

3. Lorsque le traitement à la Con A débute après l'attachement et l'étalement cellulaire, peu de détachements se produisent, toutefois des rétractions cytoplasmiques plus ou moins prononcées sont observées.

4. Nous avons noté parmi les cellules étalées des cultures traitées un plus grand nombre de polycaryons que dans les cultures témoins (fig. 8, 9). Un traitement préalable à la trypsine^{2,11} n'a pas particulièrement favorisé l'accentuation de ce phénomène.

Analyse statistique. Nous avons noté parmi les cellules étalées des cultures traitées, un plus grand nombre de polycaryons que dans les cultures témoins. Compte tenu du faible pourcentage de ces cellules dans les deux cas, un recours à une étude statistique a été nécessaire, afin de déterminer si le traitement à la Con A avait un effet éventuel sur la formation de polycaryons dans le système étudié.

L'analyse de variance concernant les cellules témoins et traitées montre une grande homogénéité à l'intérieur des séries de traitement et des différences hautement significatives entre ces séries (tableaux 1 et 2).

Discussion, conclusion. Nous avons, par ce travail, abordé les effets morphologiques de la Con A au niveau de la cellule isolée. Nous avons ainsi constaté que:

– La Con A a une action sur la différenciation cellulaire puisqu'elle perturbe la différenciation des neurones et, à un degré moindre, celle des myoblastes et des autres catégories de cellules cultivées. Nous retrouvons donc au niveau cellulaire, les effets inhibiteurs décrits par d'autres auteurs^{5,6,13} sur l'embryon *in toto*. Notons que cette action, sans doute indirecte, montre le lien étroit qui existe entre les différents «compartiments» cellulaires, membrane cellulaire, cytoplasme et noyau, puisque une action au niveau de la membrane se répercute aussi bien sur le cytoplasme que sur le noyau.

– La Con A entraîne des modifications des organites nucléaires, altérations nucléolaires (vacuolisation, fusion) et chromatinienues (condensation) et un ralentissement du métabolisme cellulaire.

– L'action de la Con A sur la membrane cellulaire¹² se traduit ici, par une perturbation de l'adhésivité des cellules au support de culture.

– La Con A provoque dans les cellules d'Amphibiens la formation de polycaryons. Une analyse statistique conclut à un effet positif de cette protéine sur leur formation. Dans l'état actuel de nos recherches, il ne nous est pas possible de dire si ces polycaryons sont des hybrides cellulaires ou le résultat d'aberrations mitotiques dues à la présence de la lectine dans le milieu de culture. Il faut cependant noter que leur taux dans les cellules traitées (environ 5%) est du même ordre que celui des hétéro-caryons obtenus chez la *Drosophile*⁴. La présence de polycaryons dans les cultures témoins (2%) pourrait être attribuée entre autres facteurs, à l'emploi de l'E.D.T.A. comme agent de dissociation des explants (TENCER, communication personnelle).

Ainsi donc, les effets de la Con A sur la morphogenèse de l'embryon d'amphibiens, ne s'exercent pas seulement sur un organisme déjà complexe¹³ mais se retrouvent au niveau cellulaire. Les résultats de nos expériences soulignent une fois de plus l'interdépendance étroite qui existe à ce niveau entre membrane cellulaire, cytoplasme et noyau.

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Arabinose Nucleoside Triphosphates are no Inhibitors for DNA-Dependent RNA Polymerases

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Summary. 1- β -D-arabinofuranosylcytosine-5'-triphosphate and 9- β -D-arabinofuranosyladenosine-5'-triphosphate were found to have no inhibitory potency for both mammalian DNA-dependent RNA polymerase II and *E. coli* DNA-dependent RNA polymerase.

It is well established that 1- β -D-arabinofuranosylcytosine-5'-triphosphate (ara-CTP) and 9- β -D-arabinofuranosyladenosine-5'-triphosphate (ara-ATP) inhibit DNA-dependent DNA polymerases from eukaryotic cells¹⁻⁴. Ara-CTP has been shown not to inhibit mammalian RNA polymerases¹; ara-ATP does not inhibit DNA-dependent RNA polymerases I and II from quail oviduct⁴ and this compound exerts also no effect on bacterial RNA polymerase³. However, in a recent study⁵ it was published that the 2 arabinose-compounds inhibit both DNA-dependent RNA polymerase II, isolated from chicken leukaemic cells and DNA-dependent RNA polymerase from *E. coli*. It is the aim of this note to clarify this obvious discrepancy.

The following enzymes were used: 1. DNA-dependent RNA polymerase II from quail oviduct with a specific activity of 7.6 nmoles [³H] ATP per mg protein and 20 min⁶; 2., DNA-dependent RNA polymerase from Rous Sarcoma Virus induced sarcoma of the breast muscle of

quails⁷ isolated by the same procedure⁶, the specific activity was 10.4 nmoles [³H] ATP per mg protein and 20 min; and 3., DNA-dependent RNA polymerase from *E. coli*⁸ purchased from Miles, Kankakee (USA). Ara-CTP and ara-ATP came from Terra-Marine Bioresearch, La Jolla (USA); chromatographically pure ara-CTP was a gift from Dr D. Gauchel (Universitätsklinik, Düsseldorf, BRD). The Terra-Marine material, which has been used also by CHUANG et al.⁵, was not chromatographically pure: The ara-CTP sample contained 60% ara-CTP, 25% dCTP or CTP and 15% mono-, di- and tetraphosphates as analyzed by anionic exchange column chromatography⁹; the ara-ATP sample contained 54% ara-ATP and 36% ATP as analyzed by TLC with solvent 2⁴. The Terra-Marine material was purified; ara-CTP according to CHOU et al.⁹ and ara-ATP according to MÜLLER et al.⁴.

The enzyme assays (volume 100 μ l) were composed as follows: 20 mM *tris*-HCl (pH 7.8), 40 mM KCl, 100 mM (NH₄)₂SO₄, 6 mM MgCl₂, 5 mM dithiothreitol, 0.1 mM

Influence of impure and purified ara-ATP and ara-CTP on different RNA polymerases. For kinetic studies to determine the Michaelis constants, concentrations of the labelled triphosphate in the range between 1.6 and 15 μM were added to the assays. The inhibitor constants were calculated¹¹ using 2 different normal substrate concentrations.

Enzyme	Source of the enzyme	Michaelis constant (μM)		Inhibitor constant (μM)			
		ATP	CTP	Impure ara-ATP	Purified ara-ATP	Impure ara-CTP	Purified ara-CTP
DNA-dependent RNA polymerase II	Quail oviduct	3.4 $\pm$ 0.7	2.6 $\pm$ 0.7	64.6 $\pm$ 10.3	no inhibition	21.3 $\pm$ 7.1	no inhibition
	RSV-induced sarcoma	5.1 $\pm$ 0.9	4.9 $\pm$ 0.8	74.0 $\pm$ 12.1	no inhibition	35.8 $\pm$ 7.5	no inhibition
DNA-dependent RNA polymerase	<i>E. coli</i>	8.7 $\pm$ 1.3	2.1 $\pm$ 0.6	93.7 $\pm$ 14.8	no inhibition	27.3 $\pm$ 7.6	no inhibition

each of the unlabelled ribonucleoside triphosphates and varying amounts of [³H] CTP (150 cpm/pmol) in the case of the ara-CTP studies and [³H] ATP (250 cpm/pmol) (The Radiochemical Centre, Amersham) in the case of the ara-ATP studies, 100 μg native herring sperm DNA/ml and 20 μl enzyme preparation. The mixture was incubated for 20 min at 37°C; the acid-insoluble radioactivity was collected on GF/C filters as described¹⁰.

The sensitivity of the different RNA polymerases toward impure and purified ara-ATP and ara-CTP was determined, and the results are summarized in the table. It was found that, if impure ara-ATP or ara-CTP is used, the different enzymes are inhibited; the inhibition is of the competitive type. However, if purified ara-ATP or ara-CTP is used in the enzyme assays, no inhibition is observed. The reason for this would appear to be in the contaminations present in the purchased material. This conclusion can be seen in an example of the ‘ara-ATP inhibition’ of oviduct RNA polymerase II as follows:

Using the equation $V = V_{\text{max}} \cdot \left[1 + \frac{K_m}{S} \cdot \left(1 + \frac{i}{K_i} \right) \right]^{-1}$ and taking $V_{\text{max}} = 100\%$, $K_m = 3.4 \mu\text{M}$, $S = 5 \mu\text{M}$, $K_i = 64.6 \mu\text{M}$ and $i = 30 \mu\text{M}$ ATP (as impurity in 83 μM ‘ara-ATP’) and calculating V, a value of 50% is obtained;

this means that the ATP contamination in the ara-ATP preparation provokes a 50% inhibition.
Thus, at present, we see no experimental evidence for an inhibition of RNA polymerases by ara-CTP or ara-ATP.

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Intracisternal A Type Particles of the Extraocular Muscle of Hereditary Muscular Dystrophy Mouse

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Summary. Intracisternal A type virus (IA) particles were observed in the extraocular muscle fiber of hereditary muscular dystrophy mouse. The particles appeared approximately 65–75 m μ in diameter, with electron lucent cores.

Recent studies on the cytoplasm of normal and neoplastic tissue in various strains of mice, such as C3H, BALB/c, C57BL, PBR and their hybrids, have reported the ultrastructural observation of IA particles and also include an unique description of the presence of the IA particle in skeletal muscle tissue of the normal BALB/c mouse¹. In skeletal or extraocular muscle tissue of the hereditary muscular dystrophy (HDM) mouse, however, electron microscopic investigations have not confirmed the presence of this particle^{2,3}. This paper reports the initial finding of the IA particle in the extraocular muscle of the HMD mouse.
HMD mice of the C57BL/j-dy (dy-dy homotype) strains were originally obtained from the Jackson Institute, Maine, USA. Dr. H. Matushita of the Department of

Physiology, Wakayama Medical College, kindly provided two mice of this strain for the experiment. This strain of mouse develops a disorder of the fine structure of muscle similar to progressive muscular dystrophy seen in humans^{2,4}. The two of HDM mice were 12 weeks of age, male and weighed 12.5 and 13.0 g, respectively. The tibialis anterior, intercostalis, diaphragm and extraocular
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