different from those for the control groups. It appears, therefore, that virus exposed mononucleate cells or the interferon induced by Sendai virus cannot be the explanation for the demonstrated immunogenicity of fused cells.

The possibility of direct viral oncolytic effect[12–14] on the tumour cells could be excluded in the immune reaction elicited by the fused cells, as the virus used had previously been UV-inactivated.

It is difficult to fully explain the mechanism of action of the fused cells. However, it appears clear that during tumour cell fusion by Sendai virus the coat components of the virus would become incorporated into the cell membrane and alter the structure of the cell periphery[11]. Although the modification of cell surface in the terms of molecular configuration has still not been understood, the viral lipoproteins might well be bound to the TSTA's in some way within the cell membrane. The modified fused cells are merely attenuated, in the sense that their growth rate is slowed down and they lose their transplantability, and/or render themselves more immunogenic in isogenic hosts. This principle was recently discussed by MITCHISON[1,15].

Résumé. Une immunité active au sarcome, produit par le cholanthrène de méthyle, a été obtenue en utilisant des cellules tumorales multinucléées, associées au virus de Sendai; les réactions ont été spécifiques à la tumeur. A la suite de ces injections, plus de 70% des souris furent résistantes aux trois inoculations, avec 10^5 cellules vivantes tumorales.

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Metabolism of Dimethylnitrosamine by Amphibians and Fish in vitro

Nitrosamines have a toxic and carcinogenic effect in a wide range of animal species[1,2], including primates[3,4] and fish[5–7]. There is much evidence to show that nitrosamines are not themselves active but that a chemical reaction occurring during their decomposition causes cellular injury and tumours[2,8].

Although dimethylnitrosamine (DMN) induces tumours of the liver in rainbow trout[5], KRÜGER et al.[9] found no evidence of methylation of nucleic acids or proteins of the liver in vivo. These findings suggest that trout liver lacks the capacity to metabolise DMN and indicate that in trout the carcinogenic effect is not related to the alkylation of cellular constituents.

It was decided to investigate further the capacity of various amphibians and fish to metabolize DMN. In a series of experiments, liver slices of rainbow trout, gold fish and 3 species of amphibians (*Triturus helveticus, Triturus cristatus* and *Ambystoma mexicanum*) were incubated with (^{14}C)-DMN and the production of labelled CO_2 was measured.

The Table shows the rates of production of labelled carbon dioxide. The highest activity was observed in

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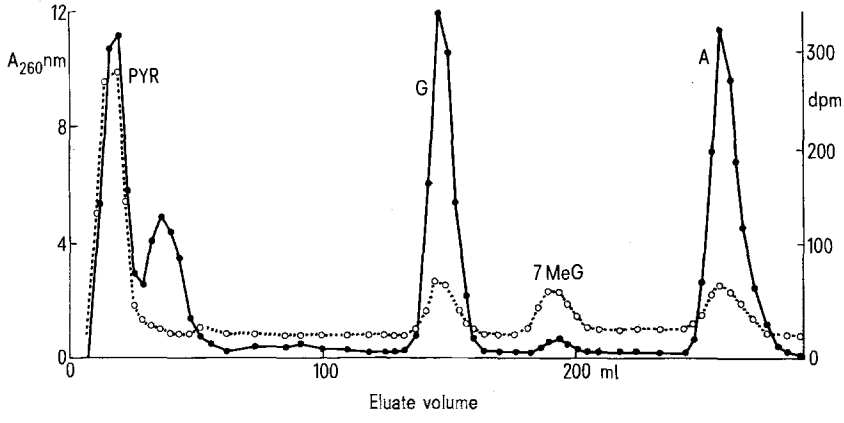

Fig. 1. Ion-exchange chromatography of nucleic acids of trout liver incubated with (^{14}C)-DMN in vitro, conditions of incubations as in the Table. Nucleic acids (DNA and RNA) were extracted from pooled slices by the SCHNEIDER procedure[17], followed by hydrolysis in 1*N* HCl at 100 °C for 1 h and ion-exchange chromatography on Dowex 50 (×12, H form) with exponential 1–4*M* HCl gradient solution, 3.6 ml fraction volume. ●, A_{260}; ○, radioactivity; PYR, pyrimidine nucleotides; G, guanine; 7-MeG, 7-methylguanine; A, adenine. Carrier 7-methyl-guanine was added to the hydrolysate.

Triturus helveticus, while in the other species a lower activity was observed. These results indicate that the livers of these species are able to metabolize the carcinogen DMN. The rate of production of labelled CO_2 observed in *Triturus helveticus* (0.3%) is slightly less than the rate observed in rat kidney slices (0.5%)[10], suggesting that a toxic and/or carcinogenic effect can be expected in the liver of this species following DMN treatment.

Methylation of the nucleic acids was observed in the liver slices of trout (Figure 1) and gold fish. The degree of methylation, which is expressed as the percentage of the guanine converted to 7-methylguanine[11], was much lower in the trout liver (0.0004%) than in the liver slices of rat or hamster[10] under comparable experimental conditions. A similar degree of methylation of nucleic acids was observed in the liver of gold fish.

Metabolism of (^{14}C)-dimethylnitrosamine by amphibian and fish liver in vitro

Species	No. of flasks	$^{14}CO_2$ production (% ^{14}C added)
Triturus helveticus	3	0.31
Triturus cristatus	3	0.07
Ambystoma mexicanum	3	0.09
Gold fish	8	0.04
Rainbow trout	12	0.16

Slices (160 mg) were cut with a tissue slicer, weighed and transferred to Warburg flasks containing 2 ml of ice-cold saline. (^{14}C)-DMN dissolve in saline (0.2 ml containing 61 µg, and 0.07 µCi) was added to each flask. Incubation was at 20–22 °C in an atmosphere of O_2, with shaking, for 90 min, O_2 uptake being measured at 10 min intervals. Radioactive CO_2 was trapped by NaOH in the centre well and its radioactivity determined after conversion to (^{14}C)-$BaCO_3$, by use of a scintillation counter, as previously described[16]. In the case of gold fish and trout DMN was used at a concentration of 62.5 µg and 2.1 µCi.

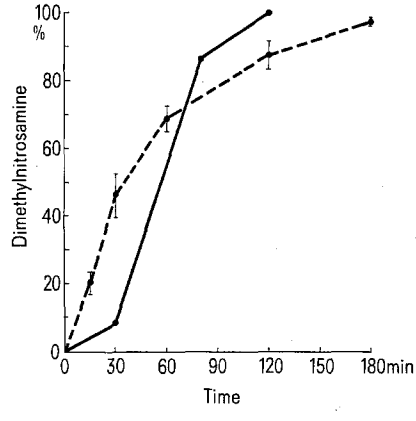

Fig. 2. Recovery of DMN in the water after injection to *Triturus helveticus* (—) and gold fish (----). For each time interval 3 or 4 determinations were performed in the gold fish experiment and 1 in the *Triturus helveticus* experiment.

In another series of experiments, the concentration of DMN was determined in water and in *Triturus helveticus* and gold fish, after i.p. injection of the carcinogen.

Each *Triturus helveticus* and gold fish received 0.05 ml of a 25% solution of DMN, corresponding to 12.5 mg, by i.p. injection. Immediately after the injection they were put in groups of 8 in beakers containing 800 ml of tap water. The newts were anaesthetized with M.S. 222 (triacene methanesulphonate) prior to injection, whereas the gold fish were not anaesthetized. At various intervals, samples of water (10 ml) were taken and after adding 1 ml of 5% (w/v) of sulphosalicylic acid, the concentration of DMN was determined by polarography. Polarographic estimation of DMN in the bodies of the newts and gold fish was performed in the supernatant of the acid homogenate after preliminary distillation[12].

Figure 2 shows the recovery of DMN in the water in these experiments. Newts had a concentration of 0.28 mg/newt 120 min after DMN treatment, when 98% of the carcinogen was found in the water. Only 8% of the DMN passed out of the newts in the first $^1/_2$ h after injection, whereas 98% had passed out after 2 h. This suggests that there was little loss of DMN through a hole left by injection. The recovery in the water of the DMN injected into the gold fish reached 20, 46, 68.8, 88.5 and 97.4% at 15, 30, 60, 120 and 180 min respectively. The percentage of recovery in the bodies of gold fish at 60, 120 and 180 min were 32.1, 8.4 and 3.7 respectively, making a total recovery of between 98 and 101%. The difference between the loss of DMN in the newt and the gold fish in the first $^1/_2$ h may have been due to the use of an anaesthetic in the case of the newt. The newts recovered from the anaesthetic between $^1/_2$ and 1 h after injection. In a control group, no DMN was detected in the newt or gold fish, nor in the water.

These findings demonstrate that DMN is eliminated by the newt and by fish relatively quickly. This rapid elimination is probably one of the reasons why no methylation of cellular constituents of trout liver was detected after i.p. injection of (^{14}C)-DMN by KRÜGER et al.[9]. Furthermore, recent studies indicate that microsomal enzymes which metabolize foreign compounds are present in fish and other marine vertebrates, although their activity is generally much lower than in mammalian species[13].

In the newt *Triturus helveticus*, it has been shown that a single i.p. injection of 16 g/kg has neither a toxic nor a carcinogenic effect. Following 6 or 7 injections at this dose level over a period of 3 weeks, however, both a toxic and a carcinogenic effect have been observed[14]. Tumours of the liver were observed in the aquarium fish *Lebistes reticulatus* exposed to DMN at a concentration of 100 ppm in water[7].

In trout, DMN induced liver tumours similar to the hepatic cell carcinomas observed in DMN-treated rats at dietary concentrations ranging from 300 to 19,200 ppm[5], which are very high when compared to the concentrations of 2 to 50 ppm used in rats[15]. Thus the rapid elimination from the body and the low capacity of trout liver to metabolize DMN are probably the two main factors which limit the detection in vivo of an alkylation of cellular constituents of the liver and require high doses of DMN to observe a carcinogenic effect.

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Résumé. On a examiné le métabolism de la (C^{14})-DMN par des coupes de foie de truite, de poisson rouge et de trois espèces d'amphibiens, ainsi que l'excretion du cancérogène après injection par voie intrapéritonéale chez le triton et le poisson rouge. La relation entre le métabolisme et l'activité toxique et/ou cancérogène est discutée.

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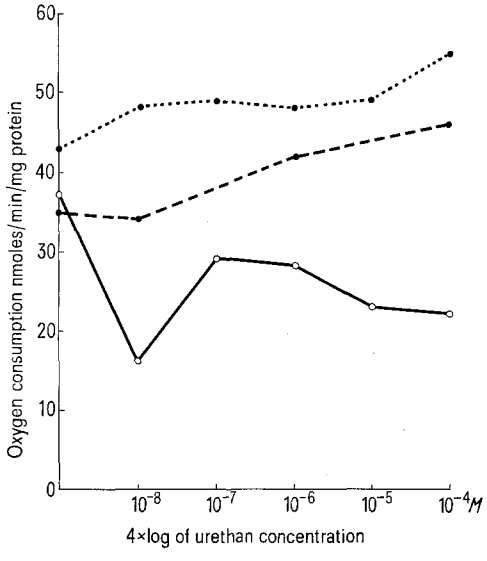
α-Tocopherol (Vitamin E): Reduction of Urethan-Induced Respiratory Depression in Mitochondria

Within the last few years, there have been several reports on the efficacy of vitamin E in retarding cancer growth. KOCUR et al.[1] reported that when vitamin E was injected i.p. to rats, the growth of transplanted tumors was inhibited and even showed complete regression in some cases. Harman found that the dimethylbenzanthracene-induced cancer was inhibited by dietary supplements of vitamin E[2].

It is also known that the chief biochemical characteristic of tumors is the increased dependence of the gycolytic pathway as opposed to the normal respiration pattern of normal cells. Recent work on the action of vitamin E has shown the usual antioxidant function of vitamin E is by no means the only function of the vitamin and recent emphasis has shifted towards the catalytic properties of tocopherols especially in regard to the role of the respiratory chain[3]. To our knowledge, the action of vitamin E on subcellular organelles in the presence of a carcinogen has not been investigated. Therefore we decided to observe the effect of vitamin E when the simple carcinogen, urethan, was added to a suspension of isolated mitochondria. As will be pointed out, the results led us to consider the in vivo action of the vitamin in the presence of urethan.



Oxygen consumption of isolated mitochondria as a function of varying urethan concentration with and without the addition of α-tocopherol. X····X, $1\times10^{-4}M$ α-tocopherol; X----X, $1\times10^{-2}M$ α-tocopherol; ·—·, zero α-tocopherol.

Methods and materials. 1. Isolated mitochondria. Male mice (CD-1, 20–30 g) were killed by cervical dislocation and the liver quickly removed. The mitochondria were isolated by the method of CARVALHO et al.[4] and finally suspended in ice-cold 0.44 *M* sucrose in 0.05 *M Tris*, pH 7.4. From this suspension, 0.5 ml aliquots were incubated with 100 µl of α-tocopherol (0.5 mg/ml of 95% ethanol) for 20 min. Then urethan in varying concentrations was added to the mitochondrial suspensions (containing usually 100 mg/ml protein) and allowed to incubate for 20 min at 20 °C. The oxygen consumption of the urethan-treated mitochondria from 6 mice was measured polargraphically in a chamber containing 4.5 ml respiration medium (sucrose 0.1 *M*, phosphate 0.003 *M*, KCl 0.02 *M*, *Tris* 0.005 pH 7.5) plus 60 µl of 0.5 *M* sodium succinate pH 7.0.

2. Urethan and vitamin E both injected intraperitoneally. Mice were given i.p. injections of vitamin E and urethan. The vitamin was injected every 24 h for 3 days at the dose of 1 international unit per g body wt. and was in the form of α-tocopherol. The urethan used was from a 10% solution and injected at the same time as the vitamin at the dose of 0.5 mg urethan per g body wt. The total urethan injection over 3 days was then 1.5 mg per g body wt. Each respiratory measurement was made on pooled mitochondria isolated from three mice approximately 24 h following the last administration of urethan and vitamin E.

A control group of mice was injected with urethan only, and killed at the end of 3 days. The mitochondria were isolated, and the oxygen consumption measured as above. The oxygen consumption of the 2 groups of mitochondria was compared on a per g mitochondrial protein basis.

3. Lipid Peroxidation (in vitro). In order to determine if lipid peroxidation was involved, we measured the amount of malondialdehyde production by the thiobarbituric acid test in the urethan-treated mitochondria incubated with and without tocopherol.

Results. Isolated mitochondria. The oxygen consumption of urethan-treated mitochondria with and without addition of tocopherol to the suspension is shown in the Figure. The data show that there was a consistent alleviation of the urethan-induced depression in oxygen consumption in the mitochondria due to the presence of the tocopherol.

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