necessarily measures the uricolytic effect. In men, especially those who suffer from gout, there may be uric acid deposits. This reserve and normal endogenous and exogenous nucleic acid breakdown tend to maintain a constant serum uric acid level. Also, the breakdown of uric acid in blood does not necessarily lead to the quantitative formation of allantoin.

From the knowledge that even purer uricase preparations than that used here may be available (*1*) and that we are still far from using toxic doses, as demonstrated with animals, we are led to hope that the enzyme may yet be an adjunct in the treatment of gout and other pathological processes in which it may be necessary to clear the bloodstream of high uric acid concentration. In the rapidly developing fields of chemotherapy and radiotherapy, there is a potential need for a means of removal of excess uric acid which accumulates from the nuclear breakdown of malignant cells.

MORRIS LONDON*
PERRY B. HUDSON
Departments of Biochemistry and Urology, Francis Delafield Hospital, and *College of Physicians and Surgeons, Columbia University, New York*

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* Present address: North Shore Hospital, Manhasset, N.Y.

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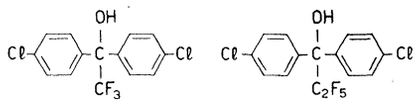
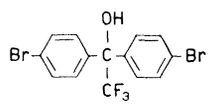
Prevention of Oviposition in the Housefly through Tarsal Contact Agents

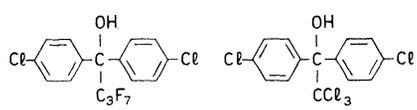
In our attempt to find ways of fighting insects, alternative to the use of contact insecticides, we have sought contact agents that would, even in sublethal doses, reduce significantly oviposition in houseflies. Among the substances tested, di-(*p*-chlorophenyl)-trifluoromethylcarbinol (I) and di-(*p*-chlorophenyl)-pentafluoroethylcarbinol (II) were outstanding. The corresponding dibromo compound (III), as well as di-(*p*-chlorophenyl)-heptafluoropropylcarbinol (IV), showed lower activity. If the chlorine atoms in I are replaced by methyl or methoxyl groups, the activity disappears completely, while replacement of the fluorine atoms in I by chlorine, which yields di-(*p*-chlorophenyl)-trichloromethylcarbinol (V) (DTMC), results in low activity. Di-(*p*-chlorophenyl)-dichloromethylcarbinol and DMC were completely inactive.



I ++++ II ++++



III ++



IV + V +

All of these compounds, which have become available only recently (*1, 2*) and have also shown interesting biological properties in other respects (*2, 3*), reduce, delay, or prevent oviposition in houseflies upon tarsal contact, when they are applied to females prior to feeding with milk.

Since compounds I (*2*) and II possess some slight contact toxicity, their oviposition-inhibiting properties are best demonstrated on a highly polyvalent resistant strain of houseflies. An extremely resistant Swiss strain (K_1) of *Musca domestica* L. (*4*) whose females are not at all affected by compounds I and II even at continuous exposure, has proved very valuable in these experiments, which have also employed a normal Swiss strain of *Musca domestica* L. and a normal and highly DDT-resistant local strain of *Musca vicina* Macq.

In general, 3-day-old females were taken from cages with mixed populations (thus fertilization was ensured) fed water and sugar only (*5*). They were then treated with compound I or II, introduced with equal numbers of untreated males into new cages, and fed with milk. Results with compounds I and II are summarized in Table 1; controls laid eggs normally.

It is thus possible to counteract the influence of continuous feeding with milk by continuous exposure to compound I or II. Smaller quantities or shorter exposure (with method 4) than those shown in Table 1 delay or drastically reduce the laying of eggs, while continuous feeding of milk, under the conditions of experiments 3 and 4 (Table 1), overcompensates the effect of compounds I and II.

On dissection of females that had been continuously exposed to compound I or II (method 5), it was found that motile spermatozoa were abundant in the spermathecae and that ovaries developed normally (same length as in control flies on milk) and contained eggs. We have thus a case of "forced retention" (*6*).

Table 1. Effect on oviposition of treatment of houseflies with compounds I and II. Three-day-old females of a highly resistant strain (K_1) of *Musca domestica* L. that had been fed only water and sugar were used. Milk was offered either daily (beginning on the fourth day of life) or only on the fourth day of life.

Expt. No. and mode of application of compounds	Milk offered	Oviposition during entire lifetime	
		Compound I	Compound II
1. Feeding in milk (0.01%)	Daily (treated)	Normal	Normal
2. Exposure to vapor	4th day	Normal	Normal
3. Topical*	4th day	None	None
4. Tarsal contact for 30 min†	4th day	None	Negligible
5. Continuous exposure‡	Daily	Very low	None

* One microgram in acetone per female.
† Females were exposed for 30 minutes to a deposit of 1 g/m² in petri dishes and then placed in the cages with the males.
‡ Filter paper of area equal to the area of one side of the cage was impregnated with 1.5 g/m² and hung in the center of the cage. (Crowding of flies in cages, which results in the covering of the compounds on the filter paper by feces, should be avoided in these experiments.)

The only data on the effect of chlorinated hydrocarbons on oviposition that have come to our knowledge are the following: Dieldrin in sublethal doses increases the reproductive potential in houseflies and *Drosophila melanogaster* Meig. (*7*), while DDT has a similar effect in *Metatetranychus ulmi* Koch (*8*). In *Drosophila* (*9*), DDT is reported to slightly reduce oviposition (*10*).

K. R. S. ASCHER*
Medical Research Laboratories, Medical Corps, Israel Defence Forces, Israel

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10. I wish to express my thanks to my colleague S. Reuter for the majority of compounds tested, and to A. Ben-Shmuel and D. Roch for excellent technical help.

* Present address: Department of Parasitology, Istituto Superiore di Sanità, Rome, Italy.

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Heat Inactivation of Catalase in Deuterium Oxide

We have measured the kinetics of heat inactivation of the enzyme catalase in H_2O and D_2O over a range of temperatures. The results show a considerable difference in both the heat and the entropy of activation, both being higher for heating in D_2O.

The enzyme (*1*) was dissolved in 0.05*M* phosphate buffer, made up in either H_2O or D_2O (> 99.6 per cent) at *p*H 7.0 in H_2O. The D_2O buffer read 6.85 on the *p*H meter (*2*). The concentration of catalase during heating was 300 μg/ml. At appropriate intervals, 0.1-ml samples were taken, diluted to 15 μg/ml in buffer made in H_2O, and assayed by observing the breakdown of H_2O_2 by measurements of optical density at 2300 A in a Beckman model DU spectrophotometer (*3*). The D_2O concentration during assay was 1.4 percent. Standing in D_2O buffer for periods up to 2 weeks at 8°C had no measurable effect on the rate of subsequent inactivation in D_2O or on the absolute enzymatic activity as compared with fresh samples in H_2O.

The inactivation curves followed first-order kinetics within the precision of the data, with reaction times from a few seconds to several hours. From the observed rate constants, the free energies of activation were calculated from the theory of absolute reaction rates (*4*) according to the equation

$$k = \frac{\bar{k}T}{h} e^{-\Delta F^{\ddagger}/RT}$$

Figure 1 shows the temperature dependence of the free energy of activation ($\Delta F^{\ddagger}$). From the relation

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

we find, for heating in H_2O, $\Delta H^{\ddagger} = 87$ kcal/mole and $\Delta S^{\ddagger} = 191$ cal/mole · deg, and for heating in D_2O, $\Delta H^{\ddagger} = 145$ kcal/mole, and $\Delta S^{\ddagger} = 360$ cal/ mole · deg.

Wiberg (*5*) and Morowitz and Brown (*6*) have recently reviewed the effect of deuterium on reaction rates. Since the zero-point energy of a covalent bond to deuterium is lower than that of the corresponding one to hydrogen by about 1.2 to 1.5 kcal/mole (because of the greater mass and consequent lower freqency), reactions which involve breaking such bonds are usually slower in D_2O, and deuterated compounds in general react more slowly than hydrogenated ones. Enzymatically catalyzed reactions have been observed to go both faster and slower in D_2O, and some of the reports are conflicting. Macht and Bryan (*7*) report a "noticeable acceleration" of the action of catalase on H_2O_2 in 0.05- to 1.0-percent D_2O, whereas Fox (*8*) reports no change in 1-percent D_2O. Our results tend to confirm Fox, although it is possible that a change of a few percent may have been unnoticed.

Caldwell, Doebbling, and Manion (*9*) reported that pancreatic amylase denatured more rapidly at 25°C in D_2O than in water, whereas Fox (*8*) reported no difference in the daily loss of catalase activity between D_2O and H_2O. It seems likely that neither of these cases represents thermal inactivation of the kind observed here. We found loss of catalase activity in H_2O at 25°C and 37°C to be much more rapid than consistent with the higher temperature data, and we suspect that bacterial growth was the cause. Extrapolation of the curve in Fig. 1 would yield a time of about 10 years at 25°C, for the same reaction in H_2O.

Morowitz and Chapman (*10*) reported that deuterium substitutes rapidly (within 20 minutes) in all bonds of proteins except C—H. Linderstrøm-Lang (*11*) found that in some proteins there is a continued slower exchange following the initial rapid one. Our evidence suggests no further effect on heat stability of catalase between about ½ hour and 2 weeks.

The mechanism of thermal inactivation of enzymes is still obscure, although it is presumably closely related, if not identical, to denaturation. The high values of the heat and entropy of activation observed here are typical of enzyme inactivations and have suggested a process involving the breaking of a number of weak interchain hydrogen bonds, with accompanying high entropy changes as the molecule becomes more disordered (*12*). It may be expected that deuterium bonds are related to hydrogen bonds qualitatively as the respective covalent bonds, and that they will be somewhat stronger. It is not surprising, therefore, to find $\Delta H^{\ddagger}$ higher for inactivation in D_2O. Further, since the vibrational energy levels of a deuterium bond are more closely spaced, the partition functions are affected, and thereby the entropy terms are expected to be changed in the free energies of reactants, products, and activated complex. It is difficult, however, to predict a priori the effect of this on the $\Delta S^{\ddagger}$ of the inactivation process, except to say that it is likely to be different (*13*).

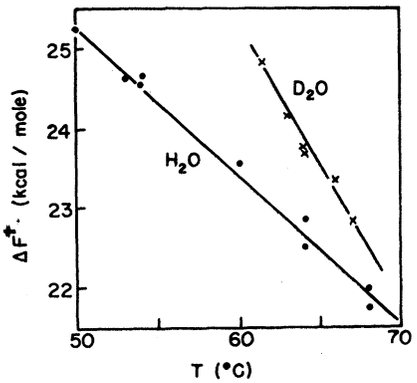

Fig. 1. The free energy of activation ($\Delta F^{\ddagger}$) for inactivation of catalase in H_2O and D_2O at various temperatures. The lines represent the equation $\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$, where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the heat and entropy of activation, respectively. For H_2O, $\Delta H^{\ddagger} = 87$ kcal/mole, $\Delta S^{\ddagger} = 191$ cal/mole · deg; for D_2O, $\Delta H^{\ddagger} = 145$ kcal/mole, $\Delta S^{\ddagger} = 360$ cal/mole · deg.

W. R. Guild
R. P. van Tubergen
Department of Biophysics,
Yale University,
New Haven, Connecticut

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13. We thank H. J. Morowitz, for suggestions concerning, and discussions of, these experiments, and E. M. McKelvey and R. M. Gibson, who performed some of the preliminary experiments.

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