R-flies brought into contact with DTMC or its DDT-"supported" combinations fall on their back and show recurrent tremors of the extended extremities ("Streck-tremor"). Flies kept in contact for long periods often have a blown-up abdomen due to swallowing of air. These symptoms of poisoning somewhat resemble those described for pyrolan[13].

Unfortunately, the action of DTMC and its combinations with DDT and related substances against resistant houseflies, compares very unfavorably with that of the newer phosphor insecticides as e.g. diazinon[6] or malathion (Table II), and it seems that the *insecticidal* properties of DTMC are of theoretical interest only. However, while this work was in progress, excellent *acaricidal* properties were claimed for α-trichloromethyl 4,4'-dichlorobenzhydrol, which seems to be the same compound as DTMC, but neither its mode of preparation, nor any physical properties have been described[14].

Table II. — Contact action of 0·25 g/sq.m. malathion on R-flies

4 × 10♀ R-flies, 2–3 days old; contact – 2 h; t = 27°C					
Age of deposit in days	1	7	14	21	28
Contact in min					
10	32	70	–	2	–
20	90	97	42	10	12
30	97	97	95	62	52
60	100	100	100	100	100
90	100	100	100	100	100
120	100	100	100	100	100
Σ % k.d	519	564	437	374	364

Note: No k.d. in untreated controls

We wish to thank Prof. G. G. MER for helpful criticism.

The excellent technical help of Miss DALIA ROCH is gratefully acknowledged.

S. REUTER[15] and K. R. S. ASCHER[16]

Medical Research Laboratories, Medical Corps, Israel Defence Forces, Israel, April 5, 1956.

Résumé

L'action des carbinols di-(*p*-chlorophenyl) trifluorométhyl et di-(*p*-chlorophenyl) trichlorométhyl (DTMC) a été déterminée sur une souche très résistante de mouches par la méthode de contact. Le premier composé semble agir probablement grâce à de faibles propriétés fumigantes, alors que le second est toxique par contact. L'action toxique de ce dernier est sensiblement augmentée par l'addition du DDT, DDE ou autre composé organique de la série du DDT.

[13] R. WIESMANN and C. KOCHER, Z. angew. Ent. *33*, 297 (1951).

[14] H. F. WILSON and J. S. BARKER, 126th Meeting of American Chemical Society, Section 29A, 1954. – D. ASQUITH, J. econ. Ent. *48*, 329 (1955).

[15] Chemical development of DTMC.

[16] Biological evaluation.

The Effect of Formaldehyde on the Mutagenic Action of X-rays in *Drosophila**

Earlier investigations showed an increase of X-ray induced mutation rates after pretreatment with cyanide and azide in immature germ cells which are characterized by peak sensitivity to the mutagenic action of X-rays[1]. This result suggested that an increased production of hydrogen peroxide, due to inhibition of the cytochrome- and catalase enzyme systems, made the genetic material more susceptible to the effects of radiation. This assumption was further supported by the observation that pretreatment with an organic peroxide (dihydroxydimethyl peroxide) likewise significantly enhanced the mutagenic action of X-rays, notably in the same sensitive stage of spermatogenesis as pretreatment with cyanide and azide[2].

Because formaldehyde is also known to act as a catalase inhibitor[3] and presumably produces mutations via the formation of an organic peroxide[4], it was thought of interest to study the effects of formaldehyde pretreatment on the rate of X-ray induced mutations.

Material and methods.— The formaldehyde used was a BDH commercial preparation. All solutions were made with a 0·7% solution of sodium chloride in distilled water. The formaldehyde concentrations ranged from 0·033–0·050 Mols per litre. 2–3 day old Oregon-K males were injected intraabdominally with 0·28 mm³ of such solutions and were afterwards tested for the incidence of sex-linked lethals by means of the Muller-5 method, the first matings being started one day after treatment. To reduce the time interval between pretreatment and irradiation, the flies were injected in batches of about 30 individuals which were then placed immediately under the X-ray apparatus, the average time interval between injection and irradiation being 11 ± 8 min. The pretreated and X-ray control flies were differently marked with indian ink on the thorax which made possible the simultaneous exposure of both groups. X-radiation was administered by a General Electric, Maximar 100 Model of the Dermatology Clinic of the University of Utrecht. It was run at 100 KVP and 5 mA, at a dose rate of 244 r per min with 1 mm Al filtration (HVL = 1·3 mm Al).

To study the sensitivity pattern in the testes, treated males were remated to 3 fresh, virgin Muller-5 females at specific time intervals. The successive broods arising in this way then represent successively younger stages of spermatogenesis at the time of treatment[5]. No controls were used, because the spontaneous mutation rate in the Oregon-K stock rarely exceeds 0·2–0·3%.

Results.— The results of experiments with 2 different concentrations of formaldehyde are presented in Table I and Figure 1. In experiment 1 pretreatment with a concentration of 0·05 Molar which is hardly capable of raising the spontaneous mutation rate, caused a significant increase of the X-ray induced mutation rates in both the first and second broods. Pretreatment with an even weaker 0·042 M formaldehyde solution in experiment 2 likewise enhanced the mutation rates produced by the irradiation with a factor of from 1·60 to 1·85 in all 3 broods.

* This paper is dedicated to Prof. Dr. JACOB SEILER, Zürich, on the occasion of his 70th birthday.

[1] F. H. SOBELS, Z. ind. Abst. Vererbungsl. *86*, 399 (1955).

[2] F. H. SOBELS, Nature *177*, 979 (1956).

[3] K. G. STERN, Hoppe-Seyl. Z. *209*, 176 (1932).

[4] F. H. SOBELS and J. W. I. M. SIMONS, Z. ind. Abst. Vererbungsl. (in press); Nature *177*, 979 (1956).

[5] CH. AUERBACH, Z. ind. Abst. Vererbungsl. *86*, 113 (1954).

Table I. Frequency of sex-linked lethals in 2–3 successive three-day broods from males which had been injected with solutions of formaldehyde prior to X-radiation, as compared with that from males which were irradiated or injected only.

Experiment	Formaldehyde concentration in Mols/l	Radiation dose in r	Sex-linked lethals – Days after treatment											
			1–4				4–7				7–10			
			n chrom.	%leth.	χ^2	*P*	*n* chrom.	%leth.	χ^2	*P*	*n* chrom.	%leth.	χ^2	*P*
F 1	0·050	–	363	0·8	–	–	360	0·3	–	–	388	0·5	–	–
R 1	–	1700	388	5·6			373	10·4			–	–		
					3·8	0·05			6·2	< 0·02			–	–
F–R 1	0·050	1700	478	9·9			268	17·5			–	–		
R 2	–	2200	338	6·5			341	9·1			306	3·6		
					5·9	< 0·02			7·4	< 0·01			1·3	0·2–0·3
F–R 2	0·042	2200	269	13·0			287	16·7			115	6·1		

For all χ^2 calculations corrections have been made allowing for the mutagenicity of formaldehyde alone in the corresponding broods.

In consideration of the low mutation rates induced by 0·05 M formaldehyde, no formaldehyde controls were taken for 0·042 M in experiment 2. The χ^2 for the difference between FR and R has been corrected for the frequency of mutations, as produced by 0·05 M formaldehyde by itself in experiment 1. Even then there remains a real excess of mutations in the first 2 broods of the pretreated series (FR_2).

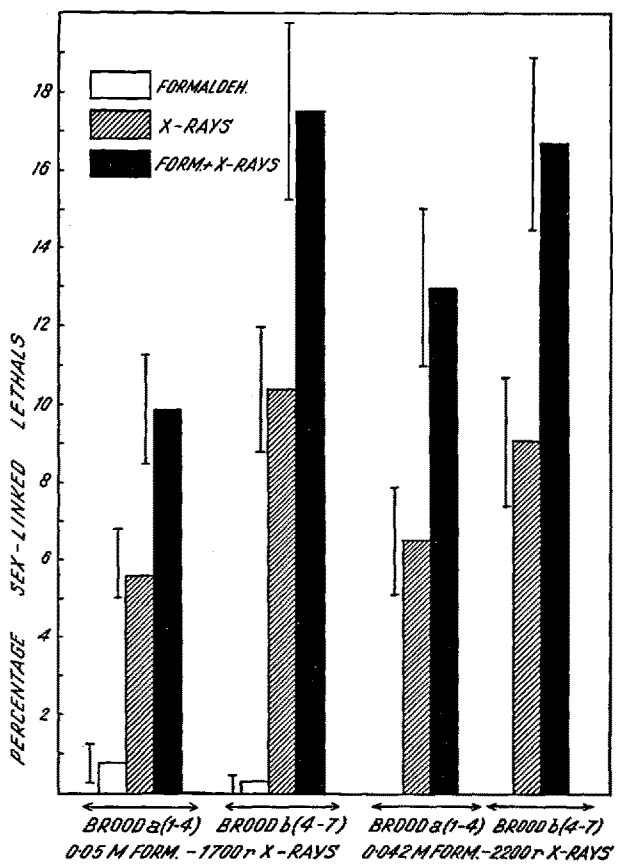

Fig. 1.—The frequency of sex-linked lethals in 2 successive broods (experiments 1 and 2) from males which had been injected with solutions of formaldehyde prior to X-radiation, as compared with that from males which were irradiated or injected only.

Formaldehyde thus resembles cyanide, azide and dihydroxydimethyl peroxide in potentiating the mutagenic action of X-rays. But its effects are different in that formaldehyde pretreatment raises the X-ray induced mutation rates in both the first and second broods, whereas in the other pretreatment experiments, the enhancement seemed specifically confined to stages sampled during the second brood only.

This finding raises the question whether formaldehyde which is known to have a mutagenic effect on mature sperm, similarly exerts a potentiating effect on X-ray induced mutation rates in this stage. Two further experiments were therefore done with daily brood changes during a period of 5 days after treatment. It was hoped that this method would result in a clearer separation of stages with different sensitivity. To ensure a maximum comparability, the two experiments were carried out within a short space of time, with males from the same sample and with the same 0·033 M formaldehyde solution. This procedure also had the advantage that one group of controls, receiving formaldehyde only, would be sufficient.

The results of the 2 separate experiments are presented in Table II. In view of their great similarity the data were pooled; this combined result is set out in Figure 2. First of all it can be seen that from the second day onwards formaldehyde is effective in causing a pronounced enhancement of the mutation rates induced by the radiation. No significant differences could be observed, however, for mutation rates of sperm sampled on the first day after treatment. It would seem therefore that fully mature sperm does not respond any more to the potentiating effect of formaldehyde on X-ray mutagenesis.

The possibility remains that the effect on the second day is different in nature from that on the later ones. BAKER and VON HALLE[6] found that after irradiation in nitrogen, there is no difference between dominant lethal rates of sperm used up during the second and on the first day. Similarly the enhancement observed with formaldehyde on the second day might be due to prevention of recovery from the irradiation effects by the pretreatment.

The sharp increase of mutation rates in the subsequent broods suggests that this mating-scheme has been highly effective in selecting stages which become increasingly more sensitive to the mutagenic action of

[6] W. BAKER and E. S. v. HALLE, Proc. nat. Acad. Sci. Washington *39*, 152 (1953).

Table II. Frequency of sex-linked lethals in 5 successive one-day broods from males which had been injected with a 0·033 M solution of formaldehyde prior to X-radiation with 1700 r, as compared with that from males which were irradiated or injected only.

Experiment	Sex-linked lethals – Days after treatment																	
	1		2				3				4				5			
	n	%l	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*	*n*	%l	χ^2	*P*
Formaldehyde controls .	365	0·3	419	1·0	–	–	469	0·2	–	–	506	1·0	–	–	430	0·5	–	–
R 3	504	5·4	409	4·6			318	4·4			332	12·3			121	17·4		
					–				6·31	<0·02			6·70	<0·01			–	
F–R 3	514	7·0	425	6·1			388	9·3			192	21·4			39	33·3		
R 4	446	4·5	436	3·0			350	3·7			325	13·9			113	27·2		
					10·16	<0·01			9·25	<0·01			–				–	
F–R 4	527	4·9	460	8·5			339	8·0			187	18·2			59	33·9		
Σ R 3 + R 4	950	5·0	845	3·8			668	4·0			657	13·1			234	22·2		
					7·19	<0·01			15·48	<0·001			7·05	<0·01			4·76	<0·05
Σ F R 3 + F R 4 . . .	1041	6·0	885	7·4			727	9·3			379	19·8			98	33·6		

For all χ^2 calculations corrections have been made allowing for the mutagenicity of formaldehyde alone in the corresponding broods.

irradiation. A comparison between the mutagenic effects in the first and in the fifth broods gives a clear

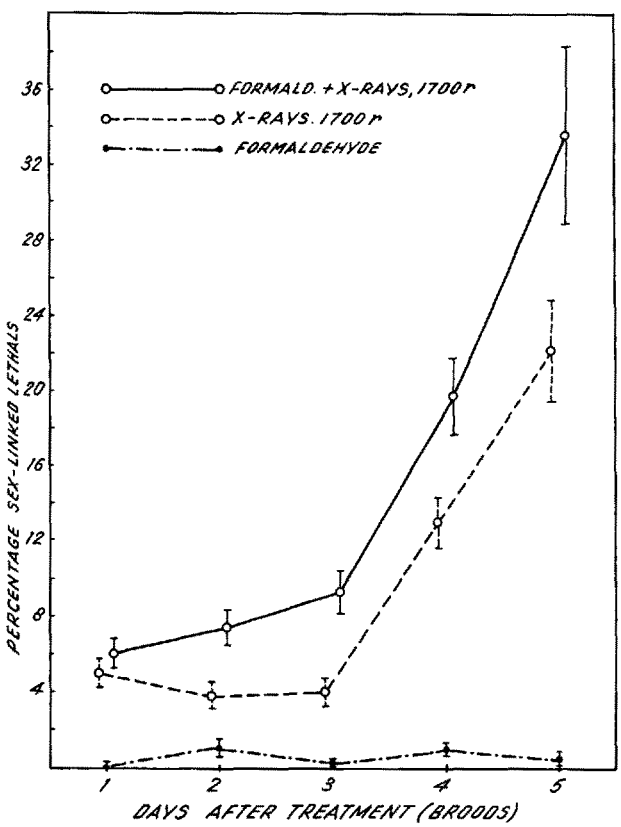

Fig. 2. – The frequency of sex-linked lethals in 5 successive one-day broods from males which had been injected with a solution of 0·033 M formaldehyde prior to X-radiation with 1700 r, as compared with that from males which were irradiated or injected only.

picture of the tremendous differences of sensitivity correlated with different stages of spermatogenesis.

It is also seen that in the X-ray controls the mutation rates in the second and third broods are slightly lower than in the first. This observation fits in with similar findings reported by a number of different workers[7]. NORDBACK recently showed that the observed decrease on the second day after the irradiation is not so much due to a further difference in sensitivity as to the effect of storing irradiated spermatozoa in the testes.

Discussion. – The data clearly show that pretreatment with low "submutagenic" concentrations of formaldehyde enhances the mutagenic action of X-rays. The possible interpretation of this finding may be considered next.

As mentioned before, it is known that formaldehyde inhibits catalase. In addition to this effect, two lines of evidence suggest that formaldehyde exerts at least part of its mutagenic action via the formation of peroxides. First, it could be shown that a given dose of formaldehyde produces a significantly greater number of mutations in males which had been pretreated with cyanide than in males which were not so pretreated. Second, it was found that dihydroxydimethyl peroxide, the organic peroxide formed by combining formaldehyde and hydrogen peroxide, acts as a fairly potent mutagen. Moreover, the pattern of sensitivity to the mutagenic action of this peroxide bears a marked resemblance to that found after injection of formaldehyde[8] (and unpublished data).

The hypothesis that an increased production of peroxides sensitizes the chromosomes to the effects of radiation thus seems further supported by the present findings on formaldehyde pretreatment. Like previous results, obtained after pretreatment with cyanide, azide and dihydroxymethyl peroxide, they suggest that the formation of peroxides may be involved in the mutagenic action of X-rays on the genetic material of *Drosophila*.

In contrast to the other chemicals, the effect of formaldehyde pretreatment is not so narrowly restricted to stages with peak sensitivity, but extends also to later stages of spermatogenesis. This finding might be con-

[7] W. BAKER and E. S. v. HALLE, Proc. nat. Acad. Sci. Washington *39*, 152 (1953). – J. D. TELFER and S. ABRAHAMSON, Drosophila Information Service *28*, 161 (1954). – J. MOSSIGE, Proc. 4th Int. Conf. Radiobiol., Cambridge, 1955 (in press). – K. G. LÜNING, Proc. 4th Int. Conf. Radiobiol., Cambridge, 1955 (in press). – K. NORDBACK, Drosophilia Information Service *29*, 150 (1955).

[8] F. H. SOBELS, Nature *177*, 979 (1956).

nected with the sensitivity of these stages to the mutagenic action of formaldehyde itself. After injection of formaldehyde in highly mutagenic concentrations, using daily brood changes, peak sensitivity was observed in sperm sampled on the second and third day after treatment (unpublished work). Another possibility is that formaldehyde speeds up sperm utilization by killing, or otherwise inactivating spermatozoa.

The author wants to express his thankfulness to Dr. CHARLOTTE AUERBACH for reading the manuscript and to Dr. SHEILA COUNCE for correcting the English. He feels much indebted to Prof. Dr. J. J. ZOON who made the X-ray apparatus of his department available for this study. The technical assistance of Miss V. VAN DEN HUL is gratefully acknowledged. The investigation was supported in part by the Radiobiological Group of the Health Research Council T.N.O.

F. H. SOBELS

Institute of Genetics of the State University, Utrecht, Netherlands, May 17, 1956.

Zusammenfassung

Vor einer Röntgenbestrahlung, deren Dosis von 1700 bis 2200 r variierte, wurde *Drosophila*-Männchen Formalinlösung in schwacher, „submutagener" Konzentration injiziert. Es zeigte sich, dass im Vergleich zu den Kontrollen, welche nur bestrahlt wurden, die Mutationsrate nach Formalin-Vorbehandlung signifikant gesteigert war. Dabei wurden spätere Entwicklungsstadien der Spermatogenese erfasst als nach einer Vorbehandlung mit Zyanid, Azid und einem organischen Peroxid; bei reifen Spermatozoen liess sich jedoch keine Zunahme der Mutationsrate feststellen. Die Befunde weisen darauf hin, dass ein erhöhter Gehalt an organischen Peroxiden in den vorbehandelten Zellen die Chromosomen empfindlicher macht für die mutagene Wirkung der Röntgenbestrahlung.

La culture de cellules tumorales sur des explants d'organes *in vitro*

Des cellules tumorales peuvent-elles envahir des organes ou fragments d'organes embryonnaires explantés *in vitro*, comme elles colonisent les tissus de l'organisme entier ? J'ai abordé ce problème au moyen de la technique de culture organotypique de WOLFF et HAFFEN[1]. Des fragments du sarcome de Souris S 180 ont été associés à des organes ou fragments d'organes embryonnaires de Poulet de 6 à 9 jours d'incubation. Les deux fragments se soudent intimement, comme le font des organes embryonnaires de Poulet et de Souris associés (WOLFF[2]).

Au contact d'explants de différents organes, les cellules tumorales prolifèrent activement. Elles peuvent entourer l'organe d'une sorte de cortex dont les cellules extérieures s'exfolient. Elles pénètrent en même temps à l'intérieur par des voies d'invasion qui empruntent généralement le stroma conjonctif. Elles se divisent activement dans les tissus de l'hôte et forment soit des nodules compacts, soit des traînées orientées qui propagent la tumeur à grande distance de son point d'entrée.

Les organes qui se sont révélés les plus aptes à la prolifération tumorale (Fig. 1) sont le mesonephros, les gonades, le metanephros, le derme, le poumon, le périoste, les mésentères et les tuniques de l'intestin, la capsule et les cloisons du foie. Le mesonephros est particulièrement favo-

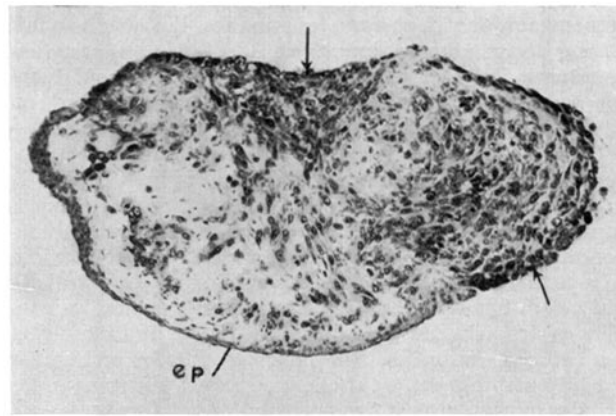

Fig. 1. Invasion de la paroi tégumentaire ventrale d'un embryon de Poulet, cultivée *in vitro*, par le sarcome S 180 de Souris. Les cellules du sarcome peuvent être facilement distinguées grâce à leur taille et à leur coloration sombre. Elles envahissent pratiquement tout l'explant dont il ne subsiste localement que l'épiderme (ep.) et des cellules conjonctives éparses. (Les régions les plus riches en cellules tumorales sont indiquées par des flèches.) Grossissement × 122.

rable à la prolifération des cellules tumorales qui s'infiltrent dans les espaces intertubulaires (Fig. 2) et entourent les canalicules urinaires qu'ils finissent par envahir, après dégénérescence de leurs cellules. Les cellules tumorales montrent un aspect de fibroblastes fuselés, lorsqu'elles sont en migration; un aspect ovoïde ou arrondi quand

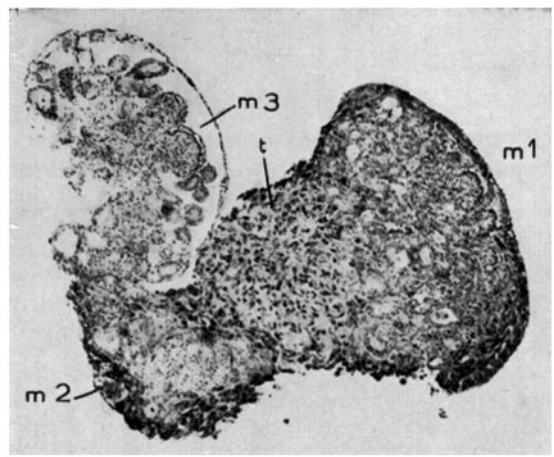

Fig. 2. Association d'un fragment de tumeur S180 de Souris avec plusieurs explants de mesonephros d'embryon de Poulet de 7 jours. L'explant tumoral se développe sur place au contact de deux des explants de mesonephros. L'invasion se fait par la périphérie des organes aussi bien que par leur face interne. Toutes les plages ou taches sombres sont des régions colonisées par des cellules tumorales. m1, m2: explants de mesonephros envahis par la tumeur. m3: explant encore intact. t: nodule tumoral développé aux dépens du fragment initial. Grossissement × 75.

elles sont groupées en amas. Les mitoses sont nombreuses, souvent aberrantes. L'organe peut être complètement envahi en l'espace de 2 à 4 jours. On peut ainsi propager

[1] ET. WOLFF et K. HAFFEN, J. exp. Zool. *119*, 381 (1952); Texas Rep. Biol. Med. *10*, 463 (1952).

[2] ET. WOLFF, Bull. Soc. zool. Fr. *79*, 357 (1954). – ET. WOLFF et J. P. WENIGER, J. Embr. exp. Morph. *2*, 161 (1954).