzyladenine, which is also related to the delay in senescence of explants, is a consequence of inhibition of the glycolytic kinases. The structural similarity of ATP, ADP, and N^6-benzyladenine offers a possible explanation for the competition between the nucleotides and N^6-benzyladenine.

V. TULI
D. R. DILLEY
S. H. WITTWER
Department of Horticulture, Michigan State University, East Lansing

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22 October 1964

Conjunctiva Contains Factor Inhibiting Growth of Candida albicans

Abstract. *Intact conjunctiva and conjunctival extracts have a strong inhibitory effect upon* Candida albicans. *The fungistatic effect is not attributable to antimetabolic action and is not caused by lysozyme or tears, neither of which inhibit growth and viability of* Candida albicans.

We have found in the normal conjunctiva a factor which inhibits the growth of *Candida albicans*. This factor may account for the rarity (*1*) of conjunctivitis caused by *C. albicans* in contrast to the relative frequency of invasion of other mucous membranes by this organism (*2*).

The normal conjunctiva has a sparse and limited microflora. In a study of 520 eyes (*3*) free of mycotic disease, cultures for fungi yielded 38 isolates, of which only two were *C. albicans*. Tears have been shown to inhibit the growth of some bacteria (*4*) by a mechanism distinct from lysozyme action, but their effect on fungi has not been reported. In our investigations pooled human tears did not affect the viability of *C. albicans*.

For our study calf palpebral conjunctiva were brought to the laboratory in chilled containers within 2 hours after slaughter. The conjunctivae were dissected, away from the Maibomian glands and down to the tarsus plate, dipped in a mixed antibiotic solution which did not inhibit *C. albicans* (*5*), and freed of excess moisture with sterile filter paper. The mucosa was tested for inhibitory activity by culturing the organism in the presence of the tissue. Cultures were prepared by applying a thin layer of Sabouraud's agar to sterile microscope slides and inoculating the agar evenly with a suspension of an 18-hour culture of *C. albicans* containing 2×10^4 spores per milliliter. The mucosal surface of the excised conjunctivae was placed on the seeded agar and incubated for 18 hours at 37°C in sterile petri dishes.

When the conjunctivae were removed, no growth was visible on the slide where the agar was in contact with the tissue. No growth could be detected microscopically in the central portions of the areas of contact. Stunted colonies were occasionally present in the periphery. A thick, viscid exudate was present in the covered areas. Growth of the yeast was normal in control areas of the slide not covered by the conjunctivae (Fig. 1).

The inhibition was not caused by anoxia, since *C. albicans* appears to grow well under anaerobic conditions.

The inhibitory effect of the intact conjunctivae toward *C. albicans* remained unimpaired after exposure of the tissues to temperatures of 4°, 22°, and 37°C for 24 and 48 hours, and after warming to 42°C for 2 hours.

An excess of nitrogenous and mineral metabolites, supplied by increas-

Table 1. Decrease in viable plate count of *Candida albicans* after 2½ hours' incubation in conjunctival extracts. The figures reported are averages of duplicates which were plated in duplicate.

Initial	2½ hours	Decrease (%)
	Conjunctival extract	
1,155,000	128,000	89
870,000	10,000	99
1,305,000	304,000	77
	Water diluent	
1,290,000	990,000	23
	50% alcohol evaporated to half-volume	
1,050,000	1,115,000	0

Table 2. Decrease in counts (units/ml) of *Candida albicans* exposed to conjunctival extracts (three preparations) in rotator at 37°C. The counts were made in a white-blood-cell chamber; budding spores were counted as one unit; a group of three spores were counted as one unit.

0 hour	2½ hours	Decrease (%)
885,000	295,000	67
1,100,000	170,000	85
1,195,000	440,000	64

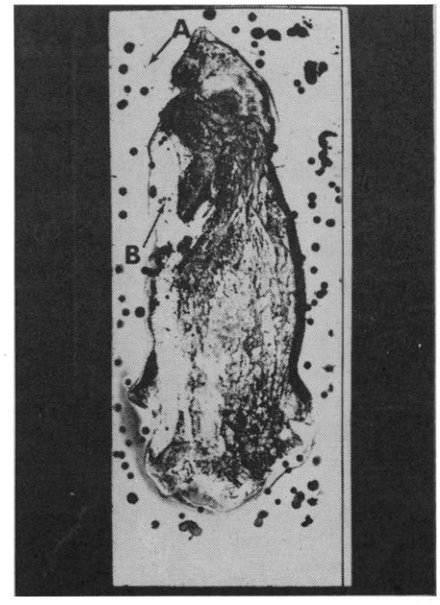

Fig. 1. Agar culture on microscope slide of *C. albicans* in presence of fresh palpebral conjunctiva (calf). A viscid exudate marks the area of contact. *A*, Normal colonies at a distance from the tissue. *B*, Stunted colonies at periphery of the tissue.

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*.

PHILIP J. KOZINN
LEONA CAROLINE
CLAIRE L. TASCHDJIAN
Department of Pediatrics, Maimonides Hospital, Brooklyn, New York, and *Department of Pediatrics, State University of New York, Downstate Medical Center, 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.