Radioprotective Effect of Hydrocortison and Prednisolon on the *Vicia faba equina*

Do corticosteroids have a protective effect against X-rays? This problem, which we consider to be of great theoretical and practical value, has not been studied sufficiently.

All our attempts[1] to demonstrate the existence of a protective effect of corticosteroids in guinea-pigs, by employing various corticosteroids, administrating them in different ways and using several tests (depilation test, acanthosis test)[2,3], were in vein. In contrast to this, with the plant *Vicia faba equina*[2-4], employing JÜNGLING's test[5] which consists of observing the appearance and development of the secondary roots after application of X-rays, we were able to demonstrate that the hemisuccinate of hydrocortison had a very distinct protective effect against X-rays. We have also shown that this protective effect was not due to the succinate part, since the succinate of sodium has a very minimal protective effect.

To test whether other corticosteroids also had a protective effect on *V. faba equina*, we slightly modified our experimental procedure in order to study the effect of prednisolon (as hemisuccinate) and compare it with that of hydrocortison. After germination in water, the shoots presenting a primary root of about 2 cm are placed in contact with 0.001 molar solutions of these corticosteroids for 3 days (solution renewed twice within 24 h) and subsequently exposed to an X-ray dose of 250 R (app. Therapix C 100 – 55 kV, 5 mA, filter 0.78 mm Al, FSO 30 cm, loc. ø 12 cm, irradiation time = 4 min 12 sec), and then replaced in water. The appearance and development of secondary roots is then observed for 30 days. The results are compared with controls, untreated and non-irradiated plants on the one hand and with plants having received 250 R without further treatment on the other.

Figure 1 (1) confirms the protective effect of hydrocortison in these new experimental conditions. (2) It shows that in an equimolecular concentration (0.001 *M*), prednisolon, which differs from hydrocortison only by the loss of 2 hydrogen atoms replaced by a double bond, has a protective effect which is superior to that of hydrocortison.

Figure 2 shows that in a 4-times higher concentration (0.004 *M*), hydrocortison has approximately the same protective effect as prednisolon in a concentration 4-times lower (0.001 *M*).

It seems that this effect corresponds to the therapeutic effect of these corticosteroids, prednisolon being about 4–5 times more active than hydrocortison (tablets of hydrocortison dosed at 20 mg and those of prednisolon at 5 mg). We consider it important to underline this parallelism[6].

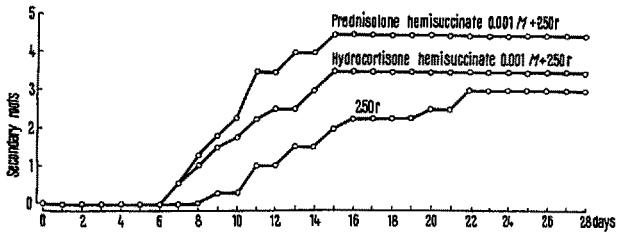

Fig. 1

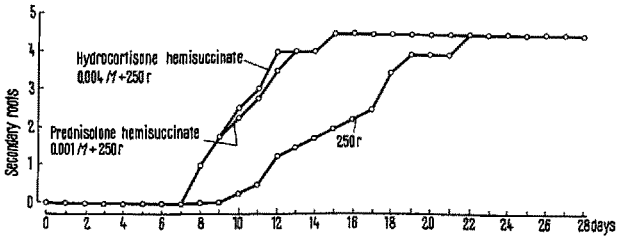

Fig. 2

Résumé. Avec le *Vicia faba* test, nous avons pu démontrer l'effet radioprotecteur de l'hydrocortisone et de la prednisolone. L'effet radioprotecteur de la prednisolone est grosso modo, 4 fois plus important que celui de l'hydrocortisone.

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University Clinic of Dermatology, Genève (Switzerland), 9 August 1967.

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Mitochondrial Swelling Induced by Diphtheria Toxin in vivo: A Comparison with the Action of some other Bacterial Toxins

In some recent papers it has been shown that diphtheria toxin induces a mitochondrial swelling in chicken embryo heart cell cultures[1] and in other primary cell cultures, while the mitochondria of some established cell lines, as HeLa cells and RC 37 cells, are unaffected[2]. This swelling is prevented by antitoxin[2] and by some blocking agents of the respiratory chain as KCN, NaN_3 and Amytal. A complete reversal effect, within certain limits, is promoted by ATP and seroalbumin[3].

These findings support the assumption that the mitochondrial swelling induced by diptheria toxin in vivo is due to toxin per se and is an active, electron transport dependent swelling[3].

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Toxin	Concentration	Mitochondrial swelling 5′	15′	30′	Cytopathic effect 5′	15′	30′	Observations
Diphtheria	1 Lf/ml	+	++	++	none	none	none	+ Thickened mitochondria
	5 Lf/ml	++	+++	+++	none	none	none	++ = Swollen mitochondria +++ = Swollen mitochondria with differences in optical density
Tetanus	1 Lf/ml	—	—	—	none	none	none	None
	5 Lf/ml	—	—	—	none	none	none	
O-streptolysin	0.01 H.U./ml	—	—	—	none	none	none	+/++ = Cytoplasmic coartation
	0.1 H.U./ml	—	—	—	none	+	++	
α-staphylotoxin	0.5 MLD/ml	—	—	—	+	+++	++++	+/++++ = Cytoplasmic vacuolation
	2.5 MLD/ml	—	—	—	++	++++	++++	

Diphtheria toxin causes a mitochondrial swelling in the liver cells of intoxicated guinea-pig, and some other bacterial toxins such as tetanus toxin and Pyocianeus toxin, induce the mitochondria of rat liver to swell[4]. In order to gather further information on the action of diphtheria toxin on the mitochondria of living cells, it seemed of interest to test whether some bacterial toxins, such as tetanus toxin, O-streptolysin and α-staphylotoxin, would provoke a mitochondrial swelling.

Materials and methods. Cell cultures. Chicken embryo heart cell cultures were obtained from 6-day-old chick embryos as previously described[1,2].

The cells were grown in Hanks' BSS containing 5% of fresh inactivated calf serum and 0.5% of lactalbumin hydrolysate (Nutritional Biochemical Corporation) in ROSE's chambers.

Bacterial toxins. Highly purified diphtheria toxin (Lilly, Lot. 00087, containing 1120 Lf/ml), highly purified tetanus toxin (Lilly, Lot. 0780, containing 960 Lf/ml), α-staphylotoxin (I.S.M., obtained from Wood 46 staphylococcus strain and containing 30 MLD/ml for rabbit) and O-streptolysin (Difco, Lot. 488100) containing 2 H.U./ml were used. All these toxins were diluted in Hanks' BSS to obtain the final concentrations (Table). The final pH of toxins' dilutions, adjusted with NaOH or HCl if necessary, was 7.3. Diphtheria toxin and tetanus toxin were exhaustively dyalized against Hanks' BSS to eliminate the preservative (merthiolate).

Experiments. After 18–22 h of incubation the nutrient medium was eliminated from the ROSE's chambers and substituted with 1 ml of toxin dilution. The ROSE's chambers were then placed under a Leitz Ortholux phase-contrast microscope equipped with a thermoregulated box (37 °C) and the cells were continuously observed for a period of 30 min. The control cultures were treated in the same manner but the nutrient medium was substituted with 1 ml of plain Hanks' BSS.

Results. The effect of toxins on the mitochondria of chicken embryo heart cells cultured in vitro and the cytopathic effect are summarized in the Table.

Diphtheria toxin, as previously noticed, causes a remarkable mitochondrial swelling but does not induce any cytopathic effect in these cells, at this concentration, within 30 min from toxin addition (Figure 2). The cells treated with tetanus toxin do not exhibit mitochondrial swelling or any cytopathic effect (Figure 1). O-streptolysin

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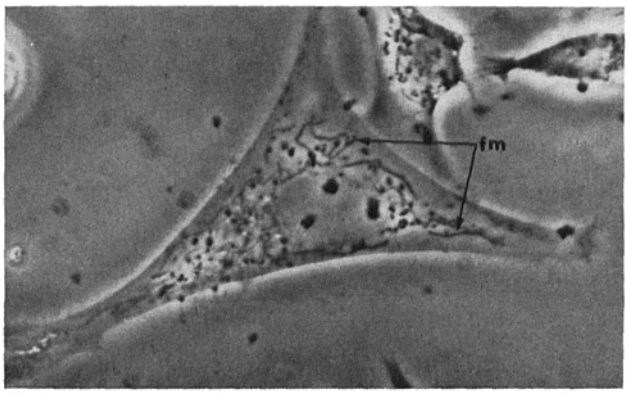

Fig. 1. Chicken embryo heart cell treated with tetanus toxin (5 Lf/ml for 30 min). Several filamentous mitochondria (fm) are visible. Phase-contrast microscope. About × 1000.

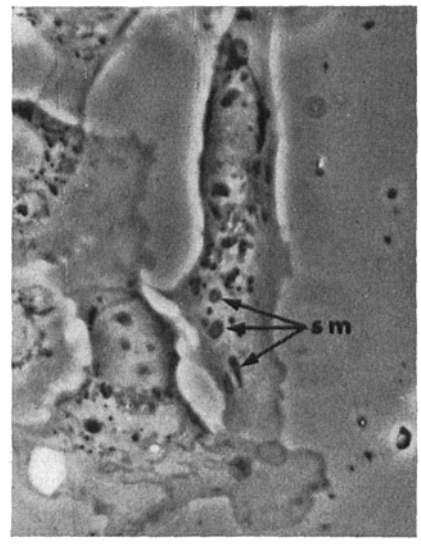

Fig. 2. Diphtheria toxin treated cell (5 Lf/ml for 15 min). sm, swollen mitochondria with differences in their optical density. Phase-contrast microscope. About × 1000.

does not cause swelling but a cytopathic effect, as slight cytoplasmic coartation, can be observed.

α-staphylotoxin-treated cells show a precocious and progressive cytoplasmic vacuolation (Figure 3). In these cells the mitochondria are not induced to swell but a mitochondrial fragmentation is often observable. In the control cultures any cytopathic effect or mitochondrial change occur.

Discussion. The effect of diphtheria toxin is comparable to that previously obtained in this laboratory[1-3].

O-streptolysin and α-staphylotoxin do not induce mitochondrial changes at these concentrations, but cause a cytopathic effect. This finding eliminates the doubt that the negative results concerning the mitochondria are due to a lack of penetration of these toxins into the cell. The cytopathic effect induced by these toxins is comparable to that described elsewhere[5-7]. Tetanus toxin does not induce any morphological change in the mitochondria but the lack of a cytopathic effect[7] does not allow the conclusion that the toxin penetrates into the cells. On the other hand, a mitochondrial swelling in the liver cells of tetanus toxin treated rats is described[4], while in mice treated with tritiated tetanus toxin the uptake of labelled toxin by the neurones is only occasionally reported[8].

In our experiments, nevertheless, the cells were entirely surrounded by the solution containing tetanus toxin, and it is probable that it reached the mitochondria in the cytoplasm and that tetanus toxin was then deprived of swelling properties in vivo[9].

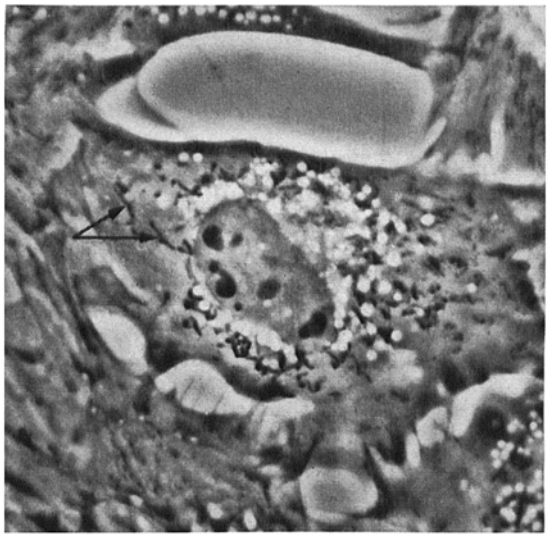

Fig. 3. α-Staphylotoxin treated cell (2,5 MLD/ml for 5 min). Note the cytoplasmic vacuolation and rod-like mitochondria (arrows). Phase-contrast microscope. About × 1000.

Riassunto. In precedenti lavori è stato studiato il rigonfiamento causato dalla tossina difterica nei mitocondri di cellule coltivate in vitro. Si è fatto un confronto fra l'azione della tossina difterica e quella di altre tossine batteriche come la O-streptolisina, la α-stafilotossina e la tossina tetanica sui mitocondri di cellule di cuore di embrione di pollo coltivate in vitro. Solo la tossina difterica ha manifestato capacità rigonfianti mentre le altre tossine, alle dosi usate e con questo tipo di cellule, sono apparse sprovviste di questa proprietà.

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Clinic of Infectious Diseases of the University, Napoli (Italy), 10 July 1967.

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[9] Acknowledgment: I wish to express my gratitude to Dr. Puccini of the Eli Lilly Co. and to Prof. De Barbieri of the Istituto Sieroterapico Milanese for the generous supply of bacterial toxins.
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Structure Activity Relationships of Compounds able to Suppress the Antipolio Action of Guanidine

Previous work from our laboratories and from others has demonstrated that the influence of guanidine on poliovirus replication is antagonized by a number of compounds; 2 methyl-donors, methionine and choline, being the most active ones (Lwoff and Lwoff[1]; Loddo and Schivo[2]).

As a working hypothesis, Lwoff has suggested that guanidine would interfere with an essential methylation of a viral structure[3]. However, since it was found that the guanidine effect is antagonized by ethionine, by 2 demethylated methionine analogs, homocysteine and α-aminobutyric acid, and by the methyl-free choline analog ethanolamine[4], the methylating hypothesis was no longer tenable.

A structure-activity relationship of compounds able to suppress guanidine effect is attempted in this paper, in order to shed some light on the mechanism by which guanidine inhibits virus replication.

The techniques used were previously described in detail[5]. In Table I are listed, in decreasing order of potency, the amino acids found active against guanidine. In Table II the inactive ones are listed. As it appears from

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