the other hand, if the drug acts to lower the set temperature the animal will avoid the heat lamp so as to 'allow' the body temperature to fall to the new level[13].

Although facilities for the measurement of regional brain histamine turnover were not available at the time of the present experiments, since McN-A-1293 has no effect alone but reduces both the fall in body temperature and the associated thermoregulatory behavior after histidine loading, it is reasonable to ascribe these phenomena to the only known effect of the compound – inhibition of histidine decarboxylase. Thus, the data confirm previous impressions[7] that the hypothermic effects of histidine loading is the result of increased brain histaminergic activity in the efferent heat loss pathways.

DDT: The Degradation of Ring-Labeled ^{14}C-DDT to $^{14}CO_2$ in the Rat

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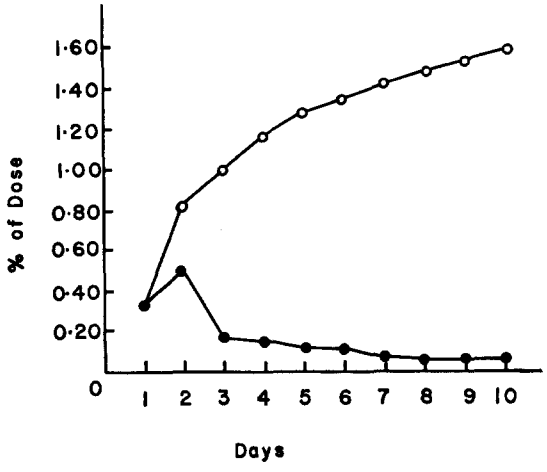
Summary. Ring fission of *p,p'*-DDT was studied in the rat following a single oral dose of 0.74 mg/kg (1.04 μCi) of uniformly ring-labeled ^{14}C-DDT. Expired air was passed through a solution of ethanolamine-ethylene glycol monomethyl ether (1:2) to trap $^{14}CO_2$. A total of 1.6% of the radioactivity administered was recovered in the expired air collected continually for 10 days, indicating that while degradation of the phenyl moiety is not a major route pf *p,p'*-DDT metabolism in the rat, it is equal to the urinary excretion. Nevertheless, these results represent the most radical change accomplished in vivo of a residual insecticide yet reported in mammals.

Increased attention has been drawn to the ecological significance of *p,p'*-DDT[2]. This growing concern is generally attributed to the stability and the subsequent persistence of DDT-type compounds especially DDE in the environment[3,4]. Because of the accumulation of *p,p'*-DDT and its metabolites in the animals, the pathways of excretion are important to the assessment of hazard since excretion is a major means of protection against accumulation and toxicity. It is surprizing, however, that despite the very high rates of usage of *p,p'*-DDT over the past 45 years, knowledge of its degradation pathways in biological and nonbiological systems is incomplete. Metabolic studies of *p,p'*-DDT have been concerned almost entirely with the loss of only 1 out of the 14 carbon atoms of the molecule[5]. This is exemplified by studies showing the conversion of *p,p'*-DDT to DBP in rats[6] and bacteria[7] and to DBP, DBH and DDM in chicks[8]. The cleavage of one of the phenyl rings of DDM to yield *p*-chlorophenyl acetate by *Hydrogenomonas* has been established[9,10]. The purpose of the present study is to determine whether the rat is also capable of degrading the phenyl ring of the *p,p'*-DDT.



Daily rate (●) and accumulated (○) $^{14}CO_2$ in the expired air from rats following a single oral dose of ring-labeled ^{14}C-DDT.

Materials and methods. White male rats (Cheek & Jones, Houston, Texas), weighing approximately 250 g, were placed in individual Cary animal cages (Glass Instruments, Inc., Pasadena, California). The Cary animal cage, designed especially for studies of expired $^{14}CO_2$ contained coarse and fine mesh screens to separate fecal material from urine which was collected in a separate flask. The expired air was drawn out through this flask by use of a water aspirator. The animals were allowed to adjust to their environment for 1 week subsequently, each of 12 rats was fed a single 0.74 mg/kg dose (1.04 μCi) of ^{14}C-DDT (specific activity of 5 mCi/mmole, New England Nuclear) in a gelatin capsule. ^{14}C-DDT had radiochemical purity of more than 99.5%. 4 similar rats were not fed *p,p'*-DDT and served as controls. The animals were returned to their cages and given free access to food and water. $^{14}CO_2$ was trapped in a solution of ethanolamine-ethylene glycol monomethyl ether (1:2 v/v). ^{14}C-radioactivity was measured by a Beckman model

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[2] Abbreviations: 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane,(*p,p'*-DDT); 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane, (*p,p'*-DDD); 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene, (DDE); 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethylene, (DDMU); 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethane, (DDMS); unsym-*bis*(*p*-chlorophenyl)-ethylene, (DDNU); 2,2-*bis*(*p*-chlorophenyl)ethanol, (DDOH); *bis*(*p*-chlorophenyl)acetic acid, (DDA); *bis*(*p*-chlorophenyl)methane, (DDM); 4,4'-dichlorobenzophenone, (DBP); and *bis*(*p*-chlorophenyl)methanol, (DBH).

[3] M. B. ABOU-DONIA, Appl. Spec. *29*, 221 (1975).

[4] D. B. MENZEL and M. B. ABOU-DONIA, in *Proc. of the Symposium on the Biological Impact of Pesticides in the Environment*, August 18–20, 1969, Oregon State University, Corvallis, p. 161.

[5] R. L. METCALF, J. agric. Food Chem. *21*, 511 (1973).

[6] J. G. PETERSON and W. H. ROBINSON, Toxic. appl. Pharmac. *6*, 321 (1964).

[7] G. WEDEMEYER, Appl. Microbiol. *15*, 569 (1967).

[8] M. B. ABOU-DONIA and D. B. MENZEL, Biochem. Pharmac. *17*, 2143 (1968).

[9] D. D. FOCHT and M. ALEXANDER, Science *170*, 91 (1970).

[10] F. K. PFAENDER and M. ALEXANDER, J. agric. Food Chem. *20*, 842 (1972).

LS 250 liquid scintillation spectrometer after adding a 1 ml aliquot of the trapping solution to 10 ml of the scintillation solution[11]. The recovery of radioactivity ranged between 98 and 100%. All analyses of labeled materials were done in duplicate using internal standards.

Results and discussion. The Figure shows the accumulated total and the daily rate of ^{14}C-radioactivity in the expired air of rats fed ^{14}C-DDT. During the period of the experiment there was a steady increase in the total radioactivity in the expired air. The daily rate of ^{14}C excreted in the expired air reached a peak on the 2nd day after administration, then sharply decreased until the 8th day and leveled off thereafter.

These results indicate that the oxidation of the phenyl groups of *p,p'*-DDT to CO_2 is not a major pathway for *p,p'*-DDT metabolism in the rat. Only a total of 1.6% of the radioactivity of the administered dose was recovered in the expired air within 10 days of ingestion at which time 10.5% of the ^{14}C remained in the tissues. Most of the ^{14}C was excreted in the feces (83.8%) and a small portion (1.6%) was recovered in the urine. Respiratory excretion was, however, of equal significance to urinary excretion.

The biotransformation of *p,p'*-DDT so far reported involves dehalogenation and hydroxylation steps that do not result in fission of the original phenyl groups. For example the major scheme for *p,p'*-DDT in the rat is the conversion to DDD and DDMU[6]. DDD is further degraded by the pathway DDD → DDMU → DDMS → DDNU → DDOH → DDA. DDA is found principally in the urine. In chicks and chick embryos a similar pathway also occurs[8]. *p,p'*-DDD appears to be the principal precursor for all other *p,p'*-DDT metabolites. The conversion of *p,p'*-DDT and DDE to DBP by the chick represents the potential degradation of *p,p'*-DDT to CO_2, but not of the phenyl groups[8]. Two other organisms have been observed to degrade phenyl groups of *p,p'*-DDT completely. Less than 1% degradation occurred with the cockroach[12] while *Hydrogenomonas* cleaved the phenyl group of DDM, a *p,p'*-DDT metabolite, to *p*-chlorophenylacetate, CO_2, H_2O and HCl[13]. Benzene ring fission most likely occurs through oxygenated intermediary products[10,14]. The degradation of the phenyl groups of *p,p'*-DDT to CO_2 represents a pathway equal to that of urinary excretion representing primarily DDA excretion. The degradation of *p,p'*-DDT to CO_2 by rats, while small, nevertheless represents a significant detoxication pathway. We plan to investigate the potential intermediary compounds suggested to exist from these observations.

[11] M. B. Abou-Donia, C. M. Lyman and S. W. Dieckert, Lipids *5*, 938 (1970).
[12] W. E. Robbins and P. A. Dohn, J. agric. Food Chem. *3*, 500 (1955).
[13] D. D. Focht, Bull. Envir. Contamin. Toxic. *7*, 52 (1972).
[14] P. J. Chapman, *Degradation of Synthetic Organic Molecules in the Biosphere* (National Academy of Science, Washington, D.C. 1972), p. 17.

Ultrastructural Autoradiographic Study of the Intracellular Fixation of ^3H-Acrolein

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Summary. The fixation of ^3H acrolein in *Dunaliella bioculata* was followed by means of quantitative autoradiography electron microscopy. A fixation was observed mostly in the nucleus where aldehyde linkage remained stable at least for 48 hours.

Previous studies have shown the cytotoxicity of acrolein (CH_2 = CH–CHO). This widely distributed aldehyde is one of the main components of the gas phase of cigarette smoke[2]. It is also found in vivo; during the enzymic oxydation of spermine and spermidine[3] and, in vitro, during the degradation of cyclophosphamide an antitumor agent[4]. Several authors have demonstrated that this aldehyde inhibits the nucleic acid synthesis of bacteria[5], of mouse kidney cells in culture[6] and also of hepatic and pulmonary tissue in partially hepatectomized rats[7].

Our previous observations have pointed out morphological modifications induced by acrolein in an unicellular alga: *Dunaliella bioculata*[8]. The major cytological effects have been observed in the nucleus. In the present work, our intent was to detect the site of fixation of the drug and to determine the stability of that linkage by means of the autoradiographic electron microscope method.

Material and methods. *Dunaliella bioculata*, a green flagellate volvocale, was grown on the mineral growth medium of Miquel[2] at 24 °C, under a 12-12 light-dark cycle, at 4000 lux. We used freshly prepared ^3H-acrolein (25 Ci/m*M* C.E.A. Saclay, 150 μCi/ml of culture or 0.6 10^{-5} m*M*/ml). At this concentration, acrolein inhibits cell division of *Dunaliella bioculata*[2].

In the first experiment, the cells in exponential growth were incubated 15, 30 or 60 min with ^3H-acrolein. In the second experiment (chase experiment), the cells were incubated 15 min with ^3H-acrolein; then, after centrifugation and washings, they were transferred into fresh medium without the drug for 90 min, 24 h or 48 h.

The algae were fixed in 4% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7) for 1 h, washed 24 h in 0.1 *M* cacodylate, then postfixed in 2% OsO_4 for 1 h. The cells were embedded in araldite and the sections were prepared for electron microscope autoradiography according to the

[1] Unité de recherches statistiques de l'Inserm, 16bis, rue Paul Vaillant Couturier, Villejuif (France).
[2] C. Izard and P. Testa, Annls Seita *6*, 121 (1968).
[3] R. A. Alarcon, Archs Biochem. Biophys. *106*, 240 (1964).
[4] R. A. Alarcon and J. Meienhofer, Nature New Biol. *233*, 250 (1971).
[5] B. W. Kimes and D. R. Morris, Biochim. biophys. Acta *228*, 235 (1971).
[6] C. Leuchtenberger, M. Schumacher and T. Haldimann, Z. Präventiv-Med. *13*, 130 (1968).
[7] N. Munsch and C. Frayssinet, Biochimie *53*, 243 (1971).
[8] F. Marano and S. Puiseux-Dao, J. Microsc. *11*, 76 (1971).