cyw; the Isolan-B strain has mutant genes that cause the production of more active oxidases; gene redundancy in the Isolan-B strain provides more sites for induction and enzyme production; and genetic differences in enzyme regulation result in more enzymes in the Isolan-B strain. The first possibility seems unlikely since, if more protein is produced by the dieldrin treatment, as reported earlier (*7*), induction would cause an effect opposite to that observed.

Although there is a precedent for the second possibility (mutant genes), in the case of organophosphate-metabolizing enzymes of resistant houseflies (*12*) this explanation is unlikely in the present case. The difference in enzyme activity seen between the Dld:cyw and Isolan-B females at 9 days (Table 1), if due to differences in enzyme structure, might be expected to be reflected in K_m values. Yet, in experimentally determining this constant by the double reciprocal plot method, using 9-day-old females of the two strains, we obtain identical values, apparent $K_m = 10.9$ μM.

Current concepts seem to accommodate both enzyme regulation and gene redundancy as explanations for the observed results. There is now experimental evidence for the latter (*13*).

L. C. TERRIERE
S. J. YU
R. F. HOYER

Department of Entomology,
Oregon State University,
Corvallis 97331

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Permeation of Dry Seeds with Chemicals: Use of Dichloromethane

Abstract. *Dichloromethane can be used to introduce chemicals into dry seeds in the absence of water. The solvent has no effect on germination or respiration. The chemicals become effective when the seeds are placed in water under normal conditions for germination. The potential of the new method is discussed.*

The effect of various chemicals on germination is usually studied by applying the compounds in aqueous solutions (*1*). Since imbibition requires time, the chemicals are not present at their site of action at zero time. This problem is particularly acute when one attempts to study the effect of metabolic inhibitors on biochemical steps during germination. Particularly when one tries to investigate the very early biochemical events, the delay of the arrival of the inhibitor at its site of action makes interpretation of results difficult. Chen (*2*) has used anhydrous acetone to introduce abscisic acid into isolated wheat embryos. We decided to try to treat entire dry seeds with solvents of various kinds (Table 1). Treatment of lettuce seeds, by almost all the solvents used, adversely affected their subsequent germination. Adverse effects were less obvious in peas. Acetone had little effect on germination, but it was not entirely effective in introducing substances into the seeds.

Additional solvents were therefore screened. Anhydrous dichloromethane had no effect on germination or on uptake of oxygen when lettuce seeds were treated for up to 24 hours with the solvent (Table 2). In contrast, anhydrous acetone distinctly depressed uptake of oxygen. When coumarin was applied together with dichloromethane, germination and uptake of oxygen were inhibited. When the seeds were rinsed in a large excess of water, with aeration, after treatment with dichloromethane and coumarin, the inhibition was completely reversed.

Dichloromethane, therefore, seems to be an ideal solvent for introducing various compounds into dry seeds. This ef-

Table 1. Effect of various solvents on germination of peas (var. Alaska) and lettuce (var. Grand Rapids). Germination percentage was examined after 24 hours in water in petri dishes. The lettuce seeds were germinated in light and the pea seeds were germinated in the dark. The solvent was removed by drying the seeds in vacuum before they were germinated.

Solvent	Percent germination after solvent treatment of			
	Lettuce treated for		Peas treated for	
	24 hours	44 hours	24 hours	44 hours
Dimethyl sulfoxide	0			
Dimethyl formamide	10			
Absolute ethanol	20	16	83	81
Bromoethane	48	0	59	60
Dichloroethane	47	13	70	70
Acetone (anhydrous)	80	77	80	60
Dichloromethane	75	72	68	
None	— 65	—	— 80	—

Table 2. Effect of treatment of lettuce seeds with nonaqueous solvents on their subsequent germination and oxygen uptake. Treatments lasted 24 hours, the coumarin concentration in each instance was $5 \times 10^{-3} M$. In all cases, germination was for 24 hours with light stimulus. After treatment with solvent the seeds were placed in a vacuum dessicator and dried until all traces of solvent had been removed. Oxygen uptake was measured with an oxygen electrode after 24 hours in germinating conditions.

Treatment	Germination conditions	Germination (%)	Oxygen uptake (μl/g per hour)
None	Water	59	2216
Dichloromethane	Water	65	2818
Acetone	Water	52	1810
None	Water + coumarin	2	890
Dichloromethane + coumarin	Water	4	955
Acetone + coumarin	Water	4	955
Dichloromethane + coumarin	Washed 3 hours, then water	51	2090
Dichloromethane + coumarin	Washed 4 hours, then water	59	2730

fectiveness of dichloromethane is presumably due to its free miscibility with polar and apolar solvents and its limited solubility in water.

The use of dichloromethane opens up new possibilities of treating dry seeds with stimulators or inhibitors of germination. A stimulator would be supplied to dry seeds if it were desired to accelerate germination. A water-soluble inhibitor would be supplied if it were desired to delay seed germination until suitable conditions occur, such as the presence of adequate rainfall. This would be useful if the inhibitor were slowly washed out of the seeds, as seems to occur with endogenous inhibitors in certain cases (*3*).

An additional use of permeating dry seeds with chemicals would be to study the metabolism of exogenously applied compounds. At present no reliable data are available on the metabolism of dry seeds.

HANNA MEYER
A. M. MAYER
Department of Botany, The Hebrew University, Jerusalem, Israel

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Aflatoxin P_1: A New Aflatoxin Metabolite in Monkeys

Abstract. *A new phenolic derivative of aflatoxin B_1, appearing mainly in conjugated form, was identified as the principal urinary metabolite of aflatoxin B_1 in rhesus monkeys. Its identification in human urine might facilitate estimation of aflatoxin exposure in human populations.*

Aflatoxin B_1 has many interesting biological and biochemical properties (*1*, *2*), including high potency as a hepatotoxic and hepatocarcinogenic agent in animals (*3*). Different species respond differently to the toxin (*1*), but susceptibility of man is unknown. Human exposure sometimes occurs (*4*, *5*), and evidence presented in case reports implicates aflatoxin in acute poisoning (*6*) and supports the hypothesis that aflatoxin exposure is associated with elevated incidence of primary liver cancer in certain populations (*7*).

Detailed knowledge of metabolism of the toxin is essential for understanding which aspects of the metabolism determine response and also for extrapolation of animal data in estimating human response. We have therefore been investigating the comparative metabolism of aflatoxin B_1 in the rat and monkey, and report here the isolation and chemical identification of a new metabolite from the urine of rhesus monkeys. The metabolite is the phenolic product of *O*-demethylation of aflatoxin B_1 and is present in urine mainly in conjugated form. Because it is a phenol, we propose the trivial name aflatoxin P_1. The major urinary metabolite of aflatoxin B_1, it represents the excretory form of about 20 percent of a single dose within 24 hours after injection.

Male rhesus monkeys (2.5 to 4.0 kg) were provided by and housed at the New England Regional Primate Research Center, Southborough, Massachusetts. Each animal was given a single intraperitoneal injection of a mixture that contained 5 μc [^{14}C]aflatoxin B_1, labeled only in the ring carbons, and nonradioactive aflatoxin B_1 (0.4 mg per kilogram of body weight), dissolved in 0.8 ml of dimethylsulfoxide.

Urine was collected continuously for 96 hours. Samples were processed according to the following scheme, with all procedures being carried out in either darkness or subdued light in order to minimize photolysis of aflatoxin metabolites. All extractions were performed with equal volumes of urine and solvent. Urine was successively extracted seven times, first with a mixture of methanol and chloroform (6 : 4, by volume), then six times with chloroform alone. The seven extracts were pooled, reduced in volume, and finally evaporated to dryness under nitrogen at room temperature (fraction 1). By this procedure we recovered 15 percent of total urine radioactivity.

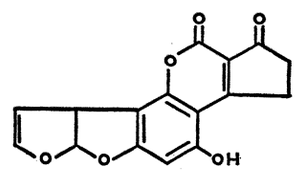

Fig. 1. Structure of aflatoxin P_1.

The remaining aqueous phase contained most (85 percent) of the total radioactivity in the urine, which we presumed to be conjugates of aflatoxin metabolites that were insoluble in chloroform. Major components of this fraction were separated and isolated as follows. The aqueous phase was recycled through an Amberlite XAD-2 (Rohm and Haas) column (4 by 50 cm) for 1.5 hours. This column, originally used for isolating steroid conjugates (*8*), retained 95 percent of the radioactivity contained in the aqueous phase. Radioactivity retained by the column was completely eluted with 1.5 liters of methanol, and the eluate was reduced to dryness (fraction 2). Two further procedures were used to isolate individual components.

In the first method, in view of the activities of the hydrolytic enzymes β-glucuronidase and sulfatase, we redissolved the residue in fraction 2 in 0.2*M* acetate buffer, *p*H 5.0, and incubated it with β-glucuronidase (Ketodase, Warner-Chilcott) for 48 hours at 37°C. The incubation mixture was extracted as described above; the extract, fraction 3, contained 60 percent of the radioactivity of fraction 2.

The aqueous residue remaining after the above treatment was reconstituted to its original volume with acetate buffer. Sulfatase type III (Sigma) was added, and the mixture was again incubated at 37°C for 48 hours. The incubation mixture was extracted and concentrated as described above; the product (fraction 4) contained 12 percent of the initial radioactivity of fraction 2.

The second method of separating components of fraction 2 consisted of column chromatography on diethylaminoethyl (DEAE) Sephadex. The procedures were adapted from those originally devised to separate certain groups of phenolic conjugates (*9*). This method avoided the interference by steroid conjugates that proved troublesome in the enzymatic procedure. A DEAE-Sephadex A-25 column (1.5 by 80 cm) eluted with a linear NaCl gradient (0.0 to 0.8*M*) was used to separate the radioactivity in fraction 2 into four subfractions. The major peak from this column contained 60 percent of the radioactivity of fraction 2; enzymatic hydrolysis with β-glucuronidase and solvent extraction suggested that most (90 percent) of the material in this subfraction was a glucuronide. In-