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.

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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

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LARRA and DROZ technic[9]. In the first experiment, the exposure time of the autoradiograms was 3 weeks, and in the second, 5 weeks. The grain count was carried out on cell pictures taken at the same magnification. The cell and organelles areas were determined by planimetry (Figure 3).

For statistical analysis, the data of the 2 experiments were separatly treated. The design of the experiments was factorial in 2 ways[10]: the first way was the nature of the structure considered (whole cell, cytoplasm, nucleus and plastid), the second way was the time. The number of replicate was 42 in the first experiment and 25 in the second. Through this statistical method it is possible to analyze separately the effect of the two factors on the labelling.

Results. For each experiment, the density of the silver grains on the nucleus, the plastid, and the cytoplasm were compared, then the evolution of the drug fixation in the 3 areas was studied.

1. Analysis of ^{3}H acrolein in relation to the cellular ultrastructures (Figures 1 and 2). In the 2 experiments, the fixation of ^{3}H-acrolein was significantly more important on the nucleus than on the plastid or on the cytoplasm ($p < 0.001$). Between these two areas, there was no significant difference in the degree of aldehyde fixation.

2. Evolution of the ^{3}H-acrolein fixation in relation to time (Figures 1 and 2). Statistical analysis demonstrated that, during the hour of the first experiment (Figure 1), the binding of acrolein was increasing significantly ($p < 0.001$). The increase was rapid during the first 15 min of the incubation time, followed by a levelling off of the fixation curve. In the chase experiment (Figure 2), the significant interaction test indicated some differences in the evolution of the labelling according to the cell structures. The statistical analysis showed that the radioactivity decreased significantly ($p < 0.001$) in the plastid and the cytoplasm during the first 90 min, then the labelling remained steady for the next 2 days. In contrast, the evolution of ^{3}H-acrolein fixation on the nucleus was not significant during the 48 h of chase.

Discussion. Our previous biochemical analysis on *Dunaliella bioculata*[11] showed that there was a progressive binding of acrolein with proteins, RNA and DNA. This is in agreement with our present autoradiographic results

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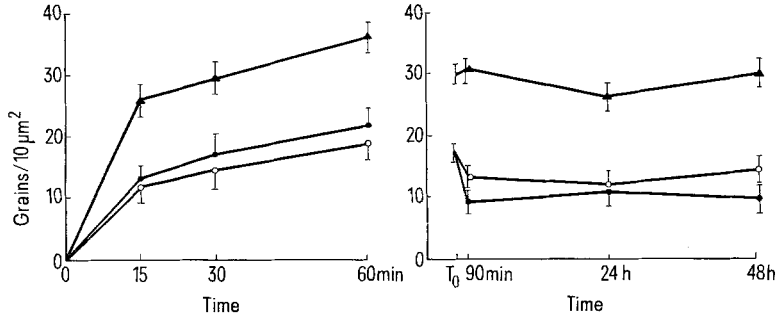

Fig. 1. Influence of the incubation time on the acrolein fixation in the cells. Grain counts on the nucleus (▲), the plastid (●) and the cytoplasm (○).

Fig. 2. Chase experiment: Evolution of the labelling on the nucleus (▲), the plastid (●) and the cytoplasm (○). After a pulse of 15 min with ^{3}H-acrolein, the cells were washed and returned on fresh medium at T_0.

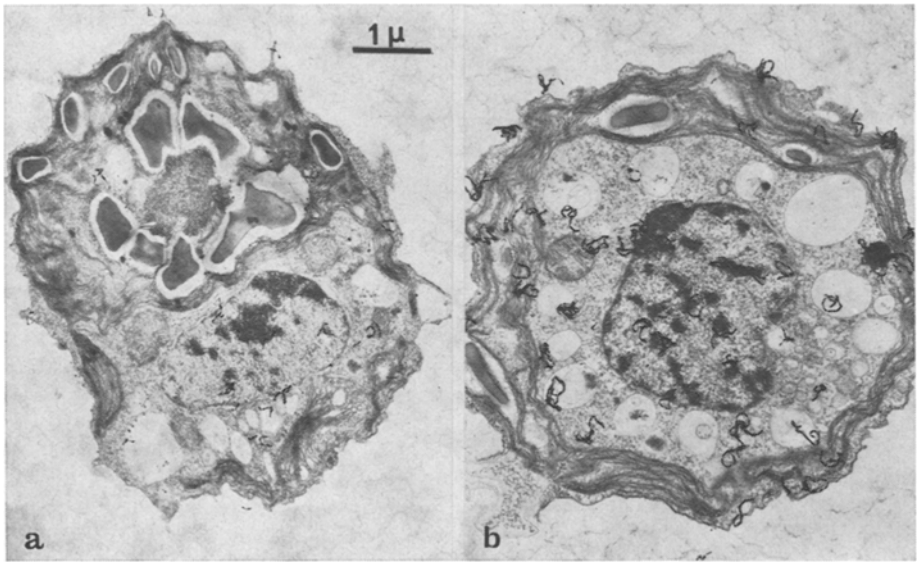

Fig. 3. Autoradiography of *Dunaliella bioculata* after a labelling with ^{3}H-acrolein. a) 15 min of labelling; b) 60 min of labelling.

concerning the increasing aldehyde fixation on all the cell structures during the 1st h of incubation. The autoradiographic analysis, however, brought additional data: the preferential area of cellular fixation for acrolein was the nucleus; this fixation was stable for at least 2 days, which is not the case for cytoplasmic or chloroplastic fixation.

These results are in agreement with those obtained on regenerating rat liver in vivo[11], where it was demonstrated that the acrolein was rapidly fixed on DNA and the fixation was stable for at least 24 h. In vitro, the acrolein had a great affinity for the DNA polymerase to which it could be irreversibly attached[12]. This preferential and stable fixation of the acrolein to the nucleus may explain the high toxicity and the irreversibility of the cytotoxic effects of that molecule. These results raise the question of the reconsideration of ALARCON's[13] hypothesis which suggests that acrolein could be an element of an universal system of control for cellular growth.

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Frequency of the Various Stages of the Seminiferous Epithelium in Different Strains of Male Mice

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Summary. The duration of the various development stages of the seminiferous epithelium in different strains of male mice was determined by scoring the frequency distribution in randomly selected tubule cross-sections. The results obtained show a difference in the duration of the various stages between the different strains.

The duration of spermatogenesis in the mouse and of the stages in the cycle of the seminiferous epithelium has been determined by OAKBERG[3].

Using the periodic acid Schiff technique, OAKBERG was able to distinguish 16 stages of spermatogenesis in the mouse, 12 of these corresponding to 1 cycle of the seminiferous epithelium. 4 cycles occur between the development of the first type A spermatogonia to the spermatozoa. According to OAKBERG, the duration of the spermatogenesis in the mouse is $34^1/_2$ days and the duration of each cycle is 207 ± 6.2 h.

The frequency distribution of tubules in randomly selected samples of non-irradiated animals was used to time each stage of the seminiferous epithelium development, and the results obtained have been of great help in the determination of the radiation sensitivity of the different kinds of spermatogonial cells. No investigations have been reported, however, to determine the tubular frequency and to check whether variations exist in different strains of mice.

Table I. Frequency of tubules by stages

Stage	C3H inbred mice			
	First scoring		Second scoring	
	No. of tubules per stage[a]	Relative frequency	No. of tubules per stage[b]	Relative frequency
I	358	0.060	264	0.067
II	298	0.050	205	0.052
III	775	0.129	476	0.120
IV	514	0.086	338	0.085
V	360	0.060	243	0.061
VI	285	0.047	195	0.049
VII	431	0.072	296	0.075
VIII	796	0.133	447	0.113
IX	769	0.128	560	0.141
X	407	0.068	268	0.068
XI	399	0.066	232	0.059
XII	613	0.102	433	0.109
Total	6005		3957	

[a] 500 randomly selected tubules scored in 8 mice + 1000 tubules scored in 2 mice. [b] About 500 randomly selected tubules scored in 8 mice.

Table II. Frequency of the various stages of the seminiferous epithelium in F_1 hybrid mice

Stage	F_1 C3H ♂ C57 Bl ♀		F_1 ARR ♂ C3H ♀	
	No. of tubules per stage[a]	Relative frequency	No. of tubules per stage[a]	Relative frequency
I	197	0.066	227	0.075
II	106	0.035	244	0.081
III	377	0.125	439	0.145
IV	193	0.064	290	0.096
V	155	0.052	232	0.077
VI	151	0.050	141	0.047
VII	193	0.064	156	0.052
VIII	454	0.151	232	0.077
IX	425	0.141	400	0.133
X	229	0.076	179	0.059
XI	222	0.074	188	0.062
XII	306	0.102	291	0.096
Total	3008		3019	

[a] 500 randomly selected tubules scored for each of the 6 mice.

Materials and methods. Male mice C3H inbred, F_1 hybrid between inbred C3H ♂ and C57 Bl ♀, and F_1 hybrid between ARR ♂ and C3H ♀, 9 to 12 weeks old, were used. The animals were killed by cervical dislocation. The testes were fixed in Orth fluid, embedded in paraffin, and then cut in 5 μm sections. The sections were

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