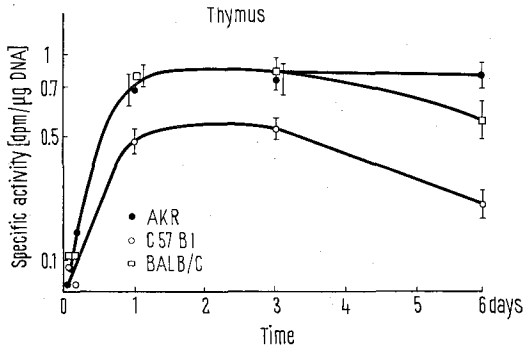

Fig. 5. Specific activity (dpm/µg DNA) as function of post-irradiation time (days) in thymus of different strains of mice (AKR ●; C 57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells (≈ 25–30×10^8 dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of $\approx$ 7%.)

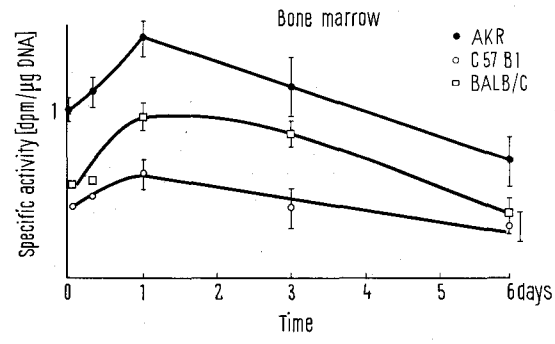

Fig. 6. Specific activity (dpm/µg DNA) as function of post-irradiation time (days) in bone-marrow of different strains of mice (AKR ●; C 57 Bl ○; BALB/c □) injected with 1×10^7 DNA labelled bone-marrow cells ($\approx 45 \times 10^8$ dpm) immediately after whole-body irradiation with 650 R. Values given represent means and standard deviation of the mean. For the 1 and 4 h period, standard deviation is not shown in order not to render the design confusing. (It is in the order of $\approx$ 7%.)

The data presented indicate, therefore, that bone-marrow cells are capable of entering spleen, bone-marrow and thymus, the organs being enumerated in order of importance of uptake. During the experimental period of 6 days a certain percentage of the cells is lost (70–90%) in spleen and bone-marrow and 50% in thymus but it is evident that cells lost by the bone-marrow and 50% in thymus but it is evident that cells lost by the bone-marrow cannot account for the labelled cells in thymus. Regeneration during the period of observation commences in spleen and is most marked for C 57 Bl and BALB/c mice[8].

Zusammenfassung. Knochenmarkszellen, durch Injektion von ^{3}H-Thymidin in syngenischen Spendern markiert, wurden in bestrahlte Empfänger von AKR-, C 57 Bl- oder BALB/c-Mäuse injiziert, wobei grösste Aktivität in der Milz, dann im Knochenmark und im Thymus gefunden wurde. Aktivitätsverlust war während der 6tägigen Beobachtungsperiode in Milz und Knochenmark am stärksten. Auch die Regeneration und die Veränderungen der spezifischen Aktivität der DNA dieser Organe waren bei C 57 Bl- und BALB/c- Mäusen am ausgeprägtesten. Die markierten Spenderzellen im Thymus scheinen nicht von Zellen zu stammen, die über das Knochenmark dorthin transportiert wurden, sondern wurden unmittelbar vom Thymus aufgenommen.

G.B. Gerber, A. Decleve, J.R. Maisin, A. Léonard and Gh. Mattelin

Euratom and Radiobiology Department, C.E.N./S.C.K., B-2400 MOL (Belgium), 26 July 1971.

[8] This is publication No. 704 of the Biology Division of the Contract Euratom-C.E.N./S.C.K. No. 078-69-1 BIAC. This work was partly supported by the 'Fonds de la Recherche Scientifique Fondamentale Collective'.

The Effect of Protein Biosynthesis Inhibitors upon the Development of Cellular Membranes Hyperpolarization

The purpose of the present work was to study the effect of injuries at various steps of protein biosynthesis process upon the development of a cellular membrane hyperpolarization.

Experiments were conducted on various kinds of cells, such as single muscle fibres and neurons of the sensomotor cortex region. The inhibition of protein biosynthesis was reached by administration of actinomycin D (inhibiting the synthesis of messenger RNA on structural genes); of puromycin (blocking the protein synthesis in ribosomes); of ribonuclease (destroying the molecules of RNA)[1].

Methods. Experiments were performed on 280 albino rats aged 8–10 months. The membrane potential of muscle fibres of the m. gastrocnemius and of neurons of the sensomotor cortical region was measured by microelectrodes in situ, according to the universally adopted methods. The hyperpolarization of m. gastrocnemius fibers was effected by i.p. administered insulin (0.16 U/100 g of body wt.), deoxycorticosterone acetate – DOCA (500 µg/100 g), estradioldipropionate – EDDP (100 µg/100 g) and by the denervation of this muscle (cross-section of the n. ischiadicus). Actinomycin D (ACM-D) was administered i.p. at a dose of 1–10 µg/100 g; puromycin at a dose of 50–100 µg/100 g; RNA-ase – 300 µg/100 g. In the experiments with reproduction of hyperpolarization of the sensomotor cortex cells, the protein biosynthesis inhibitors were applied to the investigated area with ACM-D (0.5×10^{-5}), RNA-ase (1×10^{-4}). For inhibition of various links of energy exchange we used sodium fluoride (0.01 *M*/100 g), monoiodacetate (0.005 *M*/100 g), 2, 4-dinitrophenol (0.002 *M*/100 g).

Results and discussion. Administration of insulin, estradioldipropionate, DOCA as well as denervation cause a distinct hyperpolarization of the muscular fibres (Table I). Administered doses of ACM-D, RNA-ase and

[1] These experiments were conducted in cooperation with L. A. Gromov, V. G. Korotonozhkin and O.A. Martynenko.

Table I. Effect of denervation, insulin, DOCA and EDDP administration on muscular fibres MP level in albino rats

Time	Denervation	Insulin	DOCA	EDDP
0	80.3±0.1	81.2±0.08	80.2 ±0.3	80.2±0.1
15 min	83.4±0.4	—	83.7 ±0.7	82.7±0.8
30 min	87.3±0.3	—	86.6 ±1.03	86.2±0.6
1 h	86.7±0.4	88.1±1.4	86.0 ±0.81	85.5±0.5
2 h	85.5±0.4	84.0±.88	84.8 ±0.9	83.3±0.8
3 h	83.7±0.3	82.6±0.8	85.04±1.07	83.2±1.0
4 h	82.3±0.3	—	85.1 ±1.2	81.7±0.6

Table II. Effect of protein biosynthesis inhibitors on development of hyperpolarization caused by denervation and by insulin, DOCA and EDDP administration

Protein biosynthesis	MP initial level	1 h after inhibitor administration	30 min after denervation	1 h after administration of		
				Insulin	DOCA	EDDP
RNA-ase	79,6±1,3	80,3±1.0	81.0±0.7	80.4±0.7	81.3±0.7	79.4±0.8
ACM-D	81.1±0.9	80.2±1.0	79.0±2.5	79.0±2.5	80.5±0.3	80.6±0.8
Puromycin	81.2±2.2	81.4±1.6	79.1±1.6	79.1±1.6	—	—

Table III. Development of muscular fibres hyperpolarization against the background of administration of energy metabolism inhibitors

Inhibitors of carbohydrate metabolism	Initial level of MP	MP level 1 h after inhibitors administration	MP level 30 min after denervation	MP level 1 h after insulin administration
NaF	82.6±0.6	76.2±0.9	81.8±0.8	79.5±0.75
MIA	81.6+0.6	74.6±1.0	80.1±0.2	80.3±0.6
2.4–DNPh	81.5±0.5	73.9±1.1	80.8±1.2	81.8±0.8

puromycin did not cause any change of the membrane potential within 5 h. If the animal obtained one of the protein biosynthesis inhibitors, followed 1 h later by administration of insulin, DOCA, EDDP or by muscular denervation, no hyperpolarization was observed (Table II). A similar effect – the prevention of hyperpolarization development – was noted by simultaneous use of protein biosynthesis inhibitors with insulin, DOCA, EDDP or with transsection of the nerve.

The initial value of membrane potential in the sensomotor cortex neurons was 70.6 ± 0.9 mV. After serotonin application, it increased to 80.2 ± 1.77 mV. The application of noradrenalin changed that value to 77.7 ± 0.69 mV. The action of the same substances subsequent to a preliminary application of ACM-D and RNA-ase did not increase the membrane potential, but caused a decrease of its value. Thus, after the application of ACM-D, serotonin caused a drop of membrane potential to 64.0 ± 2.84 mV, of noradrenalin to 64.1 ± 2.35 mV.

It seemed important to evaluate the extent of the specific effect of protein biosynthesis inhibitors on the hyperpolarization development. For that purpose, in the subsequent series we studied the influence of energy exchange inhibitors upon the development of the hyperpolarization of muscle fibres.

As may be seen from Table III, sodium fluoride, monoiodacetate and dinitrophenol provoked a downfall of the membrane potential. However, a transsection of the n. ischiadicus and administration of insulin following the administration of energy exchange inhibitors, caused a 6–7 mV elevation of the membrane potential.

Thus, protein biosynthesis inhibitors prevent hyperpolarization development in different cells, such as muscle fibres and neurons of the sensomotor cortex. It is noteworthy that the inhibition of protein biosynthesis process prevents hyperpolarization, caused by factors which affect the cellular metabolism in a different manner such as denervation, insulin, DOCA, EDDP, serotonin or noradrenalin.

Against the background of the effect of ribonuclease and actinomycin D, application of serotonin and noradrenalin to the sensomotror cortex area causes a neuron depolarization instead of hyperpolarization. That means that the alteration in the course of protein biosynthesis processes may substantially change the character of the electric phenomena and involve another type of cell reactions.

The development of hyperpolarization may be prevented by inhibition of various protein biosynthesis steps: by blocking the synthesis of m-RNA on structural genes;

by destroying RNA; by alteration of the protein biosynthesis in ribosomes. These data allow us to presume the changes of the cellular membrane polarization level to be associated with shifts occurring in the course of the protein biosynthesis process[2,3].

[2] V. V. Frolkis, in Adaptive Potentialities of an Ageing Organism (Kiev 1968), vol. 11, p. 40.
[3] V. V. Frolkis, Fiziol. Zh., USSR *16E*, 221 (1970).

ВЫВОДЫ. Ингибиторы биосинтеза белка (актиномицин Д, пуромицин, рибонуклеаза) предупреждают развитие гиперполяризации одиночных мышечных волокон и нейронов коры головного мозга.

V.V. Frolkis

Laboratory of Physiology,
Institute of Gerontology
Academy of Medical Sciences, USSR
Vyshgorodskaya str. No. 67, Kiev-114 (USSR),
17 August 1971.

Sur l'absence de biosynthèse des stérols et du squalène chez un coelentéré (anthozoaire), l'anémone de mer *Calliactis parasitica*

Il est connu que les insectes n'effectuent pas de biosynthèse «ex novo» des stérols à partir de l'acétate[1]; le cholestérol qui leur est indispensable provient soit de leur nourriture, soit de la dégradation de la chaîne latérale des phytostérols[1,2]. Cette situation ne peut cependant être généralisée à tous les invertébrés. Plusieurs mollusques (*Helix pomatia, Arion rufus, Planorbius corneus, Limnea stagnalis, Patella coerulea*, etc.) seraient capables de synthétiser le cholestérol[3,4]. Récemment[5], il a été montré que les gonades et l'hépatopancréas de *Aplysia depilans* synthétisent le cholestérol à partir de l'acétate et peuvent le transformer en corticostéroïdes. Les étoiles de mer *Asterias rubens* et *Solaster papposus* incorporent le mévalonate dans le squalène, le lanostérol et le 5α-cholestène-7 ol-3β[6]. Dans d'autres cas[3,4], une biosynthèse du squalène a été observée alors que l'animal ne parait pas capable de le transformer en cholestérol. Notons que le nombre des espèces étudiées est très restreint et qu'une généralisation en fonction des familles ou des classes n'est pas encore possible. Nous avons précédemment constaté l'absence de synthèse des stérols et du squalène chez l'Holothurie *Stichopus japonicus*[4]; nous reportons ici les résultats d'un travail parallèle effectué avec l'anémone de mer *Calliactis parasitica*. Rappelons que cet animal contient un pigment azoté particulier, la calliactine[7]. L'absence de biosynthèse du cholestérol et du squalène a été établie chez un autre coelentéré mais appartenant à une classe différente, la méduse *Rhizostoma* (scyphozoaire)[8].

On a injecté 0,1 mC d'acétate-1^{14}C dans la colonne de 15 anémones de mer *Calliactis parasitica*[9]; les animaux ont été maintenus en vie pendant 24 h. Les lipides ont été extraits et saponifiés suivant les procédés habituels; les stérols ont été précipités sous forme de digitonides. Après 3 cristallisations dans le méthanol, la radioactivité de la lique de l'insaponifiable (environ 1%). Il n'est toutefois pas possible, comme dans d'autres cas, d'éliminer l'existense d'une biosynthèse des stérols particulièrement lente, ou bien saisonnière, ou bien encore réprimée par leur abondance dans la nourriture.

Les stérols de cette anémone de mer ont été analysés par chromatographie sur couche mince de $Al_2O_3/AgNO_3$, par chromatographie en phase gazeuse (OV 101 1%) et par spectrométrie de masse avant et après propionylation; fraction stérolique devient nulle. Nous reportons dans le Tableau I les radioactivités des principales fractions. On remarque une incorporation importante de la radioactivité

[1] R. B. Clayton, J. Lipid Res. *5*, 3 (1964).
[2] F. J. Ritter et W. H. J. M. Wientjens, T.N.O. Nieuws *22*, 381 (1967).
[3] J. Austin, *Advances in Steroids biochemistry and pharmacology* (Ed. M. H. Briggs; Academic Press, London 1970), vol. 1, p. 73.
[4] T. Nomura, Y. Tsuchiya, D. André et M. Barbier, Bull. jap. Soc. Sci. Fisheries *35*, 299 (1969).
[5] C. L. di Prisco, F. Dessi, M. Tommasucci et C. Basile, Communication au 6ème Congrès Européen d'Endocrinologie Comparée, Montpellier 1971, p. 129.
[6] A. G. Smith et L. J. Goad, Biochem. J. *123*, 671 (1971).
[7] E. Lederer, G. Teissier et C. Huttrer, Bull. Soc. chim. fr. *7*, 5ème série, 603 (1950).
[8] H. E. van Aarem, H. J. Vonk et D. I. Zandee, Arch. int. Physiol. Biochem. *72*, 606 (1964).
[9] Nous remercions le CEA, pour des subventions ayant facilité l'achat du matériel radioactif. Les radioactivités ont été mesurées sur un appareil à scintillation Nuclear Chicago de rendement 85% pour ^{14}C.

Tableau I. Résultats de l'incorporation d'acétate de sodium 1-^{14}C (0, 1mC)[9]

Fractions	Poids (g)	Radioactivités (dpm/mg)	Radioactivités totales (dpm)
Insaponifiable	1,10	2320	$2{,}55 \times 10^6$
Acides	2,20	3550	$7{,}80 \times 10^6$
Stérols bruts	0,270	130	$3{,}50 \times 10^4$
Stérols:			
Ière cristallisation	0,211	5	$1{,}05 \times 10^3$
2ème cristallisation	0,188	3	564
3ème cristallisation	0,156	2	312