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# REVUE ROUMAINE DE BIOLOGIE

## SÉRIE DE BIOLOGIE ANIMALE

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*Hommages à l'académicien Petru Jitariu, à l'occasion de son 80e anniversaire*

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# HOMMAGE À L'ACADÉMICIEN PETRU JITARIU À L'OCCASION DE SON 80e ANNIVERSAIRE

P. Jitariu

Les biologues de Roumanie sont heureux de pouvoir renouveler leur hommage à un professeur qui fut entièrement dévoué à l'enseignement universitaire, à un homme de science qui a consacré une bonne partie de son activité à la recherche scientifique, à un dirigeant qui s'est occupé de l'organisation des conditions nécessaires à une bonne formation des biologues, à un membre de l'Académie qui a largement contribué à l'évolution de la Filiale de Iassy de l'Académie de la R. S. de Roumanie.

Né le 11 mai 1905, à Giulești (comm. de Boroaia, dépt. de Suceava) dans la famille de l'instituteur V. Jitariu, Petru Jitariu fit ses études primaires d'abord à l'école de son village natal, puis à Fălticeni (ville où son pére fut transféré) et où il fit ensuite ses études lycéales. Inscrit à la Faculté des sciences de l'Université de Iassy il y acheva en 1930 la section des sciences naturelles. Remarqué par le Prof. N. Cosmovici il fut nommé assistant (1930) au Laboratoire de Physiologie générale et comparée. Chargé des travaux pratiques il se fit remarquer par la qualité de son enseignement.

En même temps, il y commença ses recherches sur la physiologie du foie qui vont constituer sa thèse de doctorat qu'il passa à Iassy en 1938. En 1942 il va les contenuer au laboratoire du Prof. H. Rein, à Göttingen.

Promu au poste de professeur de Physiologie animale et humaine de la Faculté de Biologie de l'Université de Iassy en 1947, il va réorganiser son laboratoire (qui avait pati des suites de la guerre) et y donnera pendant près de 30 ans un cours de Physiologie, clair et bien mis à jour qui avait le don de beaucoup intéresser les étudiants. En même temps, le Professeur Jitariu va élargir son champ de recherches, en introduisant dans ses programmes des directions nouvelles, dont quelques-unes seront confiées à ses élèves, souvent comme sujets de thèses de doctorat.

Ces nouveaux sujets se réfèrent à la physiologie du milieu intérieur, à celle du cœur et en général de l'appareil circulatoire, au système nerveux,

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à la rate, à la coagulation chez les Invertébrés, à l'écophysiologie des animaux aquatiques, à l'action du champ électromagnétique sur l'organisme des animaux, enfin à la physiologie des membranes. Toutes ces recherches ont un caractère fondamental mais présentent également de nombreuses possibilités d'application pratique.

Nommé doyen de la Faculté de Biologie — Géographie de Iassy en 1953 (fonction qu'il garda pendant 13 ans), le Professeur Jitariu va s'occuper de l'amélioration des conditions de travail à cette faculté dont l'ancien local était devenu insuffisant. Des démarches longues et compliquées, appuyées par le Rectorat, aboutiront finalement à la construction d'un nouveau local où la Faculté put déménager et organiser ses laboratoires à la fin de 1963.

Se rendant bien compte que les recherches d'hydrologie et de biologie sur le lac de barrage aménagé dans les montagnes sur le cours de la rivière Bistritza (recherches nécessaires au développement de la pisciculture dans ce bassin artificiel) pourraient être réalisées en de meilleures conditions si les chercheurs pouvaient bénéficier de laboratoires situés dans son voisinage, le Professeur Jitariu milita, en sa qualité de doyen de la Faculté de Biologie et Géographie, en vue d'obtenir un bâtiment désaffecté, pour y organiser de tels laboratoires. Quand ce fut fait, une nouvelle station de recherches scientifiques de l'Université de Iassy (faisant pendant à sa Station de Zoologie marine d'Agigea fondée jadis par le Prof. Ioan Borcea) est apparue : la « Station de recherches biologiques, géologiques et géographiques "Stejarul" de Pîngărați ». Comme celle d'Agigea elle était destinée autant aux recherches scientifiques qu'à la pratique sur le terrain des étudiants.

Enfin, l'élection du Professeur Jitariu à l'Académie de la R. S. Roumanie en qualité de Membre Correspondant, va lui permettre d'organiser un Collectif de Physiologie animale, exclusivement consacré à la recherche scientifique qui, ensemble avec d'autre collectifs biologiques va entrer sous forme de Section, dans la structure de l'Institut de Biologie générale et appliquée de la Filiale de Iassy de l'Académie, (Filiale dont il était devenu le Secrétaire).

La séparation des groupes biologiques et médicaux de cet Institut aura pour effet l'apparition de deux institutions de recherches scientifiques : le Centre de recherches biologiques et l'Institut de recherches médicales.

Le Centre de recherches biologiques de la Filiale de Iassy de l'Académie, sera dirigé dorénavant par le Professeur Jitariu qui en gardera la direction jusqu'en 1977. Il s'est acquité très honorablement de la charge assez ingrate de diriger une institution multidisciplinaire, formée de plusieurs sections, secteurs et collectifs, représentant chacun une autre discipline biologique.

Elu membre titulaire de l'Académie en 1974, le Professeur Jitariu devint le Président de la Filiale de Iassy à la place du Professeur Simionescu qui devint à cette date le Vice-Président de l'Académie entière.

Comme Secrétaire de la Filiale, ensuite et surtout comme son Président, le Professeur Jitariu a contribué largement à l'adaptation de son organisation aux nouvelles conditions de travail et à ses nouvelles obligations et fonctions.



Une place importante dans ses démarches et ses préoccupations fut occupée dès lors par la construction du nouveau bâtiment de la Filiale, la réorganisation de sa bibliothèque ainsi que l'organisation des Comités, Commissions et Groupes de travail dont la fonction est de polariser l'activité des hommes de science vers certains problèmes importants, fondamentaux ou applicatifs, présentés par les spécialistes aux symposiums ou aux conférences organisés à cet effet, suivis de discussions. Actuellement, la Filiale de Iassy de l'Académie compte les formations suivantes :

— La *Commission de « l'inventique »* dont la principale fonction consiste à encourager la valorisation pratique de la création scientifique et technique ;

— *Le Groupe d'études* consacrées aux problèmes actuels de l'électroniques et des micro-ondes ;

— *Le Groupe d'études* pour l'investigation de l'avenir de la science et de la technique ;

— *La Sous-Commission* consacrée à la Révolution scientifique et technique ;

— *La Sous-Commission* consacrée aux monuments de la nature ;

— *La Sous-Commission* consacrée à la lutte contre la polution du milieu ;

— *La Sous-Commission* pour la relation « Homme-Biosphère » ;

— *Le Sous-Comité* pour l'histoire et la philosophie de la Science ;

— *La Sous-Commission* pour l'étude de la formation du peuple roumain et de sa langue ;

— *La Sous-Commission* d'Anthropologie et Ethnologie.

L'activité intense et multilatérale du Professeur Petru Jitariu lui a valu de nombreuses distinctions accordées dans notre pays et à l'étranger. Chez nous, les distinctions suivantes lui furent conférées : l'Ordre du Travail 1re classe, l'Ordre de l'Etoile de la R. S. de Roumanie 4e classe, l'Ordre du mérite scientifique ainsi que le diplome de "Professeur émérite". A l'étranger, il fut élu en 1967 membre de la Société de Pathologie comparée de Paris et, en 1972, membre de l'Association des hommes de Science des Etats-Unis.

Les biologues roumains ainsi que tous ceux qui collaborent avec l'Académie et sa Filiale de Iassy souhaitent au Professeur P. Jitariu longue vie et bonne santé, ensemble avec ceux qui lui sont chers.

*Le Comité de Rédaction*

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# L'ŒUVRE SCIENTIFIQUE DE L'ACADÉMICIEN **PETRU JITARIU**

L'activité de recherche scientifique de l'académicien P. Jitariu commence dès son entrée au laboratoire de Physiologie comparée dirigé alors par le prof. dr. Nicolae Cosmovici. Sa thèse de doctorat (1938) contient des recherches sur la glycogénogénèse et la glycogénolyse du foie des batraciens sous l'influence d'excitations bulbaires et bulbo-médulaires. Ces études ont été continuées à Göttingen chez le célèbre physiologue Rein, où il à étudié le comportement du foie dans les phénomènes de thermoréglage chez le chat. Pour ces recherches, il utilise des techniques très modernes à l'époque, tel l'enregistrement des différences de température sur les vaisseaux sanguins à l'aide d'un thermocouple et le mesurage des variations de débit de la veine porte avec le rhéomètre de Rein.

Parmi les conclusions établies, favorablement commentées dans « Naturwissenschaften Forschungen » (en 1942) et en totalité acceptées en 1959 dans la monographie sur le thermoréglage, publiée par le Journal de Physiologie — Paris, nous soulignons : le rôle stimulant du sympathique sur le métabolisme hépatique, l'augmentation de la température hépatique à l'excitation des thermo-récepteurs du museau de l'animal (mais non des autres régions du corps), la double action de l'adrénaline (directe sur la cellule hépatique et indirecte par les centres végétatifs), la stimulation prolongée du métabolisme accompagnée de l'augmentation calorique après les injections de glucose.

L'académicien P. Jitariu aborde ensuite des thèmes de recherche variés concrétisés en 130 titres publiés seul on en collaboration avec les jeunes spécialistes qu'il sait entraîner dans l'activité de recherche, en observant leurs aptitudes et aspirations, les guidant vers les directions modernes de recherche. A côté de ses prédécesseurs Leon Cosmovici, Nicolae Cosmovici et Elena Lupu, il est le créateur de l'école de physiologie de Iași.

La multilatéralité de la conception de biologue de l'académicien P. Jitariu se manifeste dans toute son œuvre scientifique. Ses recherches dans le domaine de la physiologie comparée sont axées sur la physiologie du milieu intérieur, du cœur et de l'appareil circulatoire, mettant en évidence les rapports qui s'établissent entre la tension superficielle du plasma sanguin et de la lymphe du chien, ou entre celle du liquide péricardique et le plasma chez les poissons marins. On étudie la coagulation chez les crustacées et les lamellibranches. On poursuit l'action d'extraits des fruits d'*Ecballium élaterium*. D'autres recherches poursuivent la physiologie du cœur tricaméral des amphibiens et apportent des preuves à l'appui de l'absence d'un mélange de sang oxygéné et non oxygéné au niveau du ventricule.

D'une importance particulière sont ses recherches sur l'écophysiologie des animaux aquatiques. Elles sont déterminées par la nécessité de connaître les modifications écologiques qui devaient apparaître après la construction du barrage sur le ruisseau de Bistrița. En poursuivant les migrations de ponte des truites de Lacul Roșu, lac de barrage naturel, et du ruisseau de Bistrița, avant la construction du barrage, on constate que ce déplacement s'effectuait vers les lieux riches en $H_2S$, le besoin du

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S étant expliqué par la baisse de la quantité de gluthation dans une série d'organes pendant la période de reproduction. Les chercheurs de la Station « Stejarul » de Pîngărați ont continué ces études sur les truites du lac d'accumulation de Bicaz. Si les œufs de poissons et la laitance sont mis avant la fécondation dans de l'eau avec du $H_2S$, l'éclosion est plus rapide et la mortalité des alevins réduite.

Les études sur la nutrition animale ont poursuivi le rôle des aliments végétaux iodés utilisés dans l'alimentation des rats, des poules, des chèvres et des brebis gris. Une conclusion intéressante est celle que l'affection aiguë du tympan, caractéristique pour les agneaux albinos, devient très rare si les brebis gestantes sont nourries de végétaux iodés.

Dans le domaine du système nerveux, l'académicien P. Jitariu a collaboré avec des médecins sur l'étude de l'épilepsie et a initié des recherches sur la relation du hypothalamus avec l'hypophyse, domaine de grande actualité.

La physiologie de la rate est étudiée en poursuivant les rapports spléno-gastrique et spléno-sanguin.

L'un des derniers domaines de travail de l'académicien P. Jitariu est lié à l'action du champ électromagnétique (CEM) sur l'organisme animal, il étant l'un des fondateurs de l'école de biomagnétisme de Roumanie. Les résultats de plus de 50 travaux ont été présentés au Symposium national de biomagnétisme en 1970. Basé sur les multiples résultats obtenus dans ces expériences, l'académicien P. Jitariu donne une nouvelle théorie sur le mode d'action du CEM appliqué aux animaux. Cette théorie admet, tout d'abord, l'existence des « biochamps » électromagnétiques, propres aux organismes vivants ; leur apparition a l'explication suivante ; chaque électron en mouvement produit un champ électromagnétique, or le métabolisme est un processus d'oxydo-réduction où les électrons transportés par les enzymes respiratoires spécifiques donnent naissance à des potentiels électriques d'oxydo-réduction, donc des champs électromagnétiques. On comprend donc par la notion nouvelle de « biochamp », le champ électromagnétique engendré par une structure vivante pendant son activité normale ou pathologique. L'élément qui a le rôle prépondérant dans la genèse du champ est la molécule protéique.

Les biostructures sont en général asymétriques à cause du C dont les quatre valences sont satisfaites par des radicaux différents. Les acides aminés, de même que les protéines qui en résultent sont asymétriques et polarisés. Il s'ensuit que la molécule protéique, par son extrême variété et son asymétrie est la génératrice de microchamps électriques aussi variables et aussi nombreux que les protéines respectives.

L'application du champ électromagnétique extérieur (CEM) produit la perturbation des conditions d'apparition des biochamps avec des effets qui peuvent être poursuivis dans les expériences réalisées.

Dans ces expériences on a utilisé des CEM avec pulsations, d'intensité et de fréquence réduites pour ne pas altérer les relations fonctionnelles normales. On constate aussi que le champ avec pulsations produit une sensibilité de l'organisme, chaque pulsation trouvant un substrat modifié avec un seuil d'excitation plus réduit. Mais si la dose électromagnétique atteint un certain seuil, la réponse de l'organisme reste constante, quoique



le champ augmente en intensité. D'autres observations montrent que les tissus plus actifs ont une sensibilité plus grande aux CEM.

De l'interaction des biochamps et du CEM il résulte aussi une stimulation des organes protéinoformateurs, une modification de l'ampleur des processus métaboliques et des mécanismes neuro-endocrins, une influence sur la décharge de l'ACTH, sur le métabolisme glucidique, sur le métabolisme minéral, sur la ségmentation des œufs de Tubifex.

La conclusion générale de cette nouvelle théorie est que l'action du CEM déclenche par induction la modification des paramètres du biochamp, ce qui conduit à l'activation ou non des structures qui les ont engendrées.

D'autres recherches récentes initiées par l'académicien P. Jitariu poursuivent la physiologie des membranes. Le nouveau collectif étudie les propriétés électriques des membranes cellulaires sous l'action d'une série de composés quaternaires d'amonium, d'une série d'hormones, d'ions et d'agents organiques telle la procaïne. On enregistre les potentiels de repos par la méthode des microélectrodes en verre, sur la fibre musculaire striée et sur la cellule hépatique. Les résultats sont discutés à base d'une conception nouvelle sur le rôle actif des phospholipides membranaires dans les phénomènes bioélectriques. Ces phospholipides organisés en structure micellaire globulaire et en structure micellaire laminaire peuvent changer de phase. Ces recherches prouvent qu'à la base de l'électrogènese, des modifications de potentiel et de la perméabilité se trouve l'intéraction entre phospholipides et ions.

Par tout son œuvre l'académicien Petru Jitariu s'impose comme une personnalité représentative de la science roumaine.

*Eliza Alexa*

# INFLUENCE OF THE MAGNETIC FIELD ON THE PLASMA PROTEINS AT CHICKENS

M. LAZĂR and N. NEAGA

Les auteurs ont poursuivi les modifications des protéines totales et des fractions protéiques du plasma des poulets de la race Rock blanc soumis à l'action du champ magnétique.

Les poulets âgés d'une jour ont été soumis journellement 10′, pendant 10 jours, au champ magnétique d'une intensité de 6300 Oe.

Après le traitement on a déterminé chez les poulets de diverses ages (15, 30, 45, 60, 75 jours) les protéines totales plasmatiques (par rephractométrie) et les fractions protéiques (par électrophorèse).

Le champ magnétique détermine des modifications des protéines totales et des fractions protéiques. La protéinemie et la fraction gamma-globuline augmentent d'une manière significative.

The specific plasma protein content on poultry has been less studied, as compared to mammals, that is why the literature in the field contains often inconsistent data, both on the quantitative ratio and on the nomenclature of different fractions.

Sturkie [42] shows that the protein fractions from poultry plasma can be grouped into : prealbumins, postalbumins and globulins.

Other authors [9], [43 — 45] have evidenced two prealbumin fractions in the chicken plasma.

Due to the fact that — in our experiments on white Rock chicken — the prealbumin fraction has been made visible only at few small chickens, we have not considered it any longer in our estimations.

The plasma protein content is modified as a function of age, sex, physiological condition, breed, certain physical (radiations, magnetic field) and chemical agents. By ontogenetically studying the quantitative variations of serum proteins at hens, Pora and coworkers [35] have noticed their gradual increase, accompanied by a decrease of the A/G ratio. In his experiments, performed on chickens and Leghorn hens, Ainson [1], [2] records modifications of alpha-globulins, depending on sex, and as a consequence of injecting certain hormones (adrenalin, acetylcholine etc.). Quantitative variations of the plasma proteic fractions as a function of age have been obtained by other authors, too [3 — 5], [46].

The dynamics of the total proteins and of the proteic fractions has been followed at the bursectomised chickens too, an increase of the serum albumin content, concomitantly with a decrease of the gamma-globulins having been observed [6]. Modifications of the blood protein picture, as a consequence of bursectomy, have been also evidenced in other experiments [7], [8], [28], [36].

The influence of the magnetic and electromagnetic field on plasma proteins of different animals has been extensively studied. Experimenting upon rabbits, Jitariu and coworkers [14], [15] have observed that the plasma protein image is modifying at the animals subjected to a treatment with pulsing electromagnetic field — the recorded modifications depend on the electromagnetic field type (interrupted or not).

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The variations of the quantity of serum proteins have been followed by Stavăr et al. [37 — 41] on embryos and chickens subjected to the action of a continous and interrupted electromagnetic field.

The present paper describes the influence of the magnetic field on the total plasma proteins, as well as on plasma protein fractions.

## MATERIAL AND METHODS

Experiments started on one-day white Rock chickens, divided into two groups of 60 chickens each. The first group was a control one. The second group chickens were subjected, beginning with the first day after hatching, to the action of the magnetic field, with an intensity of 6300 Oe., the treatment lasting for 10 days, 10 minutes each day.

The magnetic field was generated by a Weiss electromagnet with cylindric poles, 10 cm in diameter.

The determination of the plasma proteins was performed at different periods of ontogenetic development (15, 30, 45, 60 and 75 days).

The total proteins were determined with a Pulfrich-Zeiss immersion refractometer, while the separation of the protein fractions was accomplished by electrophoresis on paper, by using Schleicher and Schül no. 2043 filter paper, and barbiturate buffer with pH = 8.6, and a migration time of 7 hours. For the band dyeing, bromphenol blue was used, the curve registering being performed with the ERI-10 integration extinction registering equipment.

## RESULTS AND DISCUSSIONS

The obtained data (see Table 1) show that the total plasma proteins increase significantly up to the age of 60 days inclusive, in the case of chickens subjected to the magnetic field action, the increase being negligible at 75 days. At the same time, an increase of the total protein quantity — as a function of age — is to be observed at both groups.

Similar results have been obtained by us in previous studies, [16—20] experiments being made on Leghorn and white Rock chickens subjected to the action of the magnetic and electromagnetic field with different intensities and durations.

The albumin fraction exhibits unimportant quantitative variations —decreasings and increasings.

In another series of experiments, performed also on white Rock chickens, we have obtained sometimes significant increases, especially when using a 3000 Oe. magnetic field [19] [20].

In the case of Leghorn chickens, subjected to the action of a 300Oe. electromagnetic field, a negligible decrease of this fraction has been recorded [16].

By following the evolution of the alpha- and beta-globulin fractions, a constant decrease tendency is to be observed at the treated chickens, as compared to the control group, on the whole investigated period;



*Tabel 1*

Variations of total plasma proteins and of the proteic fractions at chickens treated with a magnetic field

| Group | N | Age (Days) | Total proteins g% | | | Proteic fractions % | | | | | | | | | | | | A/G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Albumine | | | Globuline | | | | | | Gamma | | | |
| | | | | | | | | | Alpha | | | Beta | | | | | | |
| | | | $\bar{X}$ | ES | P | $\bar{X}$ | ES | P | $\bar{X}$ | ES | P | $\bar{X}$ | ES | P | $\bar{X}$ | ES | P | |
| Control | 10 | 15 | 3,32 | 0,07 | — | 46.80 | 1.02 | — | 10.21 | 0.70 | — | 8.82 | 0.65 | — | 34.00 | 0.70 | — | 0.88 |
| M.F.6300 | 10 | 15 | 3.83 | 0.20 | 0.05 | 48.00 | 1.30 | — | 8.00 | 0.50 | 0.05 | 7.75 | 0.72 | — | 37.30 | 1.10 | 0.05 | 0.90 |
| Control | 10 | 30 | 3.60 | 0.10 | — | 46.85 | 1.50 | — | 9.20 | 0.82 | — | 8.10 | 0.70 | — | 35.70 | 1.50 | — | 0.88 |
| M.F. 6300 | 10 | 30 | 3.90 | 0.08 | 0.05 | 46.20 | 1.08 | — | 8.82 | 0.60 | — | 7.92 | 0.80 | — | 37.00 | 1.00 | — | 0.85 |
| Control | 10 | 45 | 3.82 | 0.15 | — | 47.10 | 1.60 | — | 8.00 | 0.90 | — | 8.35 | 0.60 | — | 36.30 | 1.60 | — | 0.89 |
| M.F.6300 | 10 | 45 | 4.30 | 0.10 | 0.05 | 47.00 | 1.30 | — | 7.60 | 0.50 | — | 6.75 | 0.72 | — | 38.65 | 0.70 | 0.05 | 0.88 |
| Control | 10 | 60 | 4.25 | 0.09 | — | 45.10 | 1.80 | — | 8.10 | 1.00 | — | 8.20 | 0.70 | — | 38.60 | 1.20 | — | 0.82 |
| M.F.6300 | 10 | 60 | 4.70 | 0.07 | 0.05 | 43.60 | 1.30 | — | 7.60 | 0.70 | — | 7.60 | 0.75 | — | 41.35 | 0.50 | 0.05 | 0.77 |
| Control | 10 | 75 | 4.40 | 0.12 | — | 44.20 | 1.70 | — | 7.90 | 1.20 | — | 8.20 | 0.50 | — | 39.70 | 1.10 | — | 0.79 |
| M.F. 6300 | 10 | 75 | 4.55 | 0.15 | — | 44.60 | 1.50 | — | 7.60 | 0.80 | — | 7.10 | 0.70 | — | 41.70 | 1.00 | — | 0.79 |

however, this diminishing reaches the noteworthiness threshold in one single case (alpha-globulins) at the 15 days age of the treated chickens.

Nevertheless, it is worth mentioning that, in various other experiments performed both on white Rock and Leghorn chickens, in which a magnetic (3000 Oe.) or an electromagnetic (300 Oe.) field has been used, we have often obtained increases of these protein fractions, [16—20] too. As with the albumins, the alpha- and beta-globulin fractions gradually decrease quantitatively at both groups, concomitantly with the chickens growing.

The magnetic field considerably influences the increase of the gammaglobulin fraction up to the age of 60 days, being obviously increased even at chickens of 75 days. There is to be mentioned that, in the great majority of our previous experiments [16], [18], [19] we have recorded the same increase of the gamma-globulin quantity — at chickens treated with magnetic field, however, in some cases being also obtained inverse results, i.e. decreases.

Both with the chickens of the control group and with those of the group subjectd to the magnetic field action, the quantity of gamma-globulins increases with the chickens growing.

The A/G ratio manifests a decreasing tendency with the chickens growing, undergoing completely unimportant decreasing or increasing variations in the case of chickens treated with magnetic field, as compared to those of the control group.

Starting from the data obtained in the present and previous investigations, as well as from those in literature, we can draw the conclusion that the dynamics of total plasma proteins and of the proteic fractions modifies itself under the action of the magnetic and electromagnetic field, both of them inducing — by their various intensities — modifications of the protein image, for quite a long period. The direction and level of these modifications depend on the type of the magnetic field, on its intensity, exposure time, animal species of breed, age, a.s.o.

The obtained results show that the magnetic field action obviously influences the organs at whose level the protein synthesis takes places — above all the liver, with an intense proteic metabolism.

Various analyses show that the hen liver produces the whole quantity of serum albumins for 13 days [26], [27]. The protein biosynthesis at the level of protein-producing organs is stimulated by the action of the magnetic field. Holodov [10 — 12] has shown that the nervous system is strongly influenced by the magnetic field, a peculiar reactivity being exhibited by the hypothalamus and the cortex.

Taking into account the close connection between the nervous and the endocrine system, effected by the hypothalamo-hypophysis system, one can suppose that the action of the magnetic field on the protein forming organs should be performed especially in this way. The hypophysis and other target glands, play— by the secreted hormons — a main role in the stimulation or inhibition of the proteic synthesis. Neither can be excluded the direct action of the magnetic field on the organs playing a role în the synthesis of plasma proteins or other substances.

A series of experimental data support these assertions. Thus, Zirra et al. [47], [48] have evidenced a series of hystological modifications in



the hypophysis of guinea pigs treated with a pulsing electromagnetic field of low intensity. Or, it is known that the hypophysis, stimulated by this physical agent, will activate — by means of the GH or of certain hormons of the target glands — the protein synthesis in different organs. In the thyroid of the treated guinea pig, the oxidase and the C vitamin content of the thyroid tissue is richer, having the aspect of a hyperfunction.

Our recent investigations (unpublished data) show a series of modifications at the level of the hypophysis tissue of the white Rock chickens, treated with a magnetic field.

The effect of the magnetic field upon other endocrine glands has been evidenced by many authors. Thus, Jitariu and coworkers [13] have found that, in the case of guinea pigs treated with an electromagnetic field, the thyroid capacity regarding the incorporation of the radioactive ion is stimulated.

In the case of chickens treated with a magnetic or an electromagnetic field of different intensities, important hystological modifications have been recorded at the level of the thyroid, thymic and gonadal tissue [21], [22], [25], [28 — 32]. The number of thyroid follicles increases, the follicular epithelium is higher a.s.o., a fact attesting a gland stimulation.

At the ovary level, an increase of the ovarian follicles and the tendency to maturation is to be observed, the modifications of the testicular tissue consisting of a greater development of the semiferous tubules, the spermatozoon evolution to maturation, a.s.o.

At the same time, the histological sections performed on the adrenal glands of the chickens treated with a 300 Oe. electromagnetic field in the embrionary period or in the first 10 days after hatching indicate an increase of the ascorbic acid quantity. It is known that the ascorbic acid shows, along with its multiple functions, a special importance in the plastic processes occurring in the organism, a close correlation existing between the increase of plasmatic proteins and the ascorbic acid level.

The ascorbic acid shortage entails the reduction of the free amino-acid and protein synthesis. By following the variation of the serum protein quantity at embryos and chickens subjected to the action of an interrupted or continous electromagnetic field, Stavăr et al. [37], [38] have found that in the 1—90 days interval, the modifications depend on the time when the treatment has been applied — during incubation or after hatching.

In the case of Rhode Island breed, we have found modifications in many relevant cases, at the level of different proteic fractions.

The interrupted magnetic field, applied to the eggs before their peing incubated, induces a stimulation of the albumin and immunoglobulin synthesis, at chickens of both sexes, for a long period, whereas the interrupted electromagnetic field, applied to the embryos on the first three days of incubation, stimulates the beta-globulin synthesis. If the treatment is applied after the chickens hatching, a stimulation of the gamma-globulin synthesis and an inhibition of the beta-globulin synthesis take place.

Our recent experiments have followed the quantitative modifications of the RNS and DNS from the muscles and liver of chickens subject

ed to a magnetic and electromagnetic field of different intensities [23 – 25].

We have observed a quantitative increase of the nucleic acids, mainly at the liver level, a fact that can be explained by the special sensibility of the hepatic tissue towards different agents, as well as by its role in the metabolism of the nucleic acids and proteins.

Our previous investigations [33], [34] have also shown that both the electromagnetic and the magnetic field, applied during the embryonic development, have a stimulating effect on the embryos, favourizing the increase of the hatching percent, a daily average growth, for a period of even 90 days, being recorded with the chickens.

In the case of the treated chickens, in the first post-hatching days there is also to be observed a stimulative effect on the growth rhythm and on the weight percent.

Our results regarding the quantitative modifications of proteins and of the proteic fractions, at the treated chickens, corroborated with those obtained by other researchers (on chickens and other animals) lead us to the conclusion that the action of the magnetic or electromagnetic field has a visible influence both on the total plasma proteins and on the different proteic fractions.

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# ASPECTS REFERRING TO THE DOSE: EFFECT RATIO IN THE HYPOLIPEMIANT ACTION OF SOME POLYENES

ȘT. AGRIGOROAEI, AL. SAUCIUC, GABRIELA AGRIGOROAEI, I. NEACȘU, CONSTANȚA BÎRCĂ, ELENA CHERA and GEORGETA NĂNESCU

The present paper investigates the effects of some antibiotics (nystatin and CM nystatin) administered in different doses, on the total lipids, triglicerides and beta-lipoproteins from animal serum, Chinchilla rabbits, which have been subjected to a mixed, atherogenic and very weak regimen (0.041 g cholesterol and 0.082 g/kg b./day, respectively)— in comparison with the chlofibrate and asclerol effects. Treatments have been applied after the preceding installation of a hyperlipemia and lasted for six weeks, during which the atherogenic regimen has been continued. Investigated polyenes proved to be more efficient than asclerol and chlofibrate as regards the seric level reduction of the lipids and beta-lipoproteins, but not of the triglicerides. The biosynthesis nystatin is more efficient than the CM nystatin. The effects of both polyenes on the lipids and beta-lipoproteins become stronger with the decrease of the administered dose.

Having in view the thorough analysis of our previous results referring to the hypolypemiant action of some biosynthesis and semisynthesis polyenes [1 — 4], we intend to achieve a more exact determination of the therapeutical doses, as well as of the application duration of the treatments. In this respect, there have been followed the effects of certain decreasing doses of nystatin and CM nystatin, respectively, concomitantly with the effects of the therapeutic doses of asclerol and chlofibrate [6], [8] on the total lipids and triglicerides, as well as on the beta-lipoptroteins from the sanguine serum of laboratory animals.

At the same time, investigations have been carried out on animals subjected to an atherogenic, very weak regimen so that the level of the serum indices had in view should be closer to that recorded for natural hyperlipemia and arteriosclerosis.

## MATERIAL AND METHODS

Experiments were made on nine groups of animals (Chinchilla rabbits) with a body weight of about 3.00 kg. All the nine groups were subjected to a mixed atherogenic very weak [7] regimen (0.041 g cholesterol/kg b. day) in the first two weeks and the last three ones and somewhat stronger (0.082 g cholesterol/kg b./day) in the four intermediary weeks.

The first group was a control one, the other ones being treated differently, for six weeks, beginning with the fourth week of atherogenic regimen in the following way : the second group — treated with asclerol, 1.53 mg active substance/kg b./day, the third group — with chlofibrate 11.73 mg/kg b./day, the fourth group with nystatin 6.66 mg/kg b./day, the fifth — with nystatin 3.33 mg/kg b./day, the sixth — with CM nystatin 1.66 mg/kg b./day, the seventh — with CM nystatin 12.50 mg/kg b./day, the eitghth — with CM nystatin 6.25 mg/kg b./day and the ninth group, with CM nystatin, 3.12 mg/kg b./day.

All agents were orally administered, their effects being followed by analyses made after three, six and nine weeks from the beginning of the experiments, by methods described in literature [5], [9], [10].

## RESULTS

### A, TOTAL SERUM LIPIDS

The normal values of the total serum lipids ranged between 145.00 — 226,25 mg % serum. After the first three weeks of atherogenic regimen, the lipemia reached values between 278.50 — 406.25 mg % serum. These values—characterizing the starting moment of the period of treatment with different agents — have been noted with 100 % for the estimation eofthe observed variations in different subsequent moments of the treatm nt period.

As it can be seen from Figure 1, after six weeks of treatment, when, at the control group there could be observed a variation of the total serum lipids of + 42.88 %, which is more and more reduced, yet positive at the 3rd, 2nd and 7th groups, and still more reduced and negative at the 4th, 8th, 9th, 5th and 6th groups.

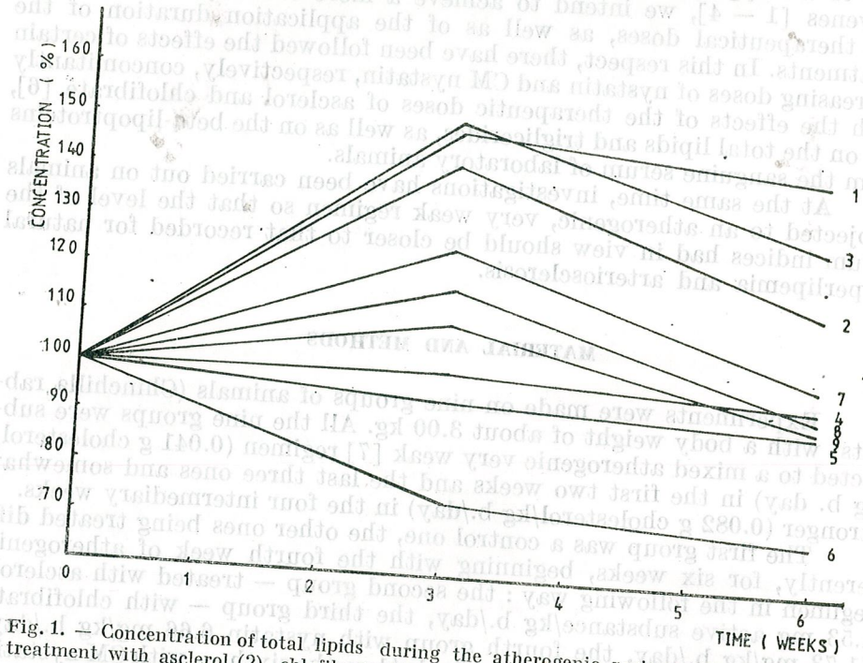


Fig. 1. — Concentration of total lipids during the atherogenic regimen (1), and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).

Estimating the efficiency of the above mentioned treatments by reporting the obtained results at the end of the experiments to the corresponding ones from the control group (considered as 100 %), the respective



results are arranged according to the degree of reduction of the serum lipids level, in the following way : 1. nystatin treatment — low dose (170.31 %), 2 — nystatin treatment — medium dose (119.26 %), 3 — CM nystatin treatment — low dose (114.71 %), 4. CM nystatin treatment — medium dose (111.12 %), 5. nystatin treatment — high dose (106.62 %), 6. CM nystatin treatment — high dose (97.74 %), 7. asclerol treatment (63.13 %) and 8. chlofibrate treatment (31.46 %).

By considering the increase depression of the index followed below the level at the beginning of the treatment as an indication on the necessary duration of its application, one can observe that nystatin in low and medium dose wholly reduces the increase of the serum lipids even in the first three weeks, while the same treatment with high dose—shortly after that time. The low and medium dose CM nystatin treatment requires almost a double time (between five and six weeks) while that in high dose — more than six weeks. Still longer periods of time are necessary in treatments with asclerol and chlofibrate.

### B. SERUM TRIGLICERIDES

The normal values of serum triglicerides ranged between 70.63 — 108.01 mg % serum. After a three week atherogenic regimen, there have been recorded variations — i.e. increases with some groups, decreases with others, the triglicerides values ranging between 45.61—115.65 mg %

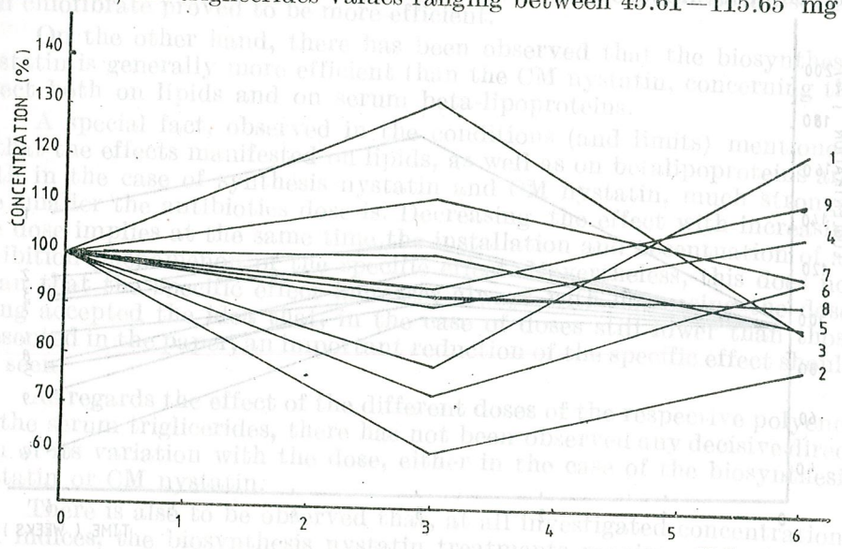


Fig. 2. — Concentration of triglicerides during the atherogenic regimen (1) and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).

serum. Generally, triglicerides have, during the atherogenic regimen, much more oscillating values than the other analyzed indices.

As it can be seen in Figure 2, after a six week treatment, when at the control group there can be observed a + 23.0 % variation of trigli-

cerides, this variation is more and more reduced, but positive at the 9th and 4th groups, and still more reduced and negative at the 7th, 6th, 8th, 5th, 3rd and 2nd groups.

According to the reduction efficiency of the serum triglicerides treatments may be arranged in the following way : 1. asclerol treatment (190.13 %), 2. chlofibrate tretament (155.69 %), 3. nystatin treatment — medium dose (154.39 %), 4. CM nystatin treatment — medium dose (132.47 %). 5. nystatin treatment — low dose (114.30 %), 6. CM nystatin treatment—high dose (113.52 %), 7. nystatin treatment—high dose (72.92% and 8. CM nystatin treatment — low dose (45.70 %).

In most of the applied treatments (with the exception of the chlofibrate and high dose CM nystatin ones) the triglicerides have rapidly decreased below the level at the beginning of the treatment, but subsequently exhibited important ascending variations, a fact that modifies the image of the final ratios.

### C. SERUM BETA-LIPOPROTEINS

The normal values of the serum beta-lipoproteins ranged between 105.28—271.70 mg % serum; after a three week atherogenic regimen, they covered the 211.64 — 477.64 mg % serum range.

There can be seen from Figure 3 that, after a six week treatment, when the beta-lipoproteins at the control group show a + 40.35 % variation as compared to the value at the beginning of the treatment, this variation is much more reduced (still, even positive) at the 2nd, 3rd and 7th groups, and yet more reduced — and negative — at the 4th, 8th, 5th, 9th and 6th groups.

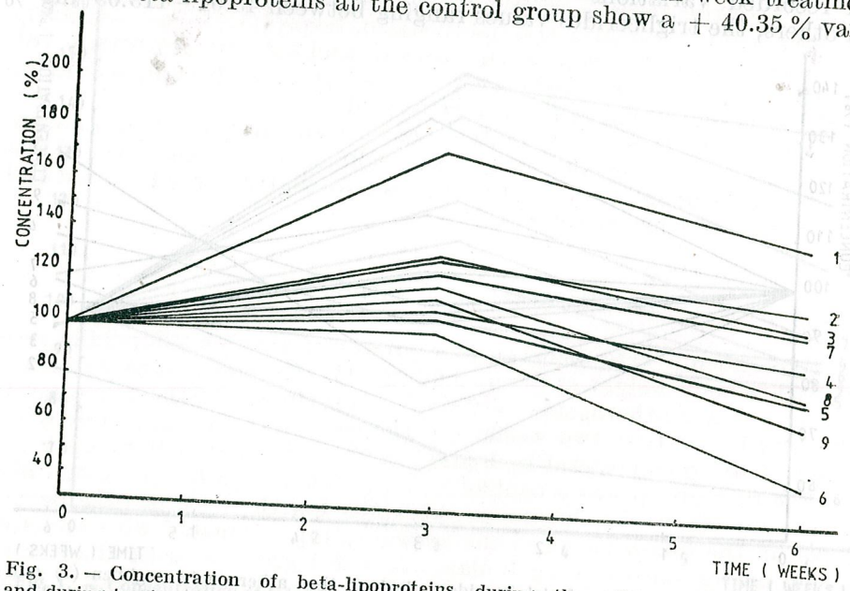


Fig. 3. — Concentration of beta-lipoproteins during the atherogenic regimen (1) and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).



From the viewpoint of the reduction efficiency of the serum beta-lipoproteins level, the treatments can be classified as follows : 1. nystatin treatment — low dose (236.53 %), 2. CM nystatin treatment — low dose (178.04 %), 3. nystatin treatment — medium dose (152.93 %), 4. CM nystatin treatment — medium dose (148.99 %), 5. nystatin treatment — high dose (119.25 %), 6. CM nystatin treatment — high dose (83.28 %), 7. chlofibrate treatment (81.05 %) and 8. asclerol treatment (61.07 %).

As regards the duration of the treatment, in the case of treatments, with asclerol, chlofibrate and high dose CM nystatin, it exceeds six weeks, while in all other cases, it ranges between 3 — 5 weeks, the shortest period qeing that of the low dose nystatin treatment.

## DISCUSSIONS AND CONCLUSIONS

Experimental results obtained show that, in the conditions of the atherogenic regimen chosen and of the polyenes doses used, the investigated polyenes are more efficient than the asclerol and chlofibrate, from the viewpoint of the reduction effects of the total lipids and serum beta-lipoproteins level, respectively. A different situation is to be met with the reduction of the serum triglicerides level, a case in which the asclerol and chlofibrate proved to be more efficient.

On the other hand, there has been observed that the biosynthesis nystatin is generally more efficient than the CM nystatin, concerning the effect both on lipids and on serum beta-lipoproteins.

A special fact, observed in the conditions (and limits) mentioned, is that the effects manifested on lipids, as well as on betalipoproteins are, both in the case of synthesis nystatin and CM nystatin, much stronger the smaller the antibiotics dose is. Decreasing the effect with increasing the dose implies at the same time the installation and accentuation of an inhibition phenomenon of the specific effect. Nevertheless, this does not mean that the specific effect increases always with decreasing the dose, being accepted the idea that, in the case of doses still lower than those presented in the paper, an important reduction of the specific effect should be seen.

As regards the effect of the different doses of the respective polyenes on the serum triglicerides, there has not been observed any decisive direction of its variation with the dose, either in the case of the biosynthesis nystatin or CM nystatin.

There is also to be observed that, at all investigated concentrations and indices, the biosynthesis nystatin treatments require approximately three weeks (or less) for the reduction of their level below that at the beginning of the treatment.

In the case of CM nystatin, the necessary duration for the reduction of the investigated indices level below the initial value is of 5—6 weeks or more.

Generally, the asclerol and chlofibrate treatments require much longer times for the reduction of the indices level below the initial value.

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*Biological Research Center*
*Iași, Calea 23 August, 20 A*
and
*Research Center for Antibiotics*
*Iași, Valea Lupului, 1*

# EFFECTS OF DIFFERENT DOSES OF HYPOCHOLESTEROLEMIANT POLYENES ON THE SERUM IONS

ELENA CHERA, ȘT. AGRIGOROAEI, AL. SAUCIUC, I. NEACȘU, GABRIELA AGRIGOROAEI, CONSTANȚA BÎRCĂ and GEORGETA NĂNESCU

Effects of 7-nystatin in high dose (6.660 mg/kg b./day), medium dose (3.330 mg/kg b./day) and low dose (1.660 mg/kg b./day) have been investigated as well as of the CM nystatin in high dose (12.500 mg/kg b./day), medium dose (6.250 mg/kg b./day) and low dose (3.125 mg/kg b./day) on the concentration of the $Na^+$, $K^+$ and $Ca^{2+}$ ions in the serum of *Chinchilla* rabbits subjected to a weak atherogenic regimen for nine weeks (0.041 g cholesterol/kg. b./day in the first two and last three weeks; 0.082 g cholesterol/kg b./day in the four intermediary ones). During the atherogenic regimen, the $Na^+$ concentration increases, while the $Ca^{2+}$ one decreases in all animals; the $K^+$ concentration is either decreasing or increasing, while the $Na^+/Ca^{2+}$ ratio increases. The 7-nystatin and CM nystatin in medium and low doses determines the reestablishment of the ion normal concentration, as well as of the $Na^+/Ca^{2+}$ ratio, their efficiency being comparable to that of the chlofibrate and asclerol.

The cholesterol administration determines the increase of the lipids and cholesterol concentration in the blood serum of the laboratory animals [1], [2], as well as the onset of atherosclerosis [1], [2], [3], [10]. These phenomena are accompanied by modifications of the ion concentration [4], [6]. It has been observed that the treatment with certain polyene antibiotics has strong hypocholesterolemiant and hypolipemiant effects [1], [2]. The present paper studies the effects of certain polyenes on the concentration of ions in the blood serum of animals subjected to an atherogenic regimen.

## MATERIAL AND METHODS

Experiments were carried on *Chinchilla* rabbits with an average body weight of 3.0 kg, which were divided in nine groups, of three animals each. All the groups had been fed on a weak atherogenic regimen for nine weeks, by administration of cholesterol in their food, as follows: 0.041 g/kg b/day in the first two weeks and in the last three ones; 0.082 g/kg b./day in the four intermediary weeks. By this regimen, modifications of the lipids, cholesterol, beta-lipoproteins, triglicerides and ions were obtained, which proved to be close to those met in natural hypercholesterolemia and atherosclerosis.

At the beginning of the experiment there were determined the normal values of the physiological parameters, investigated in all animals. In the first three weeks, the effects of the cholesterol administration were followed in all groups, by analyses carried on after the third week of atherogenic regimen. After the onset of the cholesterol effects, in the following six weeks, the groups were orally treated with different products, concomitantly with the cholesterol administration.

The first group received only cholesterol, being used as a control group. Two groups were treated with asclerol or chlofibrate, drugs frequently used in the treatment of cardiovascular diseases [5], [7], [11], [12], doses similar to those used in human therapeutics being administered. Thus, the second group was treated with asclerol 1.530 mg active substance /kg b./day, while the third one received chlofibrate — 11.733 mg/kg b./day. The other six groups were treated with polyenes, in different doses. Thus, the 4th, 5th and 6th groups were treated with 7-nystatin-high dose (6.660 mg/kg b./day), medium dose (3.330 mg/kg b./day) and low dose (1.660 mg/kg b./day), respectively, in 1 % propylene glycol as specific solvent, having no particular effects in this concentration [7]. The 7th, 8th and 9th groups, were treated with water solubilized CM nystatin-high dose (12.500 mg/kg b./day), medium dose (6.250 mg/kg b./day) and low dose (3.125 mg/kg b./day), respectively. After three, six and nine weeks from the beginning of the experiments, analyses of the concentration of the $Na^+$, $K^+$ and $Ca^{2+}$ ions from the blood serum were carried on, concomitantly with the analysis of the concentration of cholesterol, lipids, beta-lipoproteins and trigliceride, performed in other investigations. The determination of the ion concentrations was achieved by the flame-photometric method. The results obtained represent the mean value for each group of animals and are expressed in mg/100 ml serum, and as percentages to the initial value, taken as 100 %.

## RESULTS

The normal $Na^+$ concentration was of 338.70—358.70 mg/100 ml serum. During the atherogenic regimen, an increase of the $Na^+$ concentration at all groups of animals is observed. In the first group (control), the $Na^+$ concentration increases comparatively to the initial normal value over the whole duration of the treatment, the highest value being reached after three weeks of atherogenic regimen (108.01 %) (Fig. 1 A). In the second group, after three weeks of cholesterol administration, the $Na^+$ concentration increases up to 106.19 % (Fig. 1 B). The asclerol treatment determines, after three weeks of administration, a return to the initial value (99.63 %). In the third group, the $Na^+$ concentration increases after three weeks of atherogenic regimen up to 116.91 % (Fig. 1 C), comparatively to the initial value, returning to its normal value after a six weeks treatment with chlofibrate. In the 4th group, the $Na^+$ concentration increases up to 115.94 % in the initial atherogenic phase (Fig. 2 A). The high dose 7-nystatin treatment induces a reduction of the $Na^+$ concentration up to 103.04 % after three weeks, after six weeks the effect being weakened (107.40 %). In the 5th group, the $Na^+$ concentration increases up to 103.59 % (Fig. 2 B) after three weeks of atherogenic regimen. This increase is reduced almost entirely (101.33 %) after six weeks of medium dose 7-nystatin treatment. In the 6th group, the $Na^+$ concentration increases up to 103.10 % in the initial period (Fig. 2 C) and goes on increasing in the first three weeks of low dose 7-nystatin treatment (107.05 %), returning to the normal value after six weeks of antibiotics administration (101.69 %). In the 7th group, the atherogenic regimen produces a $Na^+$



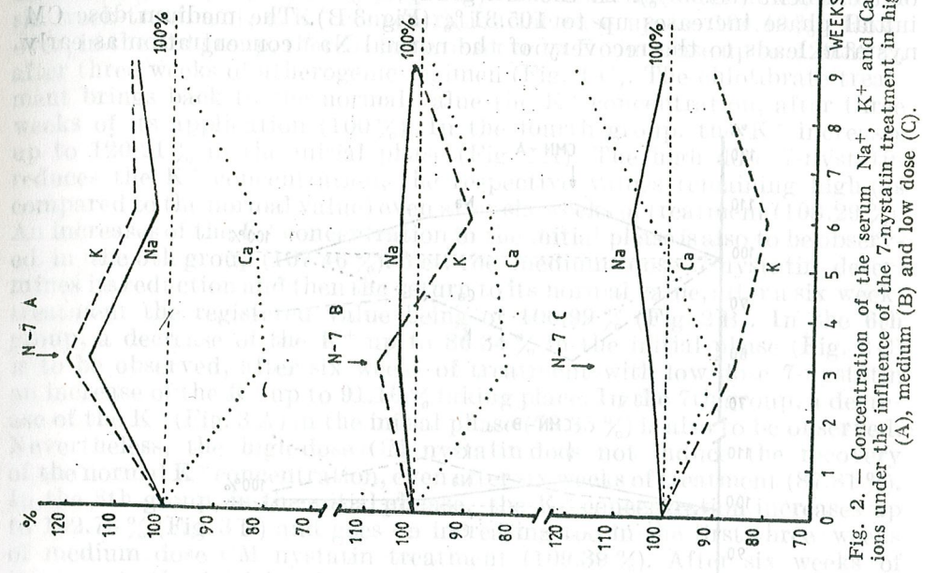


Fig. 2. — Concentration of the serum $Na^+$, $K^+$ and $Ca^{2+}$ ions under the influence of the 7-nystatin treatment in high (A), medium (B) and low dose (C).

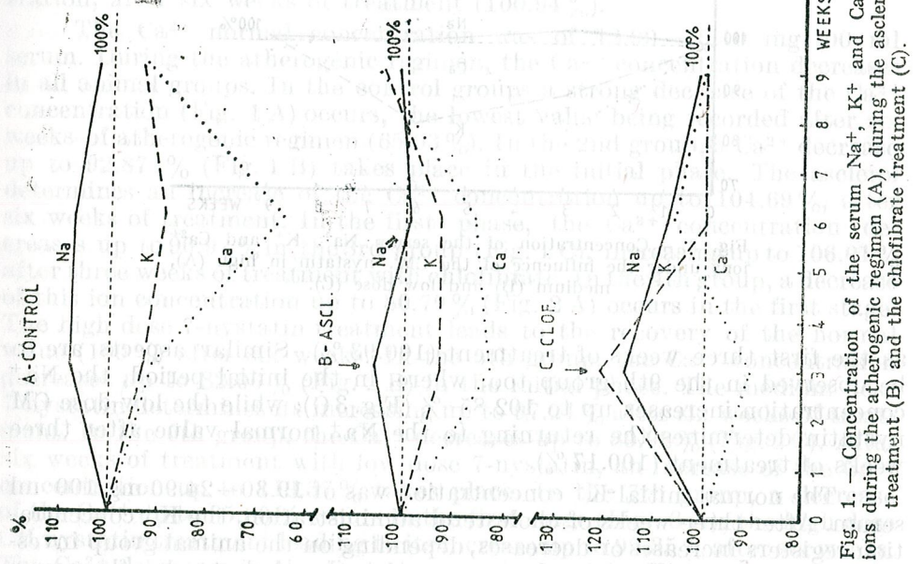


Fig. 1. — Concentration of the serum $Na^+$, $K^+$ and $Ca^{2+}$ ions during the atherogenic regimen (A), during the asclerol treatment (B) and the chlofibrate treatment (C).

increase up to 110.20 % (Fig. 3 A). The high dose CM nystatin determines the return to the normal values of the $Na^+$ concentration, after six weeks of treatment (98.55 %). In the 8th group, the $Na^+$ concentration of the initial phase increases up to 105.31 % (Fig. 3 B). The medium dose CM nystatin leads to the recovery of the normal $Na^+$ concentration as early

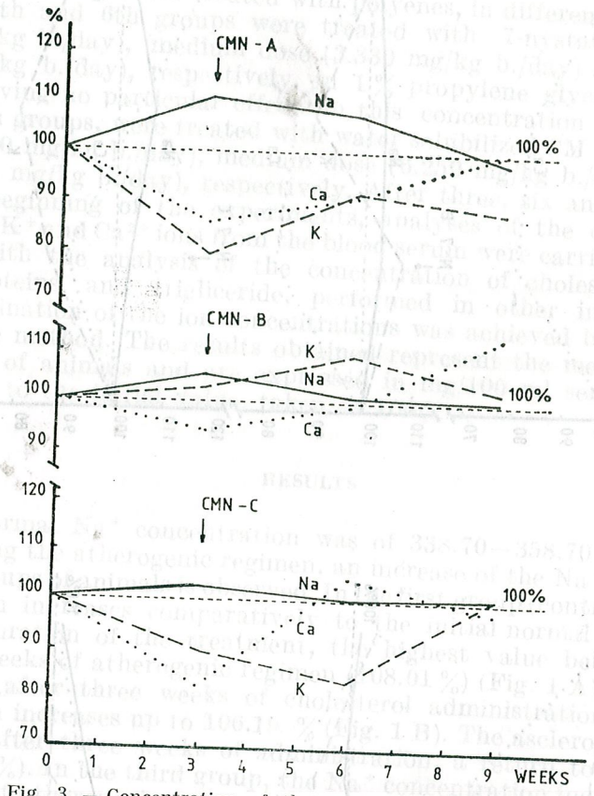


Fig. 3. — Concentration of the serum $Na^+$, $K^+$ and $Ca^{2+}$ ions under the influence of the CM nystatin in high (A), medium (B) and low dose (C).

as the first three weeks of treatment (100.93 %). Similar aspects are to be observed in the 9th group too, where, in the initial period, the $Na^+$ concentration increases up to 102.85 % (Fig. 3 C), while the low dose CM nystatin determines the returning to the $Na^+$ normal value after three weeks of treatment (100.17 %).

The normal initial $K^+$ concentration was of 19.30—24.90 mg/100 ml serum. After three weeks of cholesterol administration, the $K^+$ concentration registers increases or decreases, depending on the animal group investigated. Thus, in the control group, under the cholesterol influence, a decrease of the $K^+$ concentration occurs (Fig. 1 A), the lowest value being



recorded six weeks after the beginning of the treatment (89.40 %). In the 2nd group, in the initial phase there takes place a decrease of the $K^+$ concentration up to 92.95 % (Fig. 1 B). The asclerol determines, after a six week-treatment, a $K^+$ concentration increase up to 104.40 %. However, in the 3rd group, there is to be observed a $K^+$ increase up to 120.39 %, after three weeks of atherogenic regimen (Fig. 1 C). The chlofibrate treatment brings back to the normal value the $K^+$ concentration, after three weeks of its application (100 %). In the fourth group, the $K^+$ increases up to 120.21 % in the initial phase (Fig. 2 A). The high dose 7-nystatin reduces the $K^+$ concentration, the respective values remaining high (as compared to the normal value) even after six weeks of treatment (108.29 %). An increases of the $K^+$ concentration in the initial phase is also to be observed in the 5th group (107.46 %). Yet, the medium dose 7-nystatin determines its reduction and then the return to its normal value, after a six week-treatment the registered value being of 100.99 % (Fig. 2 B). In the 6th group, a decrease of the $K^+$ up to 86.34 % in the initial phase (Fig. 2 C) is to be observed, after six weeks of treatment with low dose 7-nystatin an increase of the $K^+$ up to 91.16 % taking place. In the 7th group, a decrease of the $K^+$ (Fig. 3 A) in the initial phase (79.35 %) is also to be observed. Nevertheless, the high-dose CM nystatin does not induce the recovery of the normal $K^+$ concentration, even after six weeks of treatment (87.81 %). In the 8th group, in the initial phaase, the $K^+$ concentration increases up to 102.76 % (Fig. 3 B) and goes on increasing too in the first three weeks of medium dose CM nystatin treatment (109.39 %). After six weeks of treatment, the initial value is restored (102.76 %). In the 9th group, a decrease of the $K^+$ (90.04 %) in the initial phase is to be observed (Fig. 3 C). The low dose CM nystatin determines a recovery of the normal $K^+$ concentration, after six weeks of treatment (100.94 %).

The $Ca^{2+}$ normal concentration was of 12.90—15.10 mg/100 ml serum. During the atherogenic regimen, the $Ca^{2+}$ concentration decreases in all animal groups. In the control groups a strong decrease of the $Ca^{2+}$ concentration (Fig. 1 A) occurs, the lowest value being recorded after six weeks of atherogenic regimen (65.03 %). In the 2nd group, a $Ca^{2+}$ decrease up to 92.87 % (Fig. 1 B) takes place in the initial phase. The asclerol determines an increase of the $Ca^{2+}$ concentration up to 104.69 %, after six weeks of treatment. In the first phase, the $Ca^{2+}$ concentration decreases up to 90.97 % in the 3rd group (Fig. 1 C), increasing up to 106.01 % after three weeks of treatment with chlofibrate. In the 4th group, a decrease of this ion concentration up to 80.79 % (Fig. 2 A) occurs in the first stage. The high dose 7-nystatin treatment leads to the recovery of the normal value (100 %) after six weeks. In the 5th group, the $Ca^{2+}$ concentration decreases up to 82.06 % (Fig. 2 B) in the initial phase. The medium dose 7-nystatin determines its increasing up to 97.24 % after six weeks of treatment. In the 6th group, the $Ca^{2+}$ decreases up to 89.92 % (Fig. 2 C). After six weeks of treatment with low dose 7-nystatin, an increase of the $Ca^{2+}$ concentration up to 119.37 % takes place. In the 7th group, a decrease of the $Ca^{2+}$ up to 85.80 % occurs in the first stage (Fig. 3 A), the high dose CM nystatin treatment leading to its increase up to 99.35 % after six weeks. The $Ca^{2+}$ decreases in the first stage up to 94.65 % (Fig. 3 B) in the 8th group, but, after three weeks of medium dose CM nystatin treatment,

its increase up to the normal value is observed, reaching a value of 113.74 % after other six weeks. In the 9th group, the $Ca^{2+}$ concentration decreases up to 82.75 % in the first stage (Fig. 3 C). The low dose CM nystatin leads to the increase of the $Ca^{2+}$ concentration up to 104.10 %, after three weeks of treatment.

## DISCUSSIONS AND CONCLUSIONS

It is known that in cardiovascular diseases, there takes place — concomitantly with hypercholesterolemia and hyperlipemia — a series of modifications of the serum ion concentrations [1], [2], [3], [10]. Our results show an increase of the $Na^+$ concentration and a decrease of the $Ca^{2+}$ concentration in all animal groups, as well as increases and decreases of the $K^+$ concentrations characteristic of each group, the decrease of this ion concentration being yet predominant. There has been found a direct correlation between the lack of balance of some organism ions, the increased consumption of NaCl, the quality of the drinking water and the frequency of cardiovascular diseases [4], [6], [8], [9]. Thus, there has been observed that tough water, rich mainly in $Ca^{2+}$, $Mg^{2+}$ and some microelements, has a protection action in cardiovascular diseases, while soft water, lacking these elements, favours the onset of such diseases [4], [6], [8], [9]. At the same time, the importance of some ionic ratios in these affections has been emphasized [6].

Nystatin and other polyenes have important hypocholesterolemiant and hypolipemiant properties [1], [2]. The present paper emphasizes the fact that these agents have positive effects too on the serum ion concentrations, which are modified by cholesterol administration. The normal $Na^+$ concentration can be re-established by the 7-nystatin, especially in medium and low dose, as well as by the CM nystatin— in high dose. In re-establishing the normal $K^+$ concentration, the highest efficiency is met with the medium dose 7-nystatin and low dose CM nystatin. The low dose 7-nystatin treatment, as well as the medium dose CM nystatin one, is the most efficient in the re-establishment of the normal serum $Ca^{2+}$ concentration. The effects of these agent doses are comparable to those of asclerol and chlofibrate. It is worth mentioning that for both polyenes the low and medium doses are generally the most efficient in the re-establishment of the normal serum ion concentration, as it is the case with their hypocholesterolemiant and hypolipemiant action. The most efficient duration of the treatment seems to be of 3 — 6 weeks.

An important effect of these hypocholesterolemiant agents consists in the re-establishment of the normal value of the serum ion ratios, which are modified in atherosclerosis [6]. Positive effects can be observed in connection with the $K^+/Ca^{2+}$ ratio, and especially with the $Na^+/Ca^{2+}$ ratio, which is particularly interesting as regards the implications of the $Na^+$ and $Ca^{2+}$ in cardiovascular diseases [4], [6]. The normal initial value of the $Na^+/Ca^{2+}$ ratio is of 23.13 — 27.44. After three weeks of atherogenic regiment, this value increases up to 27.88—33.38, while after six weeks of treatment with polyenes this value decreases up to 22.95 — 25.67.



In the control group, the values of this ratio remain high over the whole duration of the experiment (26.51 — 39.35, as compared to the initial value of 23.82). One can observe therefore that the modification of the $Na^+/Ca^{2+}$ ratio, alongside with the modification of the serum ion absolute concentration, has a special significance regarding the onset of atherosclerosis phenomena, as well as the efficiency of some agents in their treatment.

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*Biological Research Center*
*Iași, Calea 23 August, 20A*
*Research Center for Antibiotics*
*Iași, Valea Lupului, 1*

# PECULIAR ASPECTS OF THE DOSE: EFFECT RATIO IN THE HYPOCHOLESTEROLEMIANT ACTION OF SOME POLYENES

GABRIELA AGRIGOROAEI, ŞT. AGRIGOROAEI, AL. SAUCIUC, I. NEACŞU, ELENA CHERA, CONSTANŢA BÎRCĂ and GEORGETA NĂNESCU

Hypocholesterolemiant effects of nystatin and CM nystatin administered orally in three different doses, have been investigated as compared with the same effects of the asclerol and chlofibrate. Investigations have been carried on Chinchilla rabbits, fed with a mixed atherogenic and very weak regimen (0.041 g cholesterol/kg b./day in the initial and final phase and 0.082g cholesterol kg b./day in the intermediate one). Treatments have been applied for six weeks, following the preceding installation of a hypercholesterolemia. The investigated polyenes exhibited a higher efficiency than other comparative drugs. The biosynthesis nystatin also proved more efficient than the CM nystatin. Nevertheless, effects of both polyenes decrease with increasing the administered dose.

Hypocholesterolemiant properties of some biosynthesis and semisynthesis polyenes have been already described in previous papers [1—4].

Nystatin and CM nystatin effects have been further investigated, as compared with the effects of some already largely used therapeutical drugs, such as the asclerol and chlofibrate, considered as possessing specific qualities [7], [12].

The investigated antibiotics have been orally administered, each of them in three different doses, in order to follow the variation of the effect with the dose and to get more exact information on the doses to be used in therapeutics. Experiments have been conducted such as to provide estimations referring to the necessary duration of the treatment application, too.

## MATERIAL AND METHODS

The laboratory animals (9 groups of Chinchilla rabbits, with a body weight of abot 3.0 kg) were subjected to a mixed atherogenic and very weak regimen (0.041 g cholesterol/kg b./day in the initial and final stage and 0.082 g cholesterol/kg b./day in the intermediary one), [8], [10], so that the hypocholesterolemia provoked should not have an exagerated value, compared with a natural one.

In the first three weeks of atherogenic regimen, no treatment was applied to the animals. For six subsequent weeks, a group was fed further on only with cholesterol, while the other groups (whose atherogenic regimen was continued) were treated with asclerol (1.53 mg active substance kg b./day), chlofibrate (11.73 mg/kg b./day), nystatin in three different doses (6.66 mg, 3.33 mg and 1.66 mg/kg b./day respectively) or CM nystatin in three different doses (12.50 mg, 6.25 mg and 3.12 mg/kg b./day respectively).

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The total cholesterol, the free cholesterol, as well as that esterified from the animal serum was periodically determined by photocolorimetric methods [9].

## RESULTS

### A. TOTAL SERUM CHOLESTEROL (TC)

The normal values of the total serum cholesterol covered a 54.00—87.50 mg % serum range. After a three week atherogenic regimen, the cholesterol values ranged between 153.00—254.00 mg% serum.

At the end of the following six week period, the total cholesterol registered, at the control group, a variation of + 79.00% as compared to the initial value (Fig. 1); at the groups subjected to different treatments, there were recorded decreases at various increasing degrees of the total serum cholesterol (expressed as percentages to the corresponding

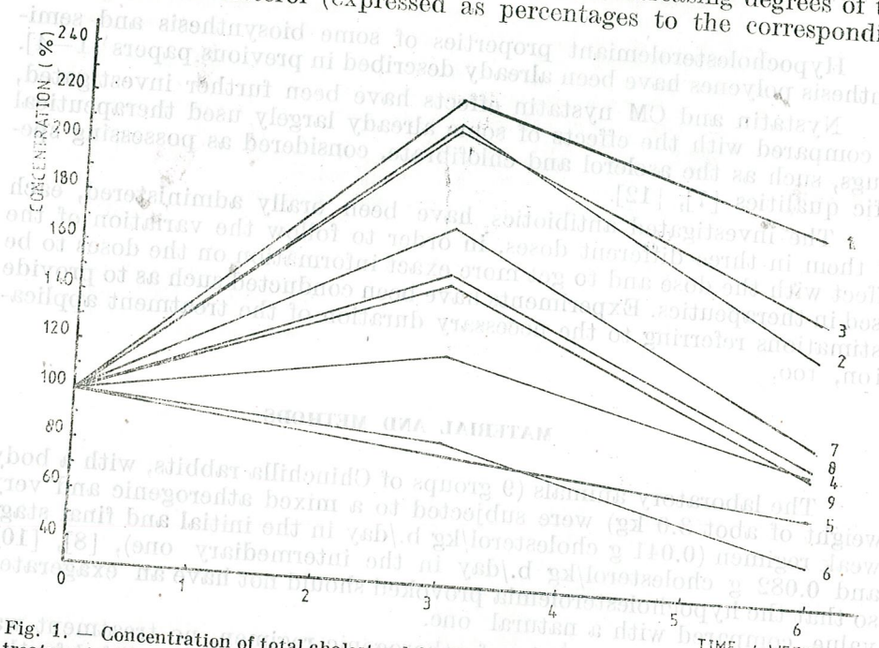


Fig. 1. — Concentration of total cholesterol during the atherogenic regimen (1) and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).

value from the control group), a fact that led to their arrangement as follows : 1. nystatin treatment — low dose (175.68%), 2. nystatin treatment — medium dose (142.98%), 3. CM nystatin treatment — low dose (123.02%), 4. nystatin treatment — high dose (122.02%), 5. CM nystatin treatment — medium dose (117.94%), 6. CM nystatin treatment — high dose (106.21 %), 7. asclerol treatment (61.36%), and 8. chlofibrate treatment (42.93 %).



There is also to be observed that the treatment with biosynthesis nystatin in low and medium doses rapidly reduces the total cholesterol level below its initial value, while the treatment with nystatin in high dose — after a 4 — 5 week period. In order to obtain the same results, in the case of treatments with CM nystatin in different doses, there are necessary longer periods of time — between five and six weeks, while treatments with asclerol and chlofibrate need even a longer time.

### B. FREE SERUM CHOLESTEROL (FC)

Starting from the free cholesterol normal values, ranging between 15.80 — 39.20 mg % serum, there were reached, after a three week atherogenic regimen values between 60.40 — 94.40 mg % serum.

After a six week treatment, at the end of which at the control group there can be reached a 60.07% increase of the free cholesterol — as compared to the initial value (Fig. 2) the groups arrange themselves, according to the efficiency of the treatment in the reduction of the free cholesterol, as follows : 1. nystatin treatment — low dose (186.68%), 2. nystatin treat-

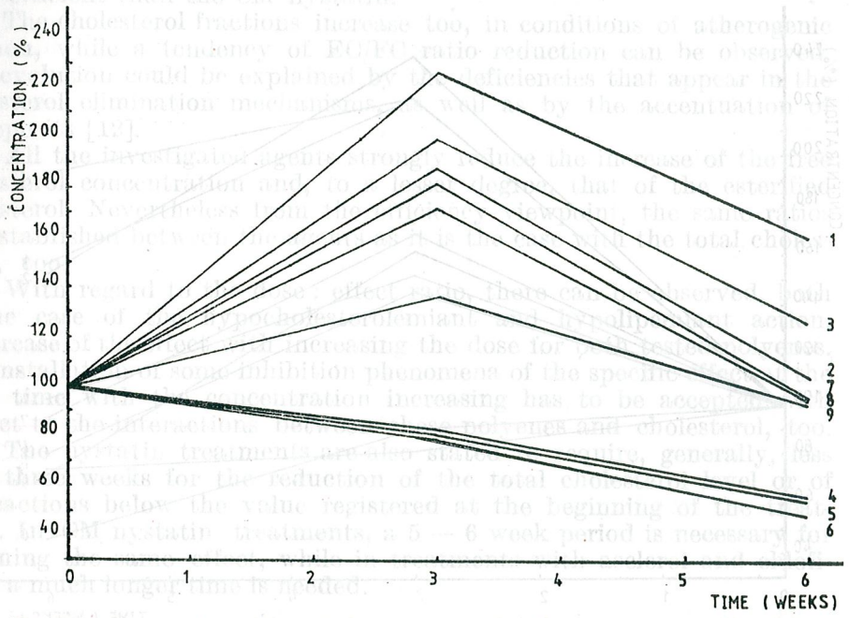


Fig. 2. — Concentration of free cholesterol during the atherogenic regimen (1) and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).

ment — medium dose (175.57%), 3. nystatin treatment — high dose (172.99%), 4. CM nystatin treatment — low dose (110.38%), 5. CM nystatin treatment — medium dose (107.70%), 6. CM nystatin treatment — — high dose (106.62%), 7. asclerol treatment (94.20%) and 8. chlofibrate treatment (57.02%).

In all concentrations used, the biosynthesis nystatin reduces very soon (starting from the first week) the level of the free cholesterol below the value at the beginning of the treatment, while the CM nystatin requires almost six weeks for lowering it slightly below the starting level. Quite a longer time is necessary in the asclerol treatment, and a much longer one in that with chlofibrate.

### C. ESTERIFIED CHOLESTEROL (EC)

The esterified cholesterol showed normal values, ranging between 35.20 — 58.70 mg% serum and—after a three week atherogenic regimen —values between 92.60 — 163.20 mg % serum.

During the following six weeks, at the control group the esterified cholesterol increased with 93.80% compared to the initial value. The depression of the esterified cholesterol increasing, recorded at the other groups arranges them, according to the treatment efficiency, in the following way : 1. nystatin treatment — low dose (156.75%), 2. CM nystatin treatment — low dose (129.28%), 3. nystatin treatment — medium dose (126.14 %), 4. CM nystatin treatment— medium dose (120.77%),

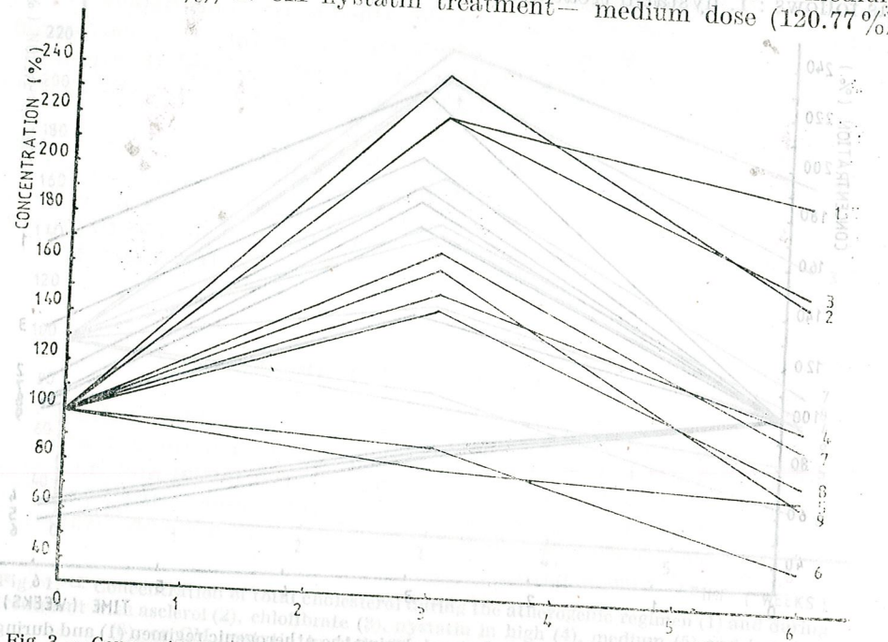


Fig. 3.— Conccentration of esterified cholesterol during the atherogenic regimen (1) and during treatment with asclerol (2), chlofibrate (3), nystatin in high (4), medium (5) and low dose (6), or CM nystatin in high (7), medium (8) and low dose (9).

5. CM nystatin treatment — high dose (105.69%), 6. nystatin treatment — high dose (95.72%), 7. asclerol treatment — (43.71%) and 8. chlofibrate (38.53%) (Fig. 3).

Only in the case of the biosynthesis nystatin, in low and medium dose there can be observed a very rapid decrease of the esterified cholesterol level, below that at the beginning of the treatment (starting even with the first week). In the case of CM nystatin, for all the three concentrations, there are necessary between five and six weeks for obtaining the same effect. Considerably longer periods of time are necessary in the case of asclerol or chlofibrate treatments.



## DISCUSSIONS AND CONCLUSIONS

Experiments made showed above all that, in conditions of atherogenic regimen, the increase of the total serum cholesterol is relatively much stronger than that of the total lipids. This fact proves a marked lack of balance between the cholesterol and the other lipidic fractions from blood, especially the cholesterol/phospholipids ratio being altered [10], [11].

As regards the reduction capacity of the total serum cholesterol level, the investigated polyenes are generally much more efficient than the asclerol and chlofibrate, the biosynthesis nystatin being — in its turn — more efficient than the CM nystatin.

The cholesterol fractions increase too, in conditions of atherogenic regimen, while a tendency of EC/FC ratio reduction can be observed. This evolution could be explained by the deficiencies that appear in the cholesterol elimination mechanisms, as well as by the accentuation of its deposits [12].

All the investigated agents strongly reduce the increase of the free cholesterol concentration and, to a lesser degree, that of the esterified cholesterol. Nevertheless from the efficiency viewpoint, the same ratios are established between the agents as it is the case with the total cholesterol, too.

With regard to the dose : effect ratio, there can be observed, both in the case of the hypocholesterolemiant and hypolipemiant action, a decrease of the effect with increasing the dose for both tested polyenes. The installation of some inhibition phenomena of the specific effect at the same time with the concentration increasing has to be accepted with respect to the interactions between these polyenes and cholesterol, too.

The nystatin treatments are also stated to require, generally, less than three weeks for the reduction of the total cholesterol level or of its reactions below the value registered at the beginning of the treatment. In CM nystatin treatments, a 5 — 6 week period is necessary for obtaining the same effect, while in treatments with asclerol and chlofibrate a much longer time is needed.

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*Biological Research Center*
*Iaşi, Calea 23 August, 20A*
and
*Research Center for Antibiotics*
*Iaşi, Valea Lupului, 1*

# AGE-DEPENDENT EFFECT OF CALCIUM CHLORIDE ON INTRAVENOUS GLUCOSE TOLERANCE IN WHITE RATS

IOSIF MADAR and NINA ŞILDAN

The dynamics of rapid intravenous glucose tolerance in 30-, 65-, 120-day-old and more than one-year-old male albino Wistar rats was studied under basal conditions as well as against the background of $CaCl_2$ administration (5 mg/100 g b.w., i.v.). In the case of immature and mature young rats (30-and 65-day-old) calcium chloride enhanced the glucose assimilation, while in adult (120-day-old) and old (more than one-year) animals it did not affect this phenomenon.

We demonstrated elsewhere that in white rats there is a direct relationship between intravenous glucose tolerance and hyperglycemia induced insulin release during postnatal ontogeny [7]. Extensive *in vivo* studies have demonstrated that calcium ion is an essential requirement for glucose stimulated insulin secretion after either oral or intravenous glucose loading [1], [5], [6], [12], [14], [15]. However, only a few studies have focused on the relation between serum calcium concentration and *in vivo* glucose assimilation, while the ontogenetic aspect of this relation is yet unknown.

In the present study we examined the influence of experimentally elevated blood calcium level on the intravenous glucose tolerance, as depending on the age of white rats.

## MATERIALS AND METHODS

Throughout the experiments 30-, 65-, 120-day-old and more than one-year-old male albino Wistar rats from the stockfarm of our laboratory were used. They were kept at a constant room temperature of about 22°C and maintained on a dry Larsen diet. Before experiments the rats were fasted 18 hours, drinking water being provided *ad libitum*.

Intravenous glucose tolerance was followed under sodium pentobarbital anaesthesia (5 mg/100 g b.w., intraperitoneally). After taking blood samples from the tail for determnation of initial glycemia level, two consecutive glucose loadings were carried out in each rat. An interval of 24 hours was allowed to elapse between two glucose loadings. The first one was carried out in basal state (glucose alone), and the second one in hypercalcemic state (calcium chlorite plus glucose). For this purpose 20% gluocse solution and 2% $CaCl_2$-containing 20% glucose solutions were used. The solutions were injected through one of the tail veins according to our technique, [8—10] glucose dose being 50 mg and that of $CaCl_2$ 5 mg/100g b.w., respectively.

After glucose or calcium-glucose loadings blood samples were taken at 5-minute intervals from the tail blood vessels during a period of 25 minutes. Blood glucose levels were estimated enzymatically according to

the method of Werner et al. [13], by using GOD-Perid Test-Combination-Glucose Kit ("Boehringer", GmbH, Mannheim, Germany).

Glucose tolerance was evaluated by calculating the assimilation coefficient of glucose (K) on the basis of semilogarithmic representation of hyperglycemia curves, using the formula of Conard et al. [4] and the procedure of Christophe [2] and Madar et al. [10], [11].

The effect of elevated blood calcium level upon the rate of excess blood glucose removal was evaluated by comparing the values of assimilation coefficients obtained in basal state ($K_1$) and against the background of calcium chloride administration ($K_2$).

The results were tested for statistical homogeneity of the means, applying the criterion of Chauvenet. The mean values of $K_1$ and $K_2$ in the same age-groups were compared according to Student's $t$ test, the limit of significance being accepted at $P = 0.05$.

## RESULTS AND DISCUSSIONS

It has been established that successive intravenous glucose tolerance values are stable in a given white rat [3] and that repeated intravenous glucose tolerance test in this species is reliable for the study of rapid-acting substances on *in vivo* glucose assimilation [8], [9], [11].

One can see from our observations (Table 1) that $CaCl_2$ injected simultaneously with glucose, in the case of 30- and 65-day-old animals

*Table 1*

Glucose assimilation coefficients in white rats of various ages, in basal state ($K_1$) and against the background of $CaCl_2$ administration ($K_2$)

| Age | $K_1$ | $K_2$ | % increase in $K_2$* | P |
|---|---|---|---|---|
| 30 days | 2.48±0.23 (18) | 3.25±0.30 | 31.05 | <0.05 |
| 65 days | 2.91±0.17 (9) | 3.66±0.20 | 25.17 | <0.02 |
| 120 days | 3.52±0.16 (10) | 3.58±0.25 | 1.70 | >0.50 |
| 365 days | 2.76±0.21 (8) | 2.88±0.24 | 4.35 | >0.50 |

Results are expressed as means ± S.E. The number of experiments is given in parentheses. *Percentage increase in $K_2$, as compared to $K_1$.

enhanced markedly the rate of glucose penetration from the blood into the tissues. The values of glucose assimilation coefficients against the background of $CaCl_2$ administration ($K_2$) in these age-groups were increased by 31.0% ($P < 0.05$) and 25.1% ($P < 0.02$), respectively, as compared



to those found in basal state ($K_1$). On the contrary, in 120-day and more than one-year-old animals $CaCl_2$ does not influence appreciably the rate of excess blood glucose removal (+1.7% and +4.3%, respectively; $P > 0.50$). These data suggest the possibility that the age of white rats is an important conditioning factor in the stimulatory effect of experimentally elevated calcemia on *in vivo* glucose assimilation.

Taking into consideration that in white rats the rate of glucose assimilation during intravenous glucose tolerance test is strongly dependent on the degree of hyperglycemia induced insulin release [7], one may assume an age-related potentiation by the elevated blood calcium level of the insulinogenic response to hyperglycemia. In fact, Pento et al. [12] have pointed out that in white rats hypercalcemia stimulated the *in vivo* insulin secretion to hyperglycemia stimulus. On the other hand, Yasuda et al. [14] have observed that insulin responses after oral glucose loading in primary hyperparathyroidism induced hypercalcemia were increased, and those in idiopathic hypoparathyroidism induced hypocalcemia were reduced significantly, as compared to normal subjects.

In conclusion, in immature and mature male albino young rats, calcium chloride injected concomitantly with glucose stimulates the rate of glucose assimilation during intravenous glucose tolerance test, while in adult and old rats it does not affect this phenomenon.

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*Biological Research Center*
*Cluj-Napoca, Clinicilor 5 — 7*

# NEW EXPERIMENTAL DATA ON THE CHARACTERIZATION OF THE BIOSYNTHESIS ANTIBIOTIC PREPARATION A 37.4 AS AN ACTIVE CANCEROSTATIC AGENT

P. ROTINBERG, SMARANDA KELEMEN and AL. SAUCIUC*

The existence of a dose-response relationship was established by *in vivo* testing on rats bearing ascitic tumor of the antitumoral activity of the different doses of antibiotic A 37.4, which was used in a combined therapy together with the polyene NsMC $L_2$ 1980.

The comparative following of the antitumoral activity induced by A 37.4 and Girostan IOB, respectively, revealed an enhanced therapeutic efficiency of the metabolite A 37.4.

These results complete the preclinical experimental evidences which recommend the new antibiotic of biosynthesis A 37.4 as an active cancerostatic agent, imposing at the same time its inclusion in the clinical screening.

The discovery of a new antitumoral drug and its use in the human antineoplastic chemotherapy is the result of a complex investigation. The chemotherapeutic screening programs devoted to this purpose, require an initial laboratory preclinical stage devised to identify new cancerostatic agents. Their pharmacotherapeutic significance will be appreciated by the clinical trial [1 — 3], [7 — 9].

In previous papers we reported the cytostatic effect of a new biosynthesis antibiotic preparation A 37.4 L I 1981, on HeLa cell cultures as well as its antitumoral activity on rats bearing various tumoral lines [5], [6].

The characterization of a new drug as an antitumoral agent — the final purpose of a preclinical chemotherapeutic screening program — is based not only on the evidence of its pharmacotherapeutical effects on experimental models, but requires additional proofs regarding the existence of a dose-response relationship and the comparison of the induced specific action with that of a standard agent [2], [3].

In the present investigation the results obtained by *in vivo* testing of the different doses of the antibiotic preparation A 37.4, used in a combined therapy with chemically modified nystatin $L_2$ 1980 (NsMC) on rats bearing ascitic tumor are exposed. At the same time a comparison between the cancerostatic efficiency of A 37.4 and that of the antitumoral agent Girostan IOB used in the human antineoplastic chemotherapy is made.

## MATERIAL AND METHODS

White Wistar femals rats, weighing 150 g, bearing of T—8 Guérin lymphotropic epithelioma, ascitic form, realized at the Oncologic Institute [4], were used as experimental animals.

The intraperitoneal treatment started 24 hours after the i.p. inoculation of $1 \times 10^5$ ascites cells and lasted until the death of the last control animal.

It should be mentioned that the combined treatment was made with a new A 37.4 preparation, namely L II 1982, in contrast to previous experiments when L I 1981 was used [5], [6]. This preparation seemed to be more cytotoxic and consequently the therapeutic program was modified, i.e. the interval between two drug administrations was extended to 8 days and the daily dose of NsMC $L_2$ 1980 was increased to 500mg/Kg b.w. in order to ensure the protective effect [5], [6].

Testing of antitumoral activity of certain different doses of A 37.4 (i.e. 0.0375, 0.050, 0.075 and 0.1 mg/kg b.w.) was made by its therapeutic association with NsMc $L_2$'80 (500 mg/kg b.w.).

This combined treatment started 24 hours after transplant by daily injection of NsMC, the antibiotic A 37.4 being administrated in the doses shown above at each 3 days after 48 hours from the transplant.

The effect of Girostan IOB on tumour development was followed in conditions of its daily administration in two different doses (i.e., 0.6 and 1.0 mg/kg b.w.).

The estimation of the antitumor activity was based on the following up of the mean survival time (MST) in the treated groups comparatively to the control.

The evaluation of antitumoral action was made by the increase of the MST and by the calculation of the statistic significance and the T/C value (where T = mean survival time for the treated group and C = = mean survival time for the control).

## RESULTS

The antitumoral activity induced by different doses of A 37.4 LII'82, used in the combined treatment with NsMC $L_2$'80, is given in table 1.

The group to which the antibiotic A 37.4 was administered in dose of 0.0375 mg/Kg b.w. exhibited a significant increase of the MST ($p < 0.02$) as compared to control animals. The induced antitumoral activity is illustrated by a 34.9 % prolongation of MST and by a T/C value of 1.35.

*Table 1*

Antitumor activity of different doses of antibiotic preparation A 37.4 L II 1982, administered in association with NsMC 1980 (500 mg/Kg. b.w./i.p./daily), on ascitic Guérin T—8 tumor.

Figures in brackets indicate the number of animals.

| Group/Treatment | MST (days) | % increase MST | Significance | T/C value | % tumoral undevelopment |
|---|---|---|---|---|---|
| CONTROL | 21.2±1.2(13) | — | — | — | — |
| A 37.4(0.035mg)+NsMC | 28.6±2.4 (9) | 34.9 | $p<0.02$ | 1.35 | — |
| A 37.4(0.050mg)+NsMc | 37.2±6.7 (6) | 75.5 | $p<0.05$ | 1.75 | 33.3 |
| A 37.4(0.075mg)+NsMC | 43.3±2.2 (6) | 104.2 | $p<0.001$ | 2.04 | 33.3 |
| A 37.4(0.1mg)+NsMc | 22.7±3.4 (7) | 7.1 | N.S. | 1.07 | 22.2 |



When the dose was increased to 0.050 mg/Kg b.w., the MST increase was 75.5 % with an associated T/C value of 1.75. Three cases of tumor undevelopment were also registered.

The maximum antitumoral activity was noticed when the antibiotic dose was of 0.075 mg/kg b.w. In comparison with the control animals, the treated group exhibited a significantly increased MST ($p < 0.001$), characterized by a prolongation of 104.2 % and a T/C value of 2.04. Again, the ascitic tumor did not developed in 3 animals.

When the maximum tested dose was administered (i.e. 0.1 mg/kg b.w.), the MST did not show a significant prolongation (only 7.1 %), but in two animals the tumor did not develop. It should be mentioned that even if the antitumoral activity seems to be minimum, 3 out of 7 animals considered in calculation, with a low MST, did not exhibit when died the abdominal hypertrophy characteristic of the ascitic tumor development.

The comparison of the antitumoral activity of the secondary metabolite A 37.4 L II'82 with that of Girostan IOB — cytostatic chosen as standard agent — required the testing of the latter in laboratory conditions.

The values of evaluation indices of antitumoral activity are listed in table 2.

*Table 2*

Antitumor activity of different doses of Girostan IOB on ascitic Guérin T—8 tumor. Figures in brackets indicate the number of animals.

| Group/Treatment | MST (days) | % increase MST | Significance | T/C value |
|---|---|---|---|---|
| CONTROL | 21.2±1.2(14) | — | — | |
| Girostan (0.6mg/kg.bw) | 23.5±0.45(10) | 10.8 | N.S. | 1.11 |
| Girostan (1.0mg/kg.bw) | 24.2±1.3(10) | 14.1 | N.S. | 1.14 |

It can be seen that — in comparison with the control group — the daily administration of the Girostan IOB in two progressively increased doses does not determine a significant increase of the MST. The values of the prolongation of MST (i.e. 10.8 % and 14.1 %) and T/C ratio (i.e. 1.11 and 1.14) reflect, at the administrated doses, a moderate antitumoral effect of the drug on this tumoral line.

## DISCUSSION

Since a new antibiotic preparation A 37.4 L II'82, was used in the present investigation, it should be mentioned that the testing made in 3 consecutive stages on different tumoral lines of the combined treatment with A 37.4 L II'82 (i.e. 0.075 mg/Kg b.w. /3 days/i.p.) and NsMC $L_2$'80 (500 mg/Kg/daily/i.p.) has confirmed the antitumoral potential of this secondary metabolite of biosynthesis (unpublished data). Thus, on rats bearing Guérin T-8 lymphotropic epithelioma, the solid subcutaneous

form, the induced antitumoral activity is shown by a significant mean tumoral regression ($p < 0.02$) of 41 % correlated with a T/C value of 0.59. In the case of ascitic form of the Guérin T-8 tumor, a significant prolongation ($p < 0.001$) of MST of 75.5 % and a T/C value of 1.75 were recorded, together with 40 % of tumoral undevelopments. The antitumoral activity, observed also on rats bearing the subcutaneous, solid from of Walker 256 carcinosarcoma, was supported by a significant mean tumoral regression ($p < 0.05$) of 45 % and a T/C value of 0.55.

These some few data show the reproducibility of the antitumoral activity characterizing the biologically active metabolite A 37.4. However, it should be emphasized that it seems that there is a difference regarding the cytotoxicity of those two preparations [5], [6]. This observation is based on the fact that the exhibition of the specific antitumoral action is conditioned by the diminution of the total dose of the preparation A 37.4 L II'82 used in treatment by the increase of the drug administration interval from 2 to 3 days, which is correlated with the augmentation of the daily dose of NsMC $L_2$'80 (the association agent which ensures the protection of normal cells against the cytotoxicity of A 37.4) from 300 to 500 mg/Kg b.w. At the same time, the increased intensity of the antitumoral effect induced by A 37.4 L II'82 on the ascitic tumoral system at a dose of 0.075 mg/kg b.w., as compared with that induced by preparation A 37.4 L I'81 (30 %), supports this appreciation.

Despite the differences observed between those two preparations A 37.4, the reproducibility of the antitumoral effect imposed the extension of investigation in order to get additional data required by the final preclinical characterization of the antibiotic preparation A 37.4 as a cancerostatic agent.

The chemotherapeutic programs of preclinical screening, elaborated by the Institute of Microbiology and Experimental Therapy from G.D.R. and the Cancer Chemotherapy National Service Center from U.S.A., in order to select new active antitumoral agents, require additional investigation stages, besides the determination of pharmacotherapeutical effect on experimental models. Among other things, these steps should give a positive answer to two experimental aspects related to the existence of a dose-response relationship at least on a tumoral system and to a comparison between the antitumoral action of the investigated drug and that of a standard agent [1 — 3].

These two last experimental aspects formed the objective of the present study, carried out in laboratory conditions using the antibiotic preparation A 37.4 L II'82.

By modifying the dose of A 37.4, used in the combined therapy of the ascitic tumor, the dependence of the antitumoral effect upon the drug dose has been followed, in the conditions of a daily administration of the same dose of NsMC $L_2$'80 (i.e. 500 mg /kg b.w.).

The analysis of the results registered in these experimental conditions demonstrated the existence of a relationship between the used therapeutic dose and the intensity of the induced cancerostatic effect.

Thus, the augmentation of the A 37.4 dose from 0.035 to 0.050 mg/kg b.w. is correlated with an intensification of the antitumoral action



of 40.6 %, the MST being at the same time twice that corresponding to the minimal dose used.

The increase of the drug dose from 0.050 to 0.075 mg/kg b.w., the usual dose in our tests, has shown a potentiation of the antitumoral effect of 28.7 %, corresponding to a MST 1.4 higher than that induced by a dose of 0.050 mg/kg b.w.

This dose-effect relationship has, however, a limited character since an administration of 0.1 mg/kg b.w. did not result in a significant increase of the antitumoral effect, even if in two animals the tumor did not develop.

The explanation of this phenomenon should have an answer in the intensification of secondary effects, related to the toxicity of the antibiotic preparation A 37.4, as the drug dose increases. In the conditions in which the drug toxicity was not sufficiently balanced by the protective effect induced by NsMC $L_2$'80 (the agent of therapeutic association), the possibility of manifestation of the antitumoral activity specific to the antibiotic preparation A 37.4 decreased.

The testing of the effect of different doses of A 37.4 L II'82, in the conditions of the combined treatment, confirmed the antitumoral potential which characterizes this secondary metabolite. The values of indices for the evaluation of the cancerostatic action induced by the different doses of the drug A 37.4, on the ascitic tumoral system, are higher than that imposed by the screening programs from G.D.R. and U.S.A. [2], [3], according to which an antitumoral agent has to determine a prolongation of MST of 30—50 % or a minimal T/C value of 1.25.

In order to compare this significant antitumoral action of the biologically active metabolite A 37.4 L II'82 with that of a standard agent, the antitumoral effect of different doses of Girostan IOB (used comparatively as a cytostatic) was followed. A moderate antitumoral action was observed in these conditions, related perhaps to low doses used daily (0.6 and 1.0 mg/kg b.w.).

A comparative analysis of antitumoral activities induced by the antibiotic preparation A 37.4 LII'82 and Girostan IOB showed that the therapeutic efficiency of the combined treatment of A 37.4 and NsMC is superior to that of Girostan IOB, even if the used doses of A 37.4 were lower than that of Girostan IOB. The comparison appears to be more favourable if it is reminded that the efficiency of the combined treatment is also assured by the undevelopment tumor cases.

The results of this investigation give a positive answer to these questions imposed by a preclinical screening program elaborated for selection of new active cancerostatic agents.

The evidence of pharmacotherapeutical antitumoral effects, the existence of a dose-effect relationship and the increased cancerostatic potential as compared to that of Girostan IOB, in the condition of preclinical screening, allow us to appreciate the secondary metabolite A 37.4 as an active cancerostatic agent and impose its clinical investigation in order to determine its practical importance in the human antineoplastic chemotherapy.

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*Biological Research Center*
*Iaşi, Calea 23 August, 20A*
**Center for Antibiotic Research*
*Valea Lupului-Iaşi*

# DIE VARIATION DER SERISCHEN PHOSPHOLIPIDE UNTER DEM EINFLUß DES ELEKTROMAGNETISCHEN FELDES BEI DEN RATTEN

G. DIMITRIU

Le champ électromagnétique détermine la variation des phospholipides du sérum des rats. Les phospholipides sériques augmentent de +21,8% jusqu'à + 31,8%, suivant le nombre des séances de traitement de 5 à 10 jours.
L'augmentation est considérée comme un effet biostimulateur, produit sous l'influence du champ électromagnétique. C'est parce que les phospholipides ont des multiples implications fonctionnelles et, à la fois, au niveau des membranes et de la perméabilité cellulaire.

Die Bedeutung, die der biostimulatorischen Aktion der physikalischen chemischen oder biologischen Faktoren beigemessen wird, und zwar in unterschiedlichem Regim, ist heute allbekannt. Ebenfalls bekannt, aufgrund von vorherigen Erfahrungen [4], [5], [6], ist die Tatsache, daß bestimmte Formen des elektromagnetischen Feldes einen stimulatorischen Einfluß auf den lebenden Organismus ausüben.

In dieser Hinsicht waren wir der Meinung, daß zwecks Erklärung des den biostimulatorischen Effekt begleitenden Mechanismus nicht ohne Bedeutung ist, die serischen Phospholipide unter dem Einfluß des elektromagnetischen Feldes zu erforschen, da allgemein bekannt ist, daß zu den zahlreichen Einflüssen der Phospholipide auch die Förderung der Verdauungsprozesse, die Resorbierung der Fette durch die Schleimhaut des Darmes, die Beförderung der Lipide im Organismus dank des hohen Gehaltes an Fettsäuren, sowie das Eindringen in die Zelle und das Verlassen derselben zählen. Außerdem sind die Phospholipide wasserlöslich und demnach saugen sie sich voll Wasser an und gewähren freien Durchgang verschiedenen wasserlöslichen Substanzen und fördern letzten Endes die Permeabilität.

Die Permeabilität der Zelle wird ebenfalls durch ihre elektrische Ladung gefördert. Durch eine innere Reaktion, an der das Oxydril der Phosphorsäure teilnimmt, entsteht ein inneres Salz, eine Reaktion also, die eine Neuorientierung der elektrischen Ladungen im Molekül bewirkt, so daß ein Dipol geschaffen wird. Dieses wiederum erfährt auch eine Neuorientierung auf dem Niveau der Trennungsflächen und zwar werden die negativen Ladungen näher an das lipide Medium gebracht und die positiven an das wässerige Medium, so daß es zu einem Potentialunterschied kommt.

All das sind Einflüsse — dazu gehören auch jene auf dem Gebiet der Enzime — die zu komplexen physiologischen Prozessen führen, welche ihrerseits auf den Metabolismus einwirken und letzten Endes zu einer besseren Verwertung der Nahrung beitragen (1).

In unseren Versuchen haben wir die Phospholipide im Blutserum der sich im elektromagnetischen Feld befindenden Ratten dosiert.



—c. 1512

Als Einwirkungsfaktor wurde ein schwaches elektromagnetisches Feld gewählt — bis zu 180 Oe —, das von einem "Magnetodiaflux" erzeugt wurde (5). Das Feld wurde täglich 5 Minuten lang eingesetzt, und zwar in unterbrochener Reihenfolge —3 Sekunden Behandlung und 1 Sekunde Pause — das ganze auf die Dauer von 5 bzw. 10 Tagen.

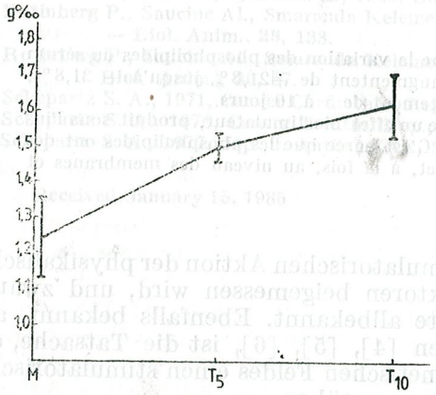


Abb.1. — Die quantitative Variation der Phospholipide ($g^0/_{00}$) im Blutserum bei elektromagnetisch behandelten Ratten.

Eine Stunde nach der letzten Behandlung wurden die Tiere geköpft und das Blut einer Zentrifuge unterworfen.

Die Phospholipide wurden laut Gomori-Methode bestimmt (2). Die Variationsreihen wurden statistisch, berechnet, auf klassischem Wege, und die statistische Bedeutung durch den "t"-Test (Student) bewertet.

Die Werte, die bei den Kontrolltieren bestimmt wurden, lagen innerhalb normaler Grenzen, also 1,238 g $^0/_{00}$.

Nach 5 Tagen elektromagnetischer Behandlung wuchsen die serischen Phospholipide um + 21,8 % (Tabelle 1 und Abbildung 1). Das Verlängern der Behandlung auf 10 Tage bewirkte auch ein stärkeres Anwachsen, bis zu + 31,84 %, ein Wert, der für die Statistik von Bedeutung ist ($\alpha < 5$).

*Tabelle 1*

Die quantitative Variation der Phospholipide (g $^0/_{00}$) im Blutserum bei elektromagnetisch behandelten Ratten

| | Kontrolle | Nach 5 Tagen Behandlung | Nach 10 Tagen Behandlung |
|---|---|---|---|
| $\bar{x} \pm ES$ | 1,238±0,114 | 1,499±0,037 | 1,624±0,089 |
| D± % | — | +21,8 | +31,17 |
| $\alpha$ | — | >5 | <5 |
| CV % | 22,53 | 6,04 | 13,84 |

Den biostimulatorischen Einfluß einiger Antibiotika verfolgend haben wir in einer früheren Arbeit [3] darauf hingewiesen, daß der tägliche Gewichtszuwachs von einem Anwachsen der serischen Phospholipide begleitet wird. Auch umgekehrt: eine Verzögerung im Wachsen bringt mit sich auch ein starkes Herabsinken der serischen Phospholipide.



Wenn man diese Daten mit den unter dem Einfluß des elektromagnetischen Feldes entstandenen Bewertungen im Zusammenhang betrachtet, ist es möglich, daß der biostimulatorische Effekt einiger von Natur aus sehr verschiedener exogener Faktoren, von der Verstärkung der Biosynthese der Phospholipide abhängt, und zwar bestimmt durch die Änderung ihres Gleichegewichts in den weiter oben besprochenen physiologischen Prozessen, und in erster Linie durch die Änderung des Gleichgewichts der Phospholipide in der Struktur der Zellhaut.

Wir schlußfolgern demnach, daß die quantitativ positive Variation der serischen Phospholipide zu den stimulatorischen Reaktionen im allgemeinen gehört, welche durch das elektromagnetische Feld in bestimmten Grenzen erzeugt den lebenden Organismus beeinflussen.

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*„Al. I. Cuza"-Universität*
*Labor für Biologie der Zelle*
*Iași, Calea 23 August 20A*

# THE EFFECTS OF OLTITOX ON THE HYPOTHALAMO-PITUITARY-ADRENAL SYSTEM ACTIVITY

V. HEFCO

Oltitox, a pesticide from the carbamates group, once administered intragastrically in doses of 1/3 $LD_{50}$, 2/3 $LD_{50}$ or $LD_{50}$, induced a hyperactivation of the hypothalamo-pituitary-adrenal system activity, recorded 24 hours after administration. There followed a decrease in activity 3 days later, after which the activity returned to normal. The effect produced in the adrenal activity was dose-dependent. The weight of the adrenals, anterior pituitary and neural lobe glands first increased, and then one can notice a tendency to revert to the normal level with time. On the basis of lethal dose value, this pesticide can be introduced in group IV of substances with low toxicity.

From a physiological point of view, each stress factor activates the hypothalamo-pituitary-adrenal system. This system represents a part of the defense mechanism and one of the corollaries of the stress response. The initial phase is the arousal and activation of the ergotropic posterior part of the hypothalamus, resulting in a number of autonomic reactions, e.g., increased heart rate, muscle vasodilatation, adrenomedullary secretion, glucose and fat mobilization, pupilary dilatation, constriction of the capillary bed of the skin, piloerection of the anterior hypothalamic area (causing the release of vasopressin and ACTH), the production of protective glucocorticoids, retention of water and salt, and a number of parasympathetic reactions. This chain of events can be considered as the basic pattern of the integrated response to stress [1].

In the present experiments, we studied the effects of different doses and different intervals after administration of a single dose of oltitox, one pesticide from the carbamates group, on the hypothalamo-pituitary-adrenal system activity.

## MATERIAL AND METHODS

Experiments were carried out on male Wistar rats, weighing about 200 g at the beginning of experiments.

Oltitox, suspended in carboxymethylcellulose 5‰, was administered intragastrically in doses of 1/3 $LD_{50}$; 2/3 $LD_{50}$ or $LD_{50}$, and the manifest changes were recorded at 24 hours, 3 days, 7 days and 12 days after pesticide administration.

Adrenal corticosteroid production in vitro was determined by the method of Van Der Vies et al. [7]. Total corticosteroid production in vitro expressed in μg/100 mg adrenal/hour served as the index of pituitary ACTH release [6].

The weight of glands was measured on torsion balance with the accuracy of 0.01 mg.

The results were statistically analysed, by means of Student's t — test.

REV. ROUM. BIOL. — BIOL. ANIM., TOME 30, Nº 2, P. 129—132, BUCAREST, 1985

## RESULTS

Experimental data are presented in Table 1.

*Hypothalamo-pituitary-adrenal activity*. 24 hours after oltitox administration in doses of 1/3 $LD_{50}$, adrenal gland activity undergoes significant increase. The same dose, 3 days after administration, evinces a significant decrease in adrenal hormone production, afterwards the activity reverts to normal, the variations registered at 7 and 12 days after pesticide administration were not significant as compared to the control group.

*Table 1*

Changes of the hypothalamo-pituitary-adrenal system activity after administration of a single dose of oltitox. The doses used and the time of recording are indicated under groups. (Mean ± S.E. Number of rats is given in brackets. The percentage is calculated as compared to control values). NS-non-significant; LD-lethal dose; days-days after pesticide administration.

| Groups | Adrenal wt mg/100 g b.w. | Pituitary wt. mg/100g b.w. | | Corticosteroid production in vitro | |
|---|---|---|---|---|---|
| | | Adenohypophysis | Neural lobe | µg/100mg/hour | µg/100mg/100g b.w. |
| Control (11) | 15.59± 0.5 | 2.3415± 0.1123 | 0.5183± 0.033 | 8.6947± 0.24 | 4.12± 0.16 |
| Treated 1/3$LD_{50}$ 24 hours (8) | 19.80± 0.52 <0.001 26% | 2.65± 0.26 NS 13.17% | 0.5449± 0.07 NS 5.1% | 9.9143± 0.4 <0.02 14% | 5.19±0.44 <0.05 25.97% |
| Treated 1/3$LD_{50}$ 3 days (8) | 18.08± 0.635 <0.01 15.2% | 2.586± 0.14 NS 10.4% | 0.56± 0.0547 NS 8% | 6.99± 0.5896 <0.02 −19.61% | 3.18± 0.3 <0.02 −22.8% |
| Treated 1/3$LD_{50}$ 7 days (8) | 16.87± 1.4 NS 7.5% | 2.60± 0.1 NS 11% | 0.634± 0.058 NS 22% | 8.733± 0.499 NS 0.4% | 4.60± 0.32 NS 11.6% |
| Treated 1/3$LD_{50}$ 12 days (8) | 16.4± 0.4 NS 4.5% | 2.26± 0.1 NS −3.5% | 0.52± 0.02 NS 0.3% | 8.70± 0.25 NS 0.06% | 4.30± 0.2 NS 4.36% |
| Treated 2/3$LD_{50}$ 12 days (8) | 16.9± 0.5 NS 7.7% | 2.39± 0.16 NS 2.07% | 0.52± 0.015 NS 0.3% | 8.18± 0.30 NS −6% | 4.015± 0.18 NS −2.35% |
| Treated $LD_{50}$ 12 days (8) | 19.55± 1.2 <0.02 24.6% | 2.90± 0.327 NS 23.8% | 0.57± 0.04 NS 9.97% | 7.35± 0.42 <0.02 −15.47% | 3.80± 0.23 NS −7.8% |

The effects produced in adrenal activity are dose-dependent. Thus, changes recorded in adrenal hormone production after administration



of one dose of 2/3 $LD_{50}$ revert to the normal level 12 days later, while the pesticide dose of $LD_{50}$ evinces one inhibitory effect also 12 days after administration.

*The weight of glands*. The weight of the adrenal glands registered 24 hours and 3 days after pesticide administration, was significantly increased. The increase in adrenal weight recorded at 7 and 12 days was not significant. 12 days after pesticide administration in a dose of 2/3 $LD_{50}$, the adrenal glands weight tends to revert to the normal weight. Conversely, the higher dose of pesticide (equal to $LD_{50}$) evinces an increased adrenal weight which also remains significant 12 days post-administration.

Neural lobe weight gradually increases on the first 7 days after pesticide administration and after 12 days, the weight reverted to normal. Also, the adenohypophysis weight increases insignificantly after pesticide administration.

## DISCUSSION

The results obtained by us have shown that the hypothalamo-pituitary-adrenal system reacts to pesticide actions by a significant hyperactivation, registered 24 hours after treatment, followed by a significant decrease of the system activity 3 days after treatment, after which it returns gradually to the control level. The higher level of pesticide manifests one evident effect also 12 days after treatment.

As a rule, stress induces a very rapid increase in blood levels of ACTH which reaches a maximum within 2 to 2.30 minutes. Corticotropin concentrations in the pituitary remain unchanged during this time, although a significant depletion of pituitary ACTH stores develops subsequently [8]. This depletion of pituitary ACTH is already evident 1 hour after exposure to stress [4] and may still be observed 48 hours later. After this fall, pituitary stores start increasing; they may reach concentrations which are ten to fifteen times greater than those found in unstressed rats [2]. Increased adrenal hormone production, observed in our experiments 24 hours after pesticide administration, can be attributed to stress-induced hyperactivation of the corticoliberin-corticotropin-adrenal system. The decreased system activity, registered in our experiments 3 days after pesticide administration, can be attributed to feedback action of high blood level of endogenous corticoids brought about by the stress, and not by the destructive action of pesticide on the pituitary-adrenal system structures, because the weight of glands was not diminished. Also, 3 days after pesticide administration, the energy metabolism of the rats significantly increased, which suggests the presence of intact structures able to increase the metabolic processes (unpublished data).

Gradual increase in neural lobe weight can perhaps be attributed, to the increased hypothalamic hormone production stored in neural lobe. An increasing weight of the neural lobe was also registered in the rats treated with dibutox, case in which the vasopressin-like activity of the neural lobe was increased [3]. The nature of our experiments cannot solve the question whether the increased neural lobe weight, observed in

present experiments, is accompanied by one change in neural lobe hormones secretion.

In conclusion, having in mind that the organism is able to normalize the pituitary- adrenal system function after a single administration of the studied doses of pesticide, we consider that this substance can be utilized for agricultural purpose. It is necessary to study the effect of this pesticide in subacute or chronic experiments in order to realize if long action of this pesticide does not lead to the atrophy of adrenal gland activity, because, as a rule, long acting stressors induce the atrophy of this vital gland both in man and animals.

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*The "Al. I. Cuza" University*
*Department of animal physiology*
*Iași, Calea 23 August 20 A*

# THE INFLUENCE OF PROCAINE, GEROVITAL AND ASLAVITAL ON CELLULAR CHOLESTEROL-LOADED MEMBRANES

ION NEACȘU

Investigations have been performed on the frog sartorius fiber membrane, by the method of glass intracellular microelectrodes. The keeping of the fibers in a cholesterol medium causes the increase of the cholesterol proportion in the membrane, as well as its tight binding in the structure and the hyperpolarization of the membrane. Procaine and the procaine-based drugs (Gerovital and Aslavital) as well as the Clofibrate (a drug used in the therapy of cardiovascular diseases) determine the removal of the cholesterol deposited in the membrane and the recovery of the structure and of the normal resting potential. The Gerovital and Aslavital effects are stronger than those of procaine. Data obtained show the possibility of applying the membrane potential measurement as a rapid method of selecting the agents with possible hypocholesterolemiant and antiatherosclerotic properties.

Cholesterol is an important compound of the eukaryotic cell membranes [13]. In the membrane of normal cells there has been proved to exist a certain cholesterol : phospholipids ratio [13], [21], which is however modified in some diseases e.g. the cardiovascular and hepatic ones, the osmotic fragility of erythrocytes, cancer or ageing phenomena [7], [9], [10], [13], [23], accompanied by the modification of the membrane functions [13].

When maintaining different cell types in a cholesterol-containing medium, an increase of the membrane cholesterol proportion, having certain influences on the membrane, has been recorded [13], [14], [19], [22]. Nevertheless, few data exist on the bioelectric effects of the increase of the cholesterol : phospholipids ratio and on the action of some pharmacological agents on the cholesterol-loaded membranes [17], [19].

The present paper investigates the action of some drugs used in the treatment of hypercholesterolemia, atherosclerosis and ageing phenomena, characterized by the increase of the membrane cholesterol [7], [13], on the electric behaviour of the cholesterol-loaded membranes (Clofibrate, procaine and procaine-containing drugs — Gerovital and Aslavital) [16].

## MATERIAL AND METHODS

Experiments were conducted on the frog (*Rana ridibunda* Pall.) sartorius fiber membrane, using glass intracellular microelectrodes technique. Each experiment was developed on five muscles from different animals, at room temperature. The normal Ringer solution (NR), pH = = 7.2, was prepared with bicarbonate buffer. The 2.5 mM procaine (PROC.), 2.5 mM procaine-containing Gerovital $H_3$($GH_3$—PROC. 2.5 mM) and 2.5 mM procaine-containing Aslavital (ASV—PROC. 2.5 mM) solutions were prepared by the addition of these substances to NR. The

cholesterol-containing solution (CHOLEST.) was prepared by solving cholesterol in ethylic alcohol and the addition of this solution to NR, so that a concentration of 0.1 mM cholesterol and 1 % ethylic alcohol is to be obtained. The Clofibrate solution (CLOF.) was obtained by solving the drug in dimethylformamide (DMFA) and by the addition of this solution to NR, so that a concentration of 0.02 % CLOF. and 1 % DMFA was reached. The solution of 1 % ethylic alcohol and 1 % DMFA in NR were prepared by the direct addition of the substances to NR. The statistical data treatment of the results were performed by Student's test.

## RESULTS

The normal resting potential (NRP) recorded in our experiment had an evarage value between 90.21 mV and 94.88 mV (SE about the value of 0.50 mV) (Figs. 1 — 3).

The action of the 2.5 mM procaine and the Gerovital $H_3(GH_3)$ and Aslavital (ASV) drugs, containing the same procaine concentration, on the normal membranes causes different effects on the membrane potential (MP). Thus, the 2.5 mM procaine determines a slight membrane hyperpolarization (Fig. 1 — A), with an average amplitude of 4.54 mV, easily reversible at the muscle fibres washing with NR. Thus, 2.5 mM $GH_3$ — PROC. causes a weak depolarization of the membrane, with an

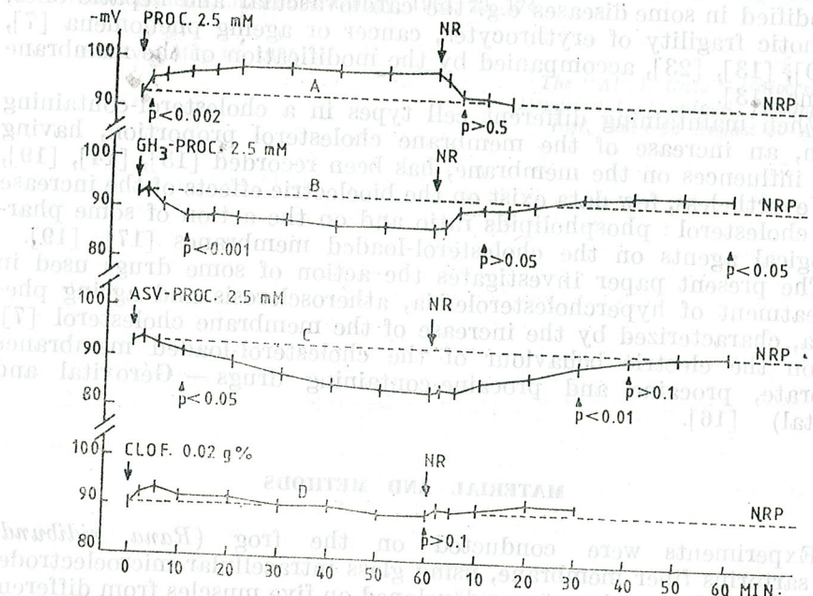


Fig. 1. — The effect of 2.5 mM procaine (A), Gerovital (B) and Aslavital (C) containing 2.5Mm procaine on the membrane potential (D = effect of 0.02 % Clofibrate).



average amplitude of 5.10 mV (Fig. 1 — B). Bringing back the fibres into NR this depolarization is easily reversible, being followed by a slight hyperpolarization (average value of 1.52 mV). The 2.5 mM ASV—PROC. determines also a membrane depolarization (Fig. 1 — C), characterized by a slow onset and an average amplitude of 5.87 mV, slowly reversible

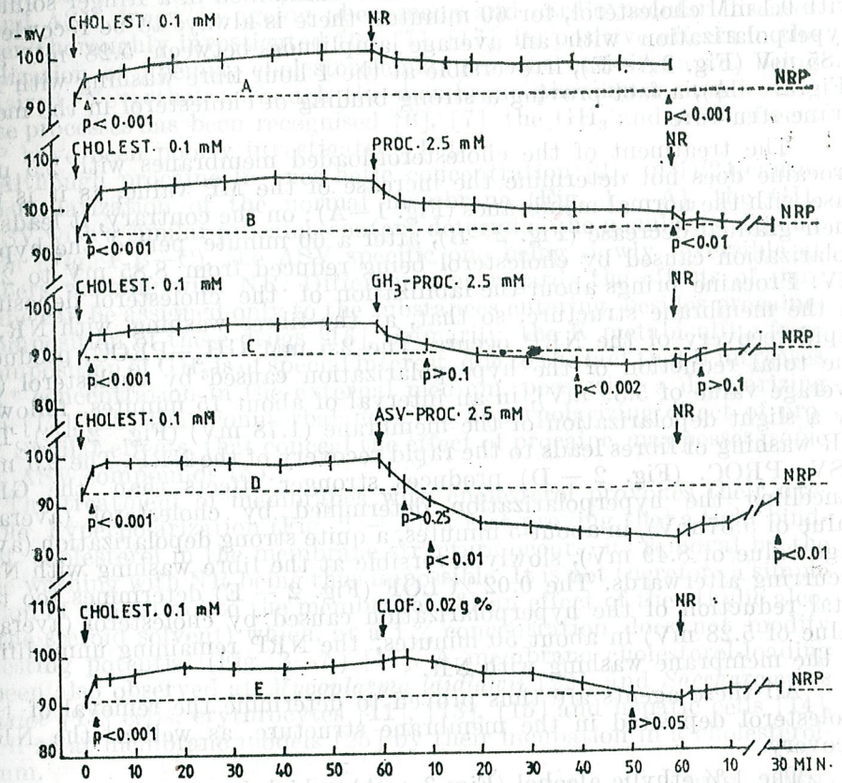


Fig. 2. — The effect of 0.1 mM cholesterol (A) on the membrane potential and the action of procaine (B), Gerovital (C), Aslavital (D) and Clofibrate (E) on cholesterol-loaded membranes.

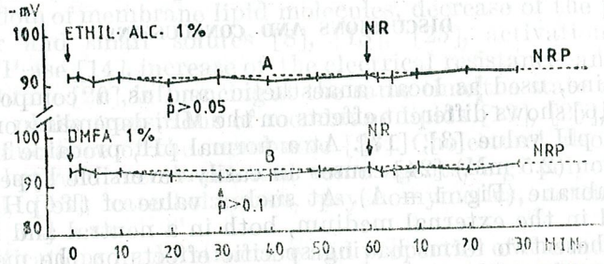


Fig. 3. — The effect of 1 % ethylic alcohol (A) and 1 % dimethylformamide (B) on the membrane potential.

when the fibres were replaced in a NR. The Clofibrate, having a concentration of 0.02 g %, similar to that used in the therapy of cardiovascular diseases [16], causes only a very weak hyperpolarization (0.89 mV) (Fig. 1 — D).

When the normal membranes were maintained in a Ringer solution with 0.1 mM cholesterol, for 60 minutes, there is always to be recorded a hyperpolarization with an average amplitude between 5.28 mV and 8.85 mV (Fig. 2 A—E), irreversible at the 1 hour fibre washing with NR (Fig. 2 — A), a fact proving a strong binding of cholesterol in the membrane structure.

The treatment of the cholesterol-loaded membranes with 2.5 mM procaine does not determine the increase of the MP value, as it is the case with the normal membranes (Fig. 1—A); on the contrary, it leads to their gradual decrease (Fig. 2—B), after a 60 minute period the hyperpolarization caused by cholesterol being reduced from 8.85 mV to 2.75 mV. Procaine brings about the labilization of the cholesterol deposited in the membrane structure, so that, at the fibres washing with NR, a rapid recovery of the NRP occurs. The 2.5 mM $GH_3$—PROC. produces the total reduction of the hyperpolarization caused by cholesterol (an average value of 5.39 mV), in an interval of about 15 minutes, followed by a slight depolarization of the membrane (1.78 mV) (Fig. 2—C). The NR washing of fibres leads to the rapid recovery of the NRP. The 2.5 mM ASV—PROC. (Fig. 2 — D) produces stronger effects than the $GH_3$, cancelling the hyperpolarization determined by cholesterol (average value of 5.87 mV), in about 5 minutes, a quite strong depolarization (average value of 8.49 mV), slowly reversible at the fibre washing with NR, occurring afterwards. The 0.02 %CLOF (Fig. 2 — E) determines too the total reduction of the hyperpolarization caused by cholesterol (average value of 5.28 mV) in about 50 minutes, the NRP remaining unmodified at the membrane washing with NR.

All these agents are thus proved to determine the removal of the cholesterol deposited in the membrane structure, as well as the NRP recovery.

The 1 % ethylic alcohol (Fig. 3 — A), which has been used at the solubilization of cholesterol, and the 1 % DMFA (Fig. 3—B), used at the Clofibrate solubilization, does not induce significant changes of the NRP.

## DISCUSSIONS AND CONCLUSIONS

Procaine, used as local anaesthetic and as a compound of $GH_3$ and ASV [16] shows different effects on the MP, depending on its concentration and pH value [3], [17]. At a normal pH, procaine in anesthetic concentration (2.5 mM) [24] causes an easily reversible hyperpolarization of the membrane (Fig. 1 — A). At such a value of the pH, procaine is to be found in the external medium, both in a neutral and in a cationic form [5], these two forms having specific effects on the membrane [3], [17]. Starting from the "2—M.S.I." concept on the membrane [1], there has been accepted that, by its interaction with membrane phospholipids



and with the $Na^+$, $K^+$ and $Ca^{2+}$ ions, procaine provokes the membrane hyperpolarization, the raising of the excitability threshold, modification of the ionic conductance and the impulse blocking during the development of the action potential [3], [17].

The complex pharmacological action of procaine, administered as $GH_3$ and ASV drugs, in ageing phenomena and cardiovascular diseases has been thoroughly investigated [6], [7], [17], its positive effects in the normalization of lipemia, cholesterolemia, nervous activity a.s.o. being emphasized. Nevertheles, though the membrane phenomena implication in these processes has been recognised [6], [7], the $GH_3$ and ASV effects on MP have been poorly investigated [17].

Although procaine in anesthetic concentration (2.5 mM)/24/causes a hyperpolarization of the normal membrane (Fig. 1 — A), the $GH_3$ and ASV with a similar procaine-content determine the membrane depolarization (Fig. 1 B—C), the ASV specific one being slowly reversible at the fibers washing with NR. Differences as regards the effects of pure procaine can be assigned only to the substances entering, besides procaine, the composition of these drugs [16]. Primarily, the K metabisulfite from the composition of $GH_3$ is of special interest, due to the fact that it increases the $K^+$ concentration in the external medium, possessing a depolarizing action [17], [18] that counterbalances the hyperpolarizing effect of procaine. Similar effects, that conceal the effect of procaine, may possess some of the ASV compounds too.

The treatment of membranes with cholesterol provokes their irreversible hyperpolarization (Fig. 2 — A), a fact proving that a tight binding of cholesterol in the membrane structure occurs, its removal by the simple washing with NR being thus impossible. It is not therefore a simple addition of cholesterol to the membrane, nor an effect of the ethylic alcohol (the steroid solvent) which, at a 1 % concentration does not modify the resting potential (Fig. 3—A) [17]. A membrane cholesterol-loading has been also observed at *Mycoplasma laidlawii* [22], and *Saccharomyces cerevisiae* [13] cells, erythrocytes [11], [13], [15] and hepatic cells [14], as well as at membrane models [25] by their incubation in a cholesterol-medium.

Starting from the studies on the X-ray diffraction, RES, RMN, calorimetry a.s.o. the complex role of membrane cholesterol has been emphasized. Thus, cholesterol has been found to determine the increase of the membrane structures packing degree [8], [25], modification of the moving freedom of membrane lipid molecules, decrease of the permeability to water and small solutes [8], [13], [25], activation of the $(Na^+—K^+)$—ATP-ase [14], increase of the electrical resistance and capacity of the membrane [20], influencing at the same time the state of liquid crystals and the phase transitions of membrane lipids [13], [25], as well as the ionic conductance of the membrane [26]. Cholesterol also assures the interaction between membrane and some agents, e.g. polyene antibiotics [2], [4], [13], catecholamines, psychomymetics, carcinogenes, the A vitamin [13] a.s.o.

Such modifications in the structure and properties of the membrane, especially the increase of the membrane structure packing degree, the permeability decrease, activation of the $(Na^+ — K^+)$-ATP-ase, modifi-

cation of the ionic conductance and of the electrical properties of the membrane — caused by the increase of the cholesterol proportion in the membrane — account for the increase of the electrochemical potential gradient and the membrane hyperpolarization. Our experiment shows that the hyperpolarization noted in such conditions is irreversible (Fig. 2—A), a fact attesting that a tight binding of cholesterol in membrane takes place, the washing with NR being not sufficient to accomplish the cholesterol removal. This presents a special biological importance, due to the fact that, during the installation of the atherogenic process, a relatively higher increase of cholesterol than that of total lipids in organism occurs, affecting thus the cholesterol : phosphilipids ratio [9], [17], with important consequences on the functions of the cellular membranes.

On the contrary, in the situation in which the cholesterol-loaded membranes are treated with procaine (Fig. 2—B) a gradual reduction of the hyperpolarization, up to the characteristic value for the 2.5 mM procaine action on the normal membrane, is to be observed. Procaine labilizes the cholesterol deposited in the membrane which can be therefore easily removed from the structure, by the fibres washing with NR. The effects of the $GH_3$ (Fig. 2—C) and ASV (Fig. 2—D) on the cholesterol-loaded membranes are of the same nature with the procaine effect, but much stronger, although these drugs contain the same concentration of procaine (2.5 mM) as in the experiments with pure procaine (Fig. 2—B). In this case, a rapid removal of the cholesterol deposited in the membrane occurs, accompanied by the NRP recovery in the first 10 — 15 minutes, and followed by the membrane depolarization — characteristic for these agents (Fig. 1 B — C) — the one determined by ASV being much ampler and slowly reversible. Differences among the effects of procaine, $GH_3$ and ASV on these cholesterol-loaded membranes are due to differences in their chemical composition [16]. Thus, it may be possible that the ASV depolarizing effects be due to a specific interaction of the "antiatherogenic factor" existent in the drug structure [16], with the membrane cholesterol. This is much ampler in the case of cholesterol-loaded membranes (Fig. 2—D) in which, between ASV and cholesterol another ratio—different from that observed in the case of normal membranes (Fig. 1—C)—is achieved.

The removal of the cholesterol deposited in the membrane structure is also observed in experiments with the Clofibrate (Fig. 2-E), a drug used therapeutically for its hypocholesterolemiant and hypolipemiant properties [16]. This effect is based on the interaction between the Clofibrate and the cholesterol deposited in the membrane, since in the case of normal membranes, neither the drug (Fig. 1—D), nor its solvent (1% DMFA) (Fig. 3-B) determines the NRP modification. The Clofibrate action is, however, slower than that of the $GH_3$ and ASV.

All these pharmacological agents, possessing hypolipemiant, hypocholesterolemiant and antiatherosclerotic properties, are thus proved to have the capacity of removing the cholesterol deposited in the membrane structure and of re-establishing the normal cholesterol : phospholipids ratio, the structure, electrical properties, permeability and NRP of the membrane. Such an effect has been also observed at some polyene antibiotics, which interact directly with the membrane cholesterol [13],



showing hypolipemiant and hypocholesterolemiant properties, experimentally demonstrated [2], [4]. Having these in mind, one can assert that the procedure of testing the removal capacity of the cholesterol deposited in the membrane structure by MP measurement, may represent a rapid method of selecting among a large series of new products, those with very probable hypocholesterolemiant and antiatherosclerotic properties, which may be subsequently checked by investigations on laboratory animals, as well as by clinical tests.

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# EFFECTS OF 2,4-DICLORPHENOXYACETIC ACID ON THE EXCITATION-CONTRACTION COUPLING IN FROG SINGLE MUSCLE FIBRES

M. ISAC and RODICA-MARIA ISAC

The effects of 2,4-diclorphenoxyactic acid (2,4-DCPA) were studied on isolated single muscle fibres of the frog. 2,4—DCPA potentiated the twitch amplitude i.e. an increase of the time to peak tension and an increased time to half relaxation. 2,4-DCPA increased the resting membrane potential; the rate of decay and the total duration of the action potential were prolonged. The muscle excitability was not affected by 2,4-DCPA. Twitch potentiation by this substance was independent of the extracellular calcium concentration. It is concluded that 2,4—DCPA potentiated the twitch response by increasing the release of activator calcium into the myofibrillar space by prolongation of the action potential. There may be a direct inhibitory action of 2,4—DCPA on the calcium re-uptake by the sarcoplasmic reticulum in frog muscle.

The effects of some drugs on the excitation-contraction coupling in striated muscle fibres or in rabbit papillary muscle were studied by Khan and Edman 1978, Wollmer. Wohlfart and Khan 1980, Paskov et al. 1973, Isac et al. 1985. The action of drugs on neuromuscular junction has been studied by Lundh and Thesleff 1977, Molgo et al. 1977.

The muscular effects of 2,4-diclorphenoxyacetic acid, seems to be a distrophic effect. It is possible to induce experimental muscular distrophy by ussing 2,4—DCPA.

## METHODS

Preparation. Single muscle fibres were isolated from the ventral head of the semitendinosus muscle of Rana temporaria. For mounting the fibres, a link of stainless steel wire (tickness : 0.1 mm) was attached to each tendon as described previously (Edman and Kiessling 1971).

Muscle chamber. Frog single fibres were mounted horizontally in a Perpex chamber (Fig. 1) between a tension transducer and an adjustable stainless steel hook, the position of which could be set by means of a micrometer screw. The chamber was 6 mm wide, 5 mm deep and contained 1.2 ml solution. The solution was changed by introducing fresh solution at the transducer end of the chamber, and it was removed by suction drain at the other end.

This caused an almost complete ($> 95\%$) exchange of the bathing solution within some 3s (Andersson and Edman 1974 b).

Temperature control. During an experiment the temperature was controlled by an Ultrakryomat TK 30 D-Thermostat which circulated an ethylene glycol-water mixture through jackets surrounding the chamber and the containers of solution. The solution containers were connected with the chamber through polyethylene tubes. The bath temperature

varied between 1 and 3.5°C and was maintained constant to within ± 0.2°C throughout any particular expt., even during exchange of solution.

Tension recording. Tension was recorded by means of an RCA-5734 mechano-electric transducer. The signals were displayed on a Physioscope TR Tönnies oscilloscope and were recorded simultaneously on paper by

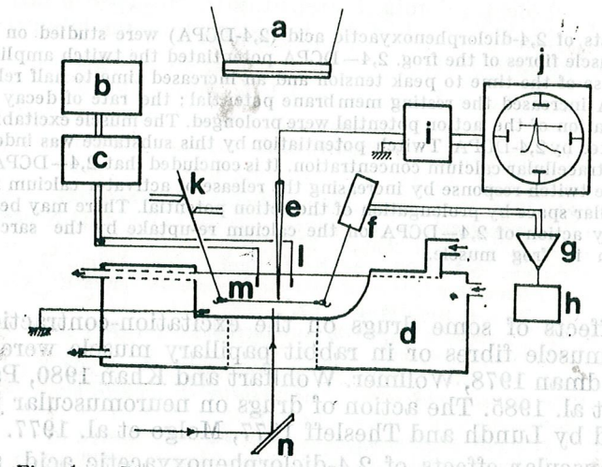


Fig. 1. — The scheme of the experimental system. a — stereomicroscope; b — biological stimulator; c — isolate unit; d — Perspex chamber: e — glass capillary electrodes; f — mechano-electric transducer; g — amplifier; h — recorder; i — amplifier; k — stainless steel hook; l — multielectrode assembly; m — single muscle fibre; n — mirror.

means of an Endim 620.02 recorder. The twitch response was interpreted through three parameters (Fig. 2): the maximum force of contraction (milinewtons — mN); the half time to peak tension (ms) $T_c$; the half time for relaxation (ms) $R_c$.

Electrical stimulation. The single muscle fibre was stimulated by means of a multielectrode assembly as described previously (Edman and

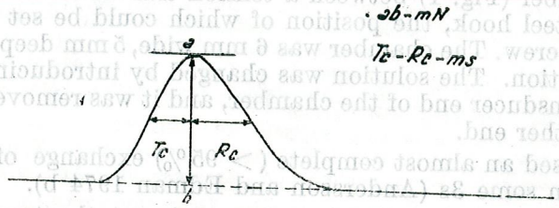


Fig. 2. — The parameters calculated for the isometric twitches.

Kieslling 1971). Pulses of 0.2 ms duration were used and the stimulation strength was adjusted to be maximal for each electrode pair. The single muscle fibre was mounted for at least 1 h before the expt., and stimulated every 2 min to record the twitch response.



Solutions. The solutions used had the following composition (mM): Ordinary Ringer solution: NaCl 115.5, KCl 2.0, $CaCl_2$ 1.8, Na-phosphate buffer 2.0, pH 7.0.

Calcium-free Ringer: NaCl 116.3, KCl 2.0, EDTA 1.0, Na-phosphate buffer 2.0, pH 7.0.

Lanthanum containing Ringer: NaCl 117.2, KCl 2.0, La 0.05, Tris-(hydroxymethyl)-aminomethane 2.0. The pH was adjusted to 7.0 by addition of $H_2SO_4$ to a final concentration of 0.94 mM.

Sucrose containing Ringer: 200 mM succrose were added to ordinary Ringer solution, in order to uncouple excitation from contraction.

All chemicals used were of analytical grade and were dissolved in double distilled water.

Drugs. 2,4-diclorphenoxyacetic acid was dissolved in Ringer solutions.

Recording of membrane potential. Conventional glass capillary electrodes (about 7 — 15 MΩ) filled with 2.5 M KCl were used for intracellular recordings of membrane potentials. The reference electrode was an Ag-AgCl electrode connected to the bath through an agar Ringer bridge. The microelectrode and the reference electrode were connected via an electrometer provided with capacitance neutralization to a Physioscope TR Tönnies oscilloscope. The signals were photographed on 35 mm film. The amplified signals were also used to modulate an audio frequency generator. Successful impalement was indicated by an abrupt change in frequency. When action potentials were recorded the fibre was stimulated only at one locus, approximately 5 mm from the tip of the microelectrode. Membrane potentials were interpreted on the basis of five parameters which were calculated (Fig. 3): resting potential — RP — mV; the overshoot of

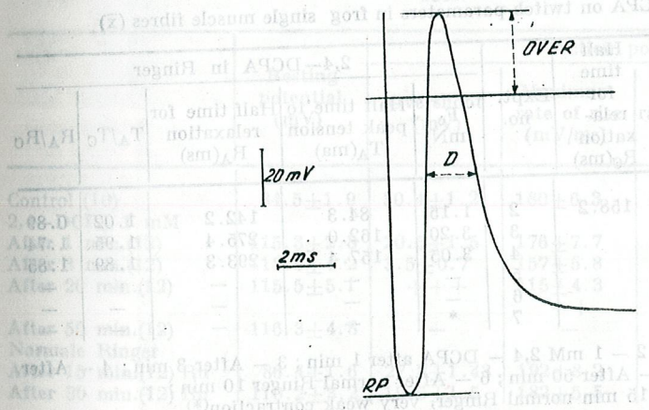


Fig. 3. — The membrane potentials interpreted through five parameters.

the action potential — Over — mV; maximum rate of rise — MRR — mV/ms; maximum rate of decay — MRD — mV/ms; the duration of the action potential at — 25 mV level — D-25 mV — ms. Results were statistic interpreted by Student's $t$ — test.

## RESULTS

2,4-diclorphenoxyacetic acid was tested on isolated muscle fibre in concentration of 1mM. 2,4—DCPA potentiated the twitch response in the first 20 minutes then the muscle fibre had no response (Fig. 4).

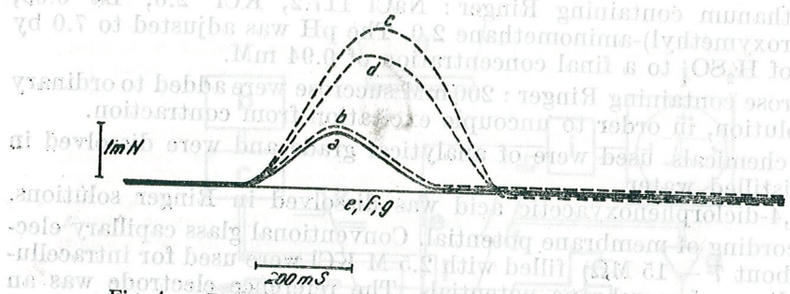


Fig. 4. — The influence of 2,4—DCPA 1 mA, on isometric twitches in single muscle fibre. a — Control; b — ImM 2,4—DCPA—1 min; c — After 3 min; d — After 20 min; e — After 50 min; f — Rn— After 1 min; g—Rn After 15 min.

This situation is not reversible even after replacement of 2,4-DCPA containing Ringer with ordinary Ringer solution (Table 1).

As illustrated in Fig. 4, 2, 4-DCPA caused a significant change in the initial rate of tension rise. The increase in twitch amplitude was asso-

*Table 1*

Effects of 2,4—DCPA on twitch parameters in frog single muscle fibres ($\bar{x}$)

| Expt. no. | $F_c$ (mN) | Normal Ringer Half time to peak tension $T_C$(ms) | Half time for relaxation $R_C$(ms) | Expt. no. | 2,4—DCPA in Ringer $F_c$ (mN) | Half time to peak tension $T_A$(ma) | Half time for relaxation $R_A$(ms) | $T_A/T_C$ | $R_A/R_C$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.08 | 83.6 | 158.2 | 2 | 1.15 | 84.3 | 142.2 | 1.02 | 0.89 |
| | | | | 3 | 3.20 | 162.0 | 275.4 | 1.95 | 1.74 |
| | | | | 4 | 3.05 | 157.4 | 293.3 | 1.89 | 1.85 |
| | | | | 5 | — | — | — | — | — |
| | | | | 6 | — | — | — | — | — |
| | | | | 7 | * | — | — | — | — |

Expt. no.: 1 — Control; 2 — 1 mM 2,4 — DCPA after 1 min; 3 — After 3 min; 4 — After 20 min; 5 — After 50 min; 6 — After normal Ringer 10 min; 7 — After 15 min normal Ringer, very weak contraction(*)

ciated with an increase of the time to peak tension and a prolongation of the relaxation phase. The half time to peak tension and the half time for relaxation increased by about 89% of the control values. (Table 1).

The effect produced by 2,4—DCPA was not reversible. Repeated washing of the fibre in normal Ringer, had no response.



To test if the extracellular calcium plays any role in the twitch potentiation produced by 2,4—DCPA, calcium was removed from the extracellular medium by repeated washing of the fibre with a calcium-free Ringer solution. The removal of calcium from the extracellular medium made the fibre inexcitable (Edman and Grive 1961, Curtis 1963, Khan and Edman 1979).

By addition of lanthanum to the bathing solution, the resting membrane potential and the twitch response were restored. 2,4—DCPA (1mM) added to the calcium-free lanthanum-containing medium caused a potentiation of the twitch similar to normal Ringer.

## RESTING AND ACTION POTENTIALS

2,4—DCPA in a bath concentration of 1 mM, increased the resting membrane potential (Table 2).

On the other hand, it is noticed that 2,4-DCPA decreased the overshoot and prolonged markedly the maximum rate of rise, the maximum

*Table 2*

Effects of 2,4—DCPA on resting and action potentials added to normal Ringer (Rn) ($\bar{x} \pm$ S.E.). Frog muscle fibre bundles exposed only 1 min before recording the resting and action potentials. Number of experiment given within brackets. Students' $t$—test: ++, p= 0.01; +++, p = = 0001.

| | Resting potential (mV) | Action potential | | | |
|---|---|---|---|---|---|
| | | Overshoot (mV) | Maximum rate of rise (mV/ms) | Maximum rate of decay (mV/ms) | Duration at — 25mV level (ms) |
| Control (10) | 84.5±1.9 | 30.4±1.2 | 180±6.3 | 53±3.3 | 2.13±1.22 |
| 2,4—DCPA 1 mM | | | | | |
| After 1 min.(12) | 115.3±2.8 | 20.5±1.5 | 176±7.7 | 46±2.9 | 2.18±1.74 |
| After 3 min.(12) | 118.6±4.2 | 3.5±0.7 | 157±5.8 | 36±2.1 | 2.98±1.15 |
| After 20 min.(12) | 115.5±5.1 | +++ | 115±4.3 | 16±0.96 | 16.67±2.45 |
| | | | ++ | +++ | +++ |
| After 50 min.(12) | 116.3±4.8 | — | — | — | — |
| Normale Ringer | | | | | |
| After 15 min.(12) Rn | 86.4±1.6 | 27.2±1.43 | 192±8.2 | 25±1.7 | 3.50±1.23 |
| After 30 min.(12) Rn | 110.2±3.2 | 19.6±1.5 | 182±5.5 | 29±2.5 | 4.20±1.76 |

rate of fall and the duration of the action potential (Fig. 5). After fifty minutes if was not possible to record any action potential, although the muscle fibre had an increased resting potential. By repeated washing of the fibre with an ordinary Ringer solution, the action potential was restored step by step, too.

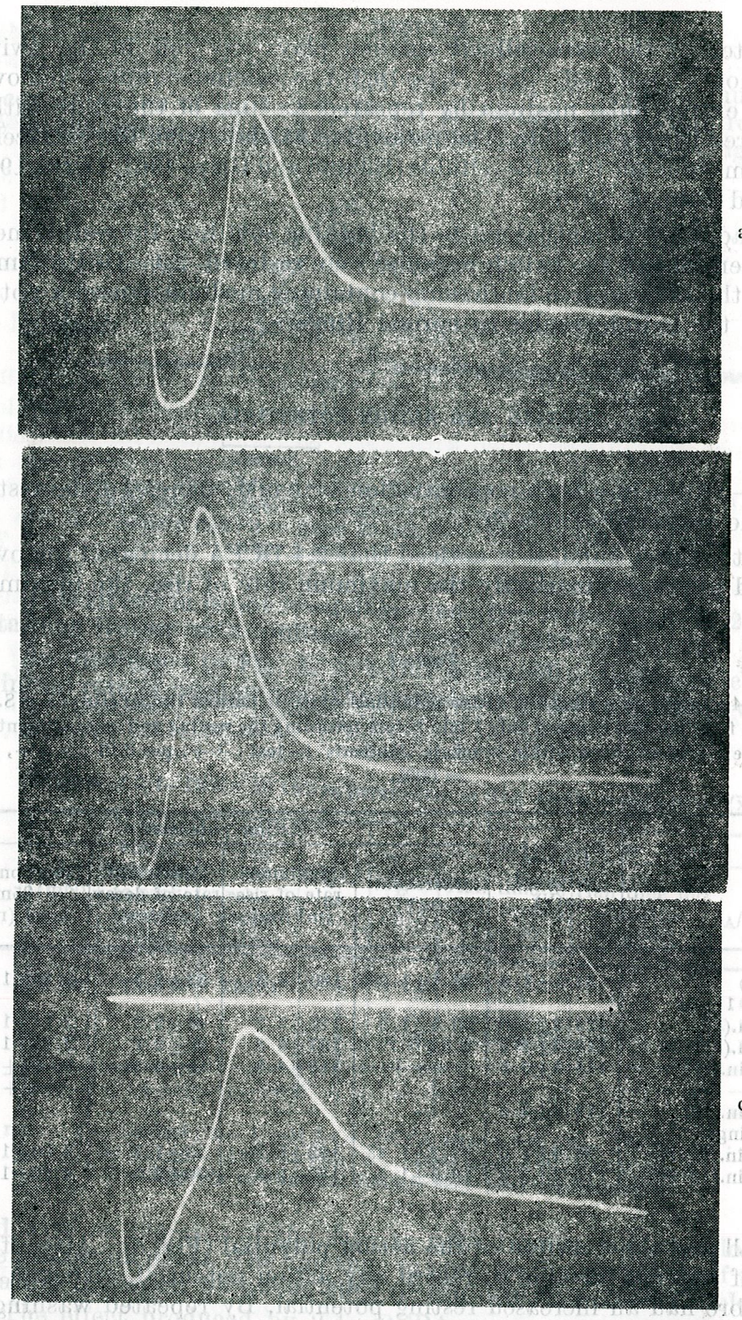


Fig. 5. — The influence of 2,4—DCPA 1 mM, on action potential of muscle fibre.
a — After 1 min ; b — After 3 min ; c — After 20 min.



## DISCUSSION

The present results on frog single muscle fibres demonstrate that 2,4—DCPA causes twitch potentiation by a direct action on the excitation-contraction coupling.

The twitch potentiation by 2,4—DCPA is characterized by an increase of the half time to peak tension and of the half time for relaxation. These changes were associated with a broadening of the action potential. As previously demonstrated on single muscle fibre (Edman et al. 1966) the duration of the mechanical activity appears to be quantitatively related to the action potential duration at the — 25 mV level. This has suggested that the action potential governs the time during which activator calcium is released into the myofibrillar space (Sandow et al. 1964, Edman et al. 1966). The longer duration of the action potential would cause a higher peak concentrtion of $Ca^{2+}$ at the contractile sites, and this in turn would require a longer time for the elimination of $Ca^{2+}$ from the myofibrils. If the peak concentration of the activator $Ca^{2+}$ were large enough to fully activate the contractile system, a further increase of the $Ca^{2+}$ concentration would merely cause a prolongation of the mechanical activity. The effects produced by 2,4—DCPA are consistent with the idea that twitch potentiation is due to the prolongation of the action potential according to the above mechanism. The results show that 2,4—DCPA also prolongs the relaxation time in frog muscle suggesting a direct inhibitory action on the resequestration of calcium in this tissue. On the other hand, the changes of the action potential suggest that 2,4—DCPA acts by predominantly decreasing the potassium conductance in frog skeletal muscle fibres. The present results suggest that extracellular calcium is immaterial for twitch potentiation produced by 2,4-DCPA.

The enhancement of the isometric twitch could be produced when there was no calcium in the extracellular medium, i.e. after replacement of calcium in the Ringer solution by lanthanum. It has been shown that the presence of lanthanum (Andersson and Edman 1974 a) prevents the membrane depolarization, and that lanthanum is unlikely to penetrate the fibre membrane (Langer and Frank 1972) and causes by itself only a slight twitch potentiation (Andersson and Edman 1974 a) in the low concentration (0.05 mM) used in the studies.

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*"Al. I. Cuza" University*
*Laboratory of Biophysics*
*6 600 Iași, Calea 23 August 20A*
*Romania*

# ANALYSE COMPARATIVE DE L'EFFICIENCE DES RÉGIMES «TROUVIT» ET «BICAZ-SALMO-0» POUR LES ALEVINS DE TRUITES ARC-EN-CIEL

## (*Salmo gairdneri* Richardson)

C. MISĂILĂ*, N. PĂSTÎRNAC** et ELENA RADA MISĂILĂ*

The paper presents the results of researches concerning the rainbow trout fry response to "Trouvit" and "Bicaz-Salmo-0" diets. The effect of these diets have been appreciated by growth rate, survival level, food conversion, valuation of alimentary proteins and by values of some biochemical and haematological parameters.

By exclusive administration "Trouvit" diet determines a supplementary growth of 11%, and reduction of mortality of about 14%. More that that, food conversion and the coefficient of proteic use are ameliorated by 24 — 31% and the coefficient of protein efficiency of 43% as against "Bicaz-Salmo-0" diet.

By alternative administration of these diets and cattle liver, fish growth and survival, as well as food conversion, coefficient of proteic use, coefficient of protein efficiency and some of haematological parameter values are comparable between the two experimental diets. The level of fish hepatic lipids was higher by 69% and the erythrocyte number/ml by 41% in "Trouvit" diet, as against the autochthonous diet.

The authors concluded that "Bicaz-Salmo-0" diet and the original method concerning alternative feeding may be applied in intensive rearing of rainbow trout fry in cages.

Pour assurer le nécessaire physiologique optimum d'éléments nutritifs pour les salmonides élevés en captivité, on applique en géneral des régimes concentrés. Ce système moderne de nutrition des truites dans l'aquaculture intensive et surintensive a remplacé peu à peu la nutrition traditionnelle qui utilisait les déchets d'abattoir et de pêcherie. Il constitue en même temps un élément principal pour assurer une production élevée de biomasse piscicole et une amélioration contrôlable de l'état physiologique général des effectifs de l'élevage.

Dans notre pays on pratique encore l'alimentation des truites à base de déchets d'abattoir qui utilise les ainsi-dits régimes humides et semi-humides. Mais il existe aussi la préoccupation de les remplacer par des aliments concentrés granulés. Les productions obtenues par des unités du Département d'Agriculture d'Etat, ayant adopté presque entièrement le nouveau système d'alimentation, confirment une série d'avantages qui recommandent son extension.

Les études sur le comportement alimentaire et sur la physiologie de la nutrition des salmonides effectuées à la Station de Recherches «Stejarul» Pîngărați, au cours de la dernière décennie ont réussi à élaborer, expérimenter, optimiser et bréveter les régimes d'aliments concentrés du type «Bicaz-Salmo-0» et «Bicaz-Salmo-1» [2 ], [6] que les performances alimentaires recommandent pour une analyse comparative avec les régimes d'importation qu'il fallait remplacer.

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*Tableau 1*

La mise en valeur métabolique des régimes « Trouvit » et « Bicaz-Salmo-0 » chez les alevins de truite arc-en-ciel

| Coefficients | Variante | Étape expérimentale I | II | III | IV | V | VI | VII | Total |
|---|---|---|---|---|---|---|---|---|---|
| CN | 1 a | 1,06 | 4,33 | 2,49 | 4,33 | 1,79 | 2,74 | 2,12 | 2,36 |
| | 1 b | 0,65 | 1,99 | 1,51 | 2,28 | 1,16 | 1,46 | 1,57 | 1,44 |
| | 2 a | 1,00 | 3,86 | 2,43 | 3,57 | 1,73 | 3,41 | 2,03 | 2,39 |
| | 2 b | 0,60 | 1,77 | 1,47 | 1,34 | 1,12 | 1,81 | 1,36 | 1,45 |
| | 3 | 0,86 | 1,13 | 1,09 | 1,42 | 1,76 | 1,57 | 1,80 | 1,43 |
| | 4 | 1,26 | 2,49 | 0,82 | 1,91 | 1,08 | 2,11 | 5,39 | 1,87 |
| CEP | 1 | 2,43 | 0,73 | 1,05 | 0,66 | 1,39 | 1,04 | 1,07 | 1,10 |
| | 2 | 2,76 | 0,85 | 1,14 | 0,85 | 1,51 | 0,88 | 1,21 | 1,16 |
| | 3 | 2,29 | 1,76 | 1,82 | 1,40 | 1,69 | 1,27 | 1,10 | 1,39 |
| | 4 | 1,43 | 0,73 | 2,21 | 0,96 | 1,68 | 0,86 | 0,34 | 1,86 |
| CUP | 1 | 2,40 | 8,15 | 5,57 | 8,83 | 4,20 | 5,62 | 5,45 | 5,15 |
| | 2 | 2,10 | 6,89 | 5,13 | 6,91 | 3,89 | 6,64 | 4,84 | 5,03 |
| | 3 | 2,50 | 3,29 | 3,29 | 3,18 | 4,13 | 3,42 | 5,24 | 4,15 |
| | 4 | 4,03 | 9,20 | 2,62 | 6,07 | 3,45 | 6,73 | 17,15 | 5,79 |
| CPC | 1 | 412 | 1.389 | 954 | 1.513 | 720 | 964 | 935 | 909 |
| | 2 | 362 | 1.178 | 877 | 1.182 | 664 | 1.135 | 827 | 8 60 |
| | 3 | 432 | 570 | 550 | 715 | 590 | 788 | 907 | 717 |
| | 4 | 701 | 1.369 | 452 | 1.047 | 595 | 1.161 | 2.964 | 1.027 |

*Explications* : a — mélange humide ; b — mélange sec ;

$$\text{CN} = \text{conversion de la nourriture} = \frac{\text{g nourriture consommée}}{\text{g accroissement ponderal des poissons}};$$

$$\text{CEP} = \text{coefficient de l'efficacité protéique} = \frac{\text{g taux d'accroissement}}{\text{g protéines utilisées}};$$

$$\text{CUP} = \text{coefficient d'utilisation protéique} = \frac{\text{g protéines utilisées}}{\text{g protéines fixées}};$$

$$\text{CPC} = \text{coefficient protéique de croissance} = \frac{\text{g protéines nécessaires pour obtenir 1kg taux d'accroissement.}}{}$$



Le Secteur de Salmoniculture de l'Entreprise Agricole d'Etat Prejmer a déjà testé le régime « Bicaz-Salmo-1 » en le comparant avec le régime « Trouvit » et maintenant on y utilise à l'échelle industrielle le régime autochtone.

Notre travail présente les résultats des premières études comparatives de la qualité nutritive des régimes « Bicaz-Salmo-0 » et « Trouvit ».

## MATÉRIEL ET MÉTHODE

Les recherches ont été effectuées à la Station I.C.A.S. Potoci. Des lots parallèles ayant chacun 1.000 alevins — le poids initial de chacun étant de 127 — 140 mg — ont été élevés dans des bassins d'alevinage bétonnés, ayant chacun une surface de 2 m². L'eau du lac de Bicaz a assuré l'irrigation des lots. Les poissons ont été nourris de la manière suivante :

— *variante 1* — « Bicaz-Salmo-0 », formule standard [6] et foie de veau, dans une alternance de 3 jours/1 jour, conformément à un procédé original [5] ;

— *variante 2* — « Trouvit » et foie, administrés comme dans la var.1 ;

— *variante 3* — « Trouvit » ;

— *variante 4* — « Bicaz-Salmo-0 ».

On a administré journellement 7 repas totalisant la ration propre à chaque variante. On a enregistré la température de l'eau et la mortalité. De 10 en 10 jours on a pesé les lots et on a recalculé les rations [4]. A la fin du test on a prélevé des échantillons de poissons pour les analyser du point de vue biochimique [1], [3] et hématologique [7]. Les données obtenues ont servi à calculer le coefficient de l'efficacité protéique, le coefficient d'utilisation protéique, ainsi que le coefficient protéique de croissance. On a calculé le coefficient de conversion de la nourriture ; pour les variantes 1 et 2, où l'on a utilisé aussi du foie de veau, on a calculé la conversion concernant tant l'humidité de la nourriture au moment de l'administration (mélange humide) que le rapport de la consommation et de l'humidité de la nourriture concentrée (mélange sec).

On a divisé la période expérimentale de 10 semaines en 7 étapes (fig. 1) et on a calculé la valeur des coefficients mentionnés pour chaque étape (tableau 1). Le tableau 2 présente une analyse synthétique de l'expérimentation, et certains résultats des analyses biochimiques et hématologiques effectuées.

## RÉSULTATS ET DISCUSSIONS

La composition théorique des formules alimentaires analysées a été la suivante :

| | « *Trouvil* » | « *Bicaz-Salmo-0* » |
|---|---|---|
| Protéines | (%) 56,0 | 50,2 |
| Lipides | (%) 8,0 | 7,9 |
| Fibres | (%) 3,0 | 4,3 |
| Cendre | (%) 12,5 | 18,3 |
| S.E.N. | (%) 20,5 | 19,3 |
| | 100,0 | 100,0 |
| Eau | (%) 10,0 | 10,3 |

Au cours de ces 10 semaines (fig. 1), on constate une croissance relativement parallèle des 4 variantes du test ; le poids moyen individuel par variante évolue de 125 — 140 mg à 3.500 — 4.000 mg, quand la température de l'eau est de 11 — 18°C (fig. 1). Entre les variantes on remar-

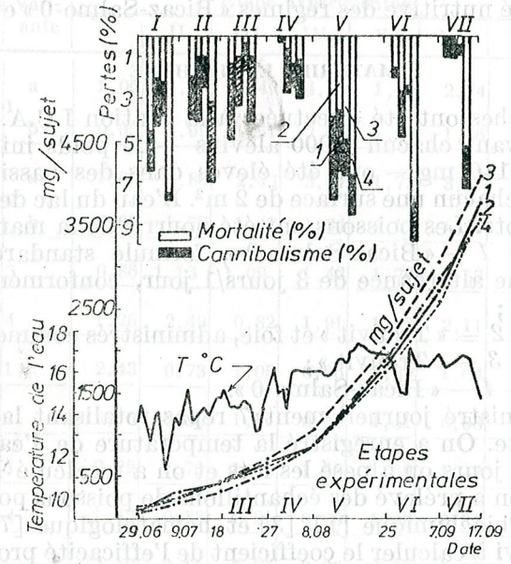


Fig. 1. — La dynamique de la croissance individuelle (mg/sujet) et des pertes d'effectif chez les alevins de truite arc-en-ciel nourris avec des régimes « Trouvit » et « Bicaz-Salmo-0 »

que cependant des différences concernant la vitesse de croissance des poissons, car le coefficient de multiplication du poids initial moyen individuel oscille entre 26,6 (var. 4) et 29,0 (var. 3). On a constaté une meilleure croissance dans la variante 3, où l'on a administré exclusivement le régime « Trouvit », qui s'explique par la composition plus complète et plus adéquate de la diète « Trouvit » due à la farine de sang, farine de hareng, extrait de poisson sec, et d'autres ingrédients. La variante 4 a enregistré la valeur la plus réduite.

Ajoutons encore le niveau comparatif des pertes d'effectif qui selon le tableau 2, est de 47,5 % pour la var. 4, par rapport à 21,5 % pour la var. 3. Si l'on analyse la provenance de ces pertes on constate que la mortalité de la var. 4 est approximativement 2, 4 fois plus grande que dans la var. 3 ; les pertes dues au cannibalisme sont, pour cette variante, 2 fois plus grande. La fig. 1 indique qu'au cours de l'expérimentation (sauf la III$^{e}$ étape) tant le taux de mortalité que celui de pertes totales ont eu des valeurs plus élevées pour la variante 4.

On retient encore (tableau 2) que la valeur des pertes dues au cannibalisme dans la variante 3 représente 50 % des valeurs des autres 3 variantes, ce qui s'explique d'une part par le développement plus homo-



*Tableau 2*

Tableau résumé des résultats de l'analyse comparative de l'efficacité des régimes « Trouvit » et « Bicaz-Salmo-0 » chez les alevins de truite arc-en-ciel.

| Paramètre analysé | VARIANTE 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Effectif initial (sujets) | 1.000 | 1.000 | 1.000 | 1.000 |
| Effectif final (sujets) | 735 | 742 | 785 | 525 |
| Mortalité (%) | 13,9 | 13,0 | 14,0 | 33,1 |
| Cannibalisme (%) | 12,6 | 12,8 | 7,5 | 14,4 |
| Poids initial (mg/sujet) | 135 | 127 | 140 | 135 |
| Poids final (mg/sujet) | 3.779 | 3.625 | 4.057 | 3.590 |
| Rapport de la multiplication du poids initial individuel (×) | 27,99 | 28,54 | 29,92 | 26,59 |
| CN a — mélange humide | 2,36 | 2,39 | — | — |
| b — mélange sec | 1,44 (100 %) | 1,45 (101,3 %) | 1,43 (76,5 %) | 1,87 (100 %) |
| CEP | 1,10 (100 %) | 1,16 (105,4 %) | 1,39 (143,3 %) | 0,97 (100 %) |
| CUP | 5,15 (100 %) | 5,03 (97,7 %) | 4,15 (71,7 %) | 5,79 (100 %) |
| CPC | 909 (100 %) | 860 (94,6 %) | 717 (69,8 %) | 1.027 (100 %) |
| Protéines carcasse (%) | 17,15 | 17,09 | 17,30 | 17,27 |
| Lipides hépatiques (%) | 9,09 | 15,38 | 9,54 | 9,58 |
| Hémoglobine (g/100 ml) | 6,49 | 6,59 | 4,51 | 6,78 |
| Érithrocytes/ml (x 1.000) | 642 | 946 | 1.116 | 1.225 |
| Hématocrite (%) | 53 | 58 | 33 | 37 |

(Pour explications voir encore tableau 1)

gène des alevins (réduisant les incidences de cannibalisme) et d'autre part par le spectre protéique plus complet de cette diète quant aux protéines d'origine animale. On constate aussi que pour la variante 3 la conversion (tableaux 1 et 2) présente des valeurs de 25 % plus avantageuses que pour le témoin (var. 4) et les valeurs de CEP, CUP et CPC (voir tableau 1) sont dans cette variante plus avantageuses que pour la variante 4(31 — 43 %).

Si l'on fait une appréciation d'ensemble sur les variantes mises en discussion jusqu'à présent (3 et 4) on constate des différences quantitatives et qualitatives en faveur du régime « Trouvit ». Mais si l'on ajoute au tableau des observations les données des variantes 1 et 2 où l'on a appliqué *le procédé de la nutrition alternative*, on remarque certains aspects différents de ce qu'on vient de montrer.

Du point de vue de la croissance individuelle, la variante utilisant le régime « Trouvit » évolue presque parallèlement à la variante 1, la valeur finale de la variante 2 étant plus basses que celle de la variante 1 ; les coefficients de l'augmentation du poids initial peuvent être également comparés. En appliquant le procédé de la nutrition alternative, la différence des rythmes de croissance entre la variante « Trouvit » et le régime autoch-

tone disparaît. De plus, la mortalité et les pertes dues au cannibalisme (tableau 2) sont comparables.

L'utilisation métabolique de la nourriture est aussi améliorée par le régime « Bicaz-Salmo-0 » par rapport à la variante 4 ; elle est semblable aux valeurs de la variante « Trouvit ». Ainsi, les valeurs de la conversion de la nourriture calculées pour toute la période sont de 1,44 (var. 1) et de 1,45 (var. 2). Les valeurs des 2 variantes sont aussi semblables dans les cas du CEP, du CUP et du CPC. La seule différence considérable se réfère au niveau des lipides hépatiques des poissons en expérience : 15,4 % (var. 2) par rappport à 9,1 % (var. 1).

Si l'on compare les 2 variantes « Trouvit » (2 et 3), on observe que dans la variante 2 qui remplace 25 % de la nourriture concentrée de la ration par du foie de veau, le taux de la croissance individuelle est plus réduit (de 11 %), ce qui est influée par la mise en valeur métabolique des protéines de la nourriture (CEP, CUP et CPC). Le taux de la mortalité diminue de 1 % dans la variante 2, le nombre d'érythrocytes baisse aussi (16 %), alors que la concentration des lipides hépatiques s'accroît de 60 % ; la concentration de l'hémoglobine augmente de 45 % et la valeur de l'hématocrite de 25 % par rapport à la variante 3.

En comparant entre elles les variantes 1 et 4 de la nourriture « Bicaz Salmo-0 », on constate que si le foie de veau remplace 25 % de la diète concentrée, conformément au procédé d'alimentation alternative, on obtient les effects suivants : le taux de mortalité est réduit de 2,4 fois et les incidences de cannibalisme de 2 % ; le rythme de croissance augmente de 9,5 % ; la valeur de la conversion se réduit de 30 % ; le nombre d'érythrocytes représente 58 % de la valeur du témoin et l'hématocrite croît de 43 %.

## CONCLUSIONS

1. Administrée en exclusivité, la nourriture « Bicaz-Salmo-0 » provoque aux alevins de truite arc-en-ciel un ralentissement de croissance de 11 % par rapport à la variante « Trouvit », le taux de mortalité augmente de 14 à 33 %, les pertes dues au cannibalisme évoluent de 7.5 à 14,4 %. On a constaté encore une réduction de 24—31 % du CN, CUP et CPC pour la variante « Trouvit » par rapport à la diète « Bicaz-Salmo-0 » et une augmentation de 43 % du CEP.

2. Lors de l'administration alternative de foie d'abattoir (3 jours/1 jour), la différence entre les 2 régimes disparaît si l'on considère la plupart des aspects analysés. Ainsi la croissance individuelle est comparable, sinon légèrement réduite dans la variante « Trouvit » et les taux des pertes (mortalité et cannibalisme) sont presque les mêmes pour les 2 régimes. La mise en valeur métabolique de la nourriture (CN, CEP, CUP et CPC) enregistre des niveaux semblables, tout comme la concentration des protéines de la carcasse, le niveau de l'hémoglobine et de l'hématocrite. Il y a des différences concernant le niveau des lipides hépatiques, qui est supérieur de 69 % à la variante « Trouvit » et le nombre d'érythrocytes/ml de sang, qui est également supérieur (47 %) par rapport au régime «Bicaz-Salmo-0 ».



3. Ayant amélioré la composition de la diète « Bicaz-Salmo-0 » on peut déjà envisager son application pratique. En utilisant le procédé d'alimentation alternative, les résultats obtenus peuvent être comparés à ceux du régime d'importation. Compte tenu du prix de revient de la nourriture « Trouvit » et du fait qu'il s'agit d'un produit d'importation, on considère comme opportune l'utilisation des 2 brevets roumains dans l'élevage industriel des alevins de truite arc-en-ciel.

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* *Institut de recherches et aménagements forestières Bucarest*
*Station de recherche et production Salmonicole Potoci*
*Potoci, département de Neamț*
et
** *Entreprise agricole d'Etat Prejmer*
*Secteur de salmoniculture*
*Prejmer, département de Brașov*

# RATE AND AMPLITUDE OF VOLUME CHANGES IN THE SWIMMING BLADDER OF *SALMO TRUTTA LACUSTRIS* L.

I. MIRON

Using an experimental model with two submerged laboratories set at depths of 0.50 m and 10 m in Lake Bicaz we could make continuous observations on the movements of *Salmo trutta lacustris* L. The fish would move through a circular tunnel long of 31.4 m placed in a vertical position. The findings have revealed that the trout made a complete tour of the tunnel in 57.5 sec. It performed continously a succession of 122 tours without being affected by changes in the bladder volume which, between 0.5 m and 10m on the vertical, kept decreasing and returning to normal in proportion of 50 per cent.

Aquaculture nowadays is concerned with identifying some new species of water organisms adaptable to rearing in captivity conditions in order to continue the ample process of domesticating and increasing the biological potential [1].

A species well represented in the salmonicolous limnofauna of big reservoirs is the lake trout, detected in Lake Tarniţa on the Someş river and in Lake Izvorul Muntelui — Bicaz. It weighs 16 kg and 8 kg, respectively. The literature reports specimens of 30 kg [2].

The choice food of *Salmo trutta lacustris* L. is *Alburnus alburnus L.* which it catches by violent attacks, delving sometimes above water surface. Rearing in captivity implies floating cages at variable depths and artificial diet consisting largely of pellets. The distribution of these pellets compels the fish to rapid and frequent movements on the vertical for reaching them.

Our study was aimed at following the way in which fish can adjust the rate and amplitude of their swimming bladder in order to easily catch the pellets in cages with depths of down to 10 meters.

Recent studies on the role of the swimming bladder in the vertical movement [3] of fish have confirmed that changes in the bladder volume are caused by their movement and not by specific muscular contraction.

## MATERIAL AND METHOD

In order to enable the fish to move on the vertical and at the same time to observe the rate, frequency and horizon of their movement, we devised a circular tunnel with a diameter of 10 meters (Fig. 1), square-shaped in cross section with a side of 40 cm; the tunnel walls were made



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of 10 mm mesh side relon network, supported by a framework of pvc tubes; the tunnel was placed in a vertical position with minimum and maximum depth sections attached to the windows of two submerged laboratories, one at 0.50 m and the other at 10 m deep. The biological material consisted of six lake trout specimens aged 1 year and six months, bred in floating cages. The trout originated from wild genitors collected from Lake Bicaz, the lake in which the present experiment was carried out. The fish were introduced in the upper part of the tunnel.

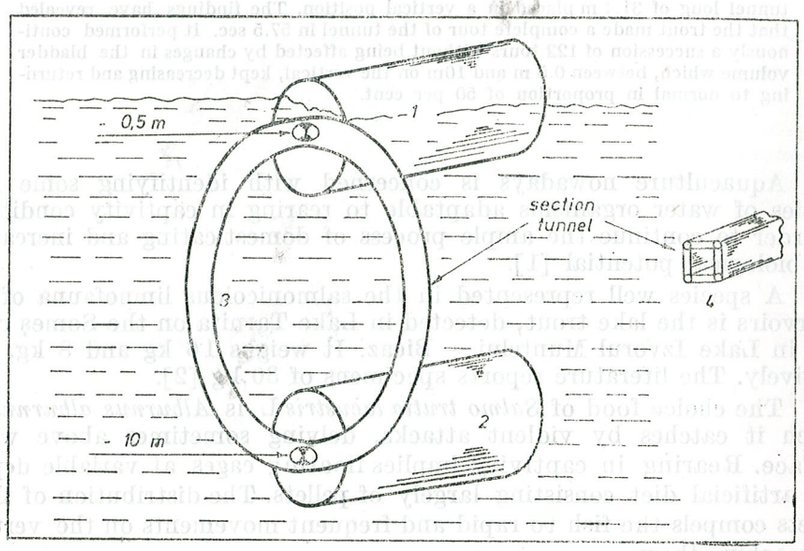


Fig. 1.— Scheme of the experiment with the two levels of observation : 1. submerged laboratory 1 ; 2. submerged laboratory 2 ; 3. circular tunnel for trout mouvement ; 4. tunnel cross-section.

## RESULTS

After a period of accommodation that lasted 18 hours from the moment the fish had been introduced into the tunnel, in which interval they moved randomly therein, we noticed an incipient relative orderly movement in the whole batch, viz. they began making some complete, counterclockwise tours of the tunnel. The average duration of a complete long tour of 31.4 m was 57.5 sec ; during one hour they performed 62.6 rotations, i.e. 1965.6 m/h ; by the end of the second hour this regularity was disturbed.

These observations indicate that within an interval of 28.75 sec, the bladder volume decreased and increased by 50 %.



In conclusion, the high frequency of swimming bladder changes proves a capacity of adaptation to rapid movements along a 10 m vertical line in the lake trout.

*Acknowledgement.* Thanks are due to Academician Petre Jitariu for promoting our submerged laboratory working method.

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*Salmonicolous Research and Production Station*
*5650 — Bicaz P.O.B.2*

# MECHANISMS OF OSMOTIC ADAPTATION OF SOME *MOLLUSCA* SPECIES TO HYPOSALINE CONDITIONS

V. CRĂCIUN, MARGARETA CRĂCIUN and I. NEACȘU

Water and $Na^+$, $K^+$ and $Ca^{2+}$ distribution in extra- and intracellular media has been determined for two marine mollusca species (the mussel and the white shell), in conditions of medium dilution. The two species have proved to be osmoconform ones, revealing certain osmoregulation possibilities, too. The $Ca^{2+}$ accumulation phenomenon in cellular organites has been evidenced as one of the mechanisms of osmotic adaptation to hyposaline media.

## INTRODUCTION

One of the main restricting factors of the aquatic organisms spreading is the osmotic one. Having in view the great variability of this factor on the Romanian coastline of the Black Sea, it has to be considered in the scientific guiding of some animal cultures of this region.

Animals adapt themselves at the osmotic variations of the medium in two ways; some of them are osmoconform ones, possessing an internal medium whose osmolarity follows exactly the osmotic variations of the medium; others are osmoregulator organisms, having the possibility of maintaining a constant osmotic internal medium, regardless of the osmotic variations of the water in which they live.

Osmoregulation having an evolutive value higher than osmoconformism, a great number of intermediary forms has normally developed between these two extremes, showing more or less efficient osmoregulation possibilities. It is the case of most mollusca in the Black Sea.

The great majority of the mechanisms by which animals accomplish their osmoregulation possibilities is to be found at the membrane level and are either passive, based on adaptive modifications of the membrane permeability, or active, requiring a metabolic energy contribution.

## MATERIAL AND METHODS

Experiments have been performed at Romanian Institute of Marine Research — Constanța with two aboriginal marine mollusca species (the mussel and the white shell). Animals have been transferred from normal marine water to less salty water, having salinities of $12‰$, $8‰$ and $6‰$, in order to measure the water and $Na^+$, $K^+$ and $Ca^{2+}$ distribution in the extra- and intracellular medium, at intervals of 1, 3 and 7 days after the salinity decrease.

The determination of the $Na^+$; $K^+$ and $Ca^{2+}$ intracellular concentrations has been performed by the flamephotometric method, the correction for the inulinic space being made.

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## RESULTS

An increase of the intracellular content of water, penetrated osmotically from the extracellular medium, has been noticed with the two species used in the experiments (Fig. 1). This phenomenon reaches its

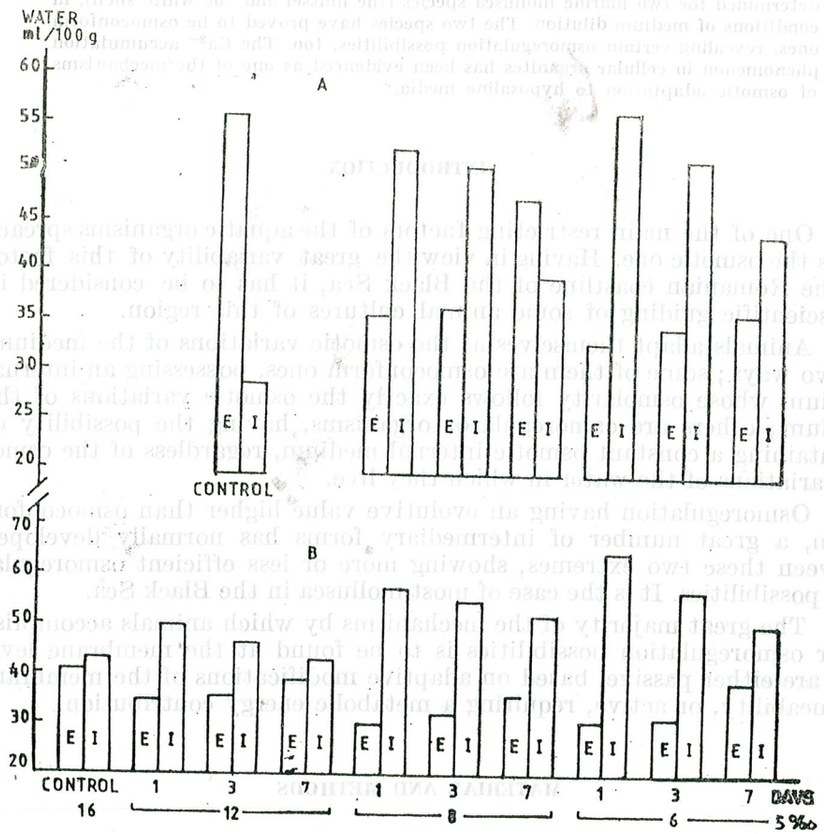


Fig. 1. — Extracellular (E) and intracellular (I) water content in mussel (A) and white shell (B) before (Control) and after the salinity ($S^0/_{00}$) diminishing.

maximum amplitude in the first day after the salinity decrease, after 3 and, respectively, 7 days, a tendency of the cellular volume to return to the initial values being observed.

The $Na^+$ intracellular content decreases with the decrease of the water salinity, especially in the first day of the experiment (Fig. 2). Nevertheless, much more pronounced is the $K^+$ loss (Fig. 3) but both the $Na^+$ and the $K^+$ loss is not as high as expected from the modifications of the concentration gradients, determined by the dilution of the external medium.



Things are altogether different as regards the calcium intracellular content, which decreases only in the first day of the experiment, a spectacular $Ca^{2+}$ accumulation in the intracellular medium — exceeding the initial values — being subsequently recorded (Fig. 4).

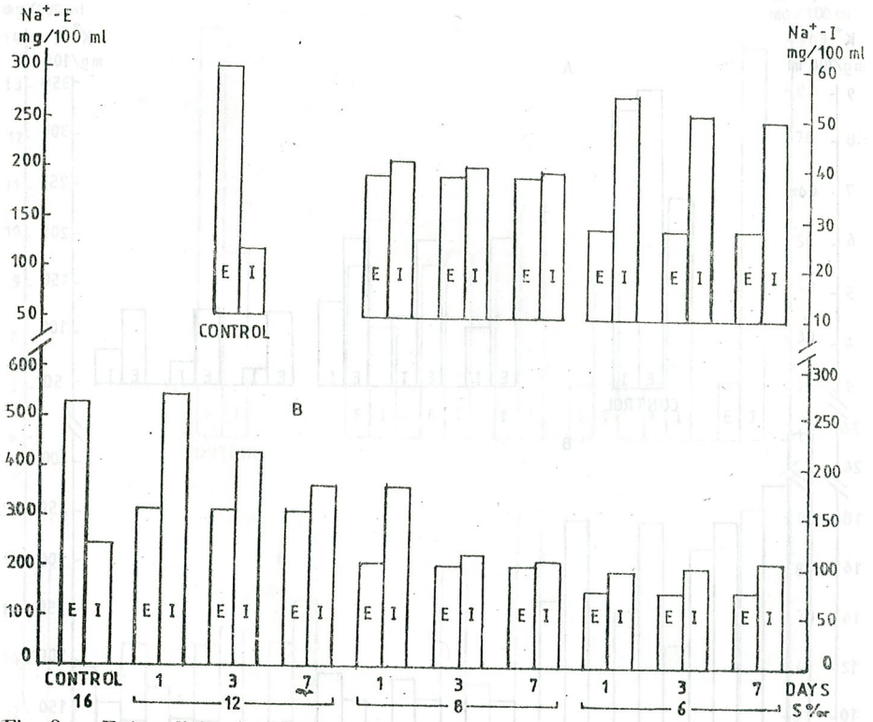


Fig. 2. — Extracellular (E) and intracellular (I) sodium concentration in mussel (A) and white shell (B) before (Control) and after the salinity ($S^0/_{00}$) diminishing.

## DISCUSSIONS

The increase of the intracellular volume as a result of the decrease of the water salinity proves that the species used in our experiments are osmoconform animals. Water penetrates in the direction of the osmotic gradient, from the extracellular medium — more diluted with the salinity decrease — to the more concentrated intracellular medium. The $Na^+$ and $K^+$ (in the first day, $Ca^{2+}$ too) ions exhibit an inverse movement, diffusing from the intracellular in the extracellular medium, according to their concentration gradient, obtained by the marine water dilution.

Nevertheless, this pasive ion movement does not lead to the annulment of the ionic gradients between the two media, due to the existence of certain membrane mechanisms, limiting the excessive losses of intracellular ions. One of these is the so-called "solvent drag" effect [1] which supposes the acceleration of the solvite flux in the direction of the osmotic

water flux and, obviously, the slowing down of the flux in the opposed way. In our case, $Na^+$, the main extracellular cation, shows an acceleration of the influx in the direction of the water movement, which invades the cells, while $K^+$, the main intracellular cation, exhibits a decrease of the efflux in the opposed way of the water movement. That is why the

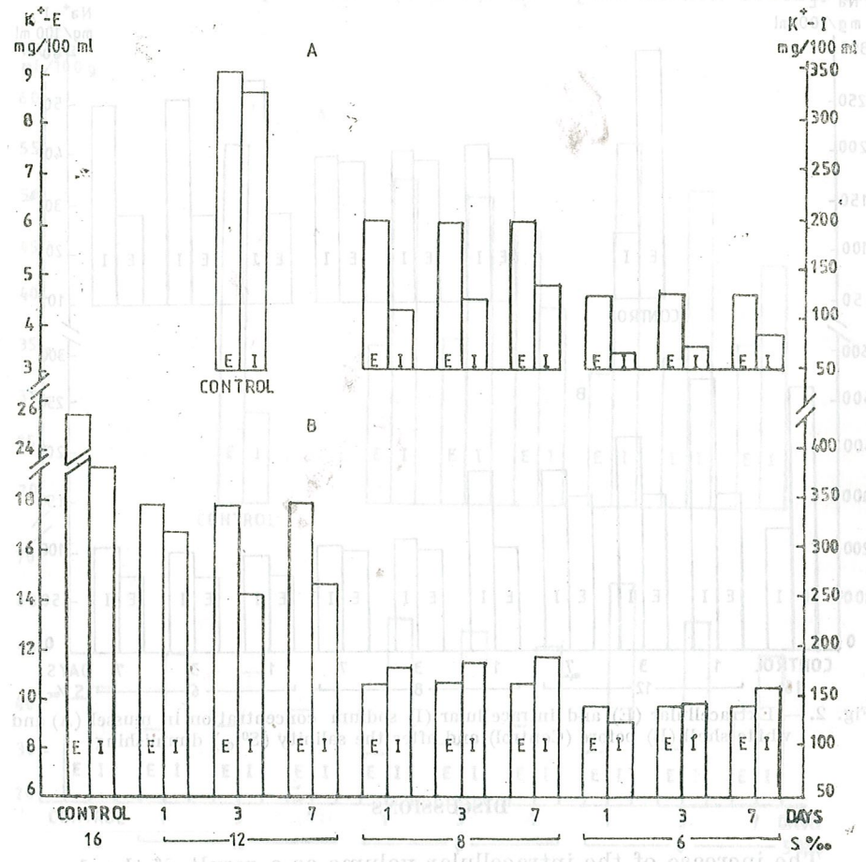


Fig. 3. — Extracellular (E) and intraccellular (I) potassium concentration in mussel (A) and white shell (B) before (Control) and after the salinity ($S^0/_{00}$) diminishing.)

dilution of the surrounding medium does not lead to a suitable dilution of the intracellular medium.

Another phenomenon is the active ion transport through cell membranes, in opposite direction to their concentration gradient. The most frequently used mechanism of active transmembrane ion transport is the $Na^+ — K^+$ pump which, as mentioned in another paper [3], is stimulated with these species in hypo-saline conditions.

By the pump activity, the $Na^+$ ion (which can be replaced — under certain circumstances — by $H^+$) is eliminated, while $K^+$ is reintroduced



in the cells. Concomitantly with the $Na^+$ (or $H^+$) elimination from cells, the excess water — osmotically penetrated in the cytoplasm — can be also eliminated by the "pump leak" phenomenon [5]. According to this model, by the $Na^+$ or another ion pumping outside the cell, this one

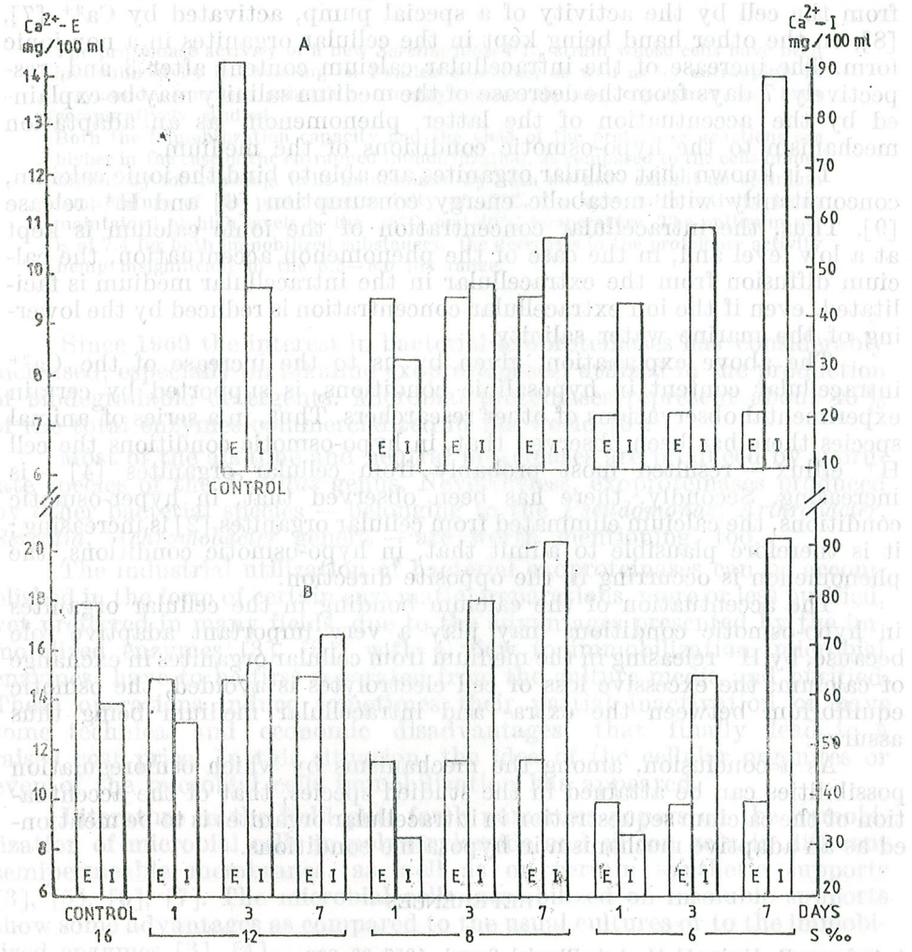


Fig. 4. — Extracellular (E) and intracellular (I) calcium concentration in mussel (A) and white shell (B) before (Control) and after the salinity ($S^0/_{00}$) diminishing.

behaves like an "impermeant" ion, counterbalancing the excess of osmotic intracellular pressure and preventing thus the exaggerated increase of the cell volume. The fact that this happens too in our experiments is proved by the tendency of reversibility in time of the cellular volume increasing, observed by us.

The intracellular calcium loss, noticed in the first day of the experiment is also a manifestation of the osmoconform behaviour of the studied species. However, the subsequent accumulation of calcium in cells

suggests the intervention of an adaptive, metabolic energy-consumer mechanism.

Usually, the intracellular concentration of free ionic calcium is maintained at very low levels, this ion being, on the one hand, eliminated from the cell by the activity of a special pump, activated by $Ca^{2+}$ [7], [8], on the other hand being kept in the cellular organites in a non-ionic form. The increase of the intracellular calcium content after 3 and, respectively, 7 days from the decrease of the medium salinity may be explained by the accentuation of the latter phenomenon, as an adaptation mechanism to the hypo-osmotic conditions of the medium.

It is known that cellular organites are able to bind the ionic calcium, concomitantly with metabolic energy consumption [6] and $H^+$ release [9]. Thus, the intracellular concentration of the ionic calcium is kept at a low level and, in the case of the phenomenon accentuation, the calcium diffusion from the extracellular in the intracellular medium is facilitated, even if the ion extracellular concentration is reduced by the lowering of the marine water salinity.

The above explanation, given by us to the increase of the $Ca^{2+}$ intracellular content in hyposalinic conditions, is supported by certain experimental observations of other researchers. Thus, in a series of animal species there has been observed that, in hypo-osmotic conditions the cell $H^+$ efflux — resulted most probably from cellular organites [4] — is increasing. Secondly, there has been observed that, in hyper-osmotic conditions, the calcium eliminated from cellular organites [2] is increasing; it is therefore plausible to admit that, in hypo-osmotic conditions, the phenomenon is occurring in the opposite direction.

The accentuation of the calcium bonding in the cellular organites in hypo-osmotic conditions may play a very important adaptive role because, by $H^+$ releasing in the medium from cellular organites in exchange of calcium, the excessive loss of cell electrolytes is avoided, the osmotic equilibrium between the extra- and intracellular medium being thus assured.

As a conclusion, among the mechanisms by which osmoregulation possibilities can be attained in the studied species, that of the accentuation of the calcium sequestration in intracellular organites is to be mentioned as an adaptive mechanism in hyposaline conditions.

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*Biological Research Center*
*Iași, Calea 23 August, 20A.*

# IMMOBILIZATION OF EXOPROTEINASES PRODUCING CELLS OF *SARCINA LUTEA*

N.D. TOPALĂ, D. C. COJOCARU and OCTĂVIȚA AILIESEI

The proteinase activity of a new *Sarcina lutea* $PI_{18}$ strain whose cells have been immobilized by ion bonding on Ponilex anionites, as well as by entrapping in acrylamide — maleic anhidride — methylenbisacrylamide copolymer has been comparatively studied.
Both the immobilization capacity and the yield of the proteinase reactions are higher in the case of the entrapped immobilization, as compared to the cells immobilized by ion bonding. Cells immobilized by both methods exhibit an optimum temperature of the proteinase activity of 37°C, the enzymatic activity being maintained at high levels in the 25°C and 40°C temperature. The optimum pH is of 7.4 for both immobilized substances, the decreases in the proteinase activity being insignificant in the 6.5—8.0 pH range.

Since 1960 the interest in bacterial exoproteinases has considerably increased, especially on alkaline exoproteinases, utilized in the production of biodegradable detergents. Microbial proteinases represent about 40% of the total enzymes commercialized in the world [9].

Most of the alkaline and neutral proteinases are produced by sporulate species of the *Bacillus* genus. Nevertheless, exoproteinases produced by other bacterial species — belonging to the *Pseudomonas*, *Arthrobacter*, *Serratia*, *Achromobacter* genera — are worth mentioning, too.

The industrial utilization of bacterial exoproteinases can be accomplished in the form of certain enzymatic preparations, more or less purified, yet preferred in many fields, due to the advantages presented by the immobilized enzymes [3], [8]; with a view to immobilization, microbial enzymes have to be first separated from the culture media and purified. These operations induce sometimes their partial inactivation or have some technical and economic disadvantages, that finally lead to a raised cost price. In this situation, the idea of the cellular organites or even of the microbial cells immobilization has appeared.

Literature in the field puts forth numerous papers on the immobilization of microbial cells in polyacrylamide and silicon gels, in different semipermeable membranes, as well as on certain synthetic supports [3], [5], [6], [7]. The microbial cells immobilized on insoluble supports show some advantages as compared to the usual cultures or to the immobilized enzymes [3], [4].

Regardless of the fact that microbial cells maintain or not their vital activity by immobilization, they keep their ultrastructure and their enzymatic equipment in active state, for a longer or shorter period of time.

The present paper presents a series of experimental data regarding the proteolytic activity of *Sarcina lutea* $PI_{13}$ cells, immobilized by ionic bonding on Ponilex anionites, as well as by entrapping in acrylamide — — maleic anhidride — methylenbisacrylamide copolymer.

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## MATERIAL AND METHODS

Experiments have been carried on *Sarcina lutea* $PI_{13}$ isolated by one of us from residual waters. At isolation with caseine on a solid medium this strain showed a remarkable exoproteinase activity.

For the quantitative determination of the proteinase activity, the *Sarcina lutea* $PI_{13}$ has been cultivated with caseine on a liquid medium, at 24 and 28°C, on a 240 r.p.m. rotative stirrer, for 72 hours, determinations of the specific activity — by the Kunitz method [2] — being performed at 24, 48 and 72 hours.

For immobilization there has been used a 48 hours-aged cellular suspension, obtained in submerged culture at 24°C and separated by centrifugation at 10,000 r.p.m. for 10 minutes. Bacterial cells have been washed several time with natrium chloride isotonic solution, then suspended in the same solution, thus inducing the appearance of the dry substance in the obtained suspension.

The immobilization by ion bonding has been performed in the "bath" system on the Ponilex AS-49 anionite, activated by a previously described method [8] and balanced with a 0.05 M phosphate buffer solution, pH — 7.4, while, in the case of entrapped immobilization, the monomer (acrylamide, maleic anhidride and methylenbisacrylamide) concentration used was of 7.5, 5.25 and 1.8% respectively, the last value being established as optimum.

The proteinase activity has been determined by the Kunitz method [2] by dosing the hydrolysis products released from caseine under enzyme action, with the Folin-Ciocâlteu reagent, after the excess caseine precipitation with trichloracetic acid. The unit of the proteinase activity of the immobilized *Sarcina lutea* $PI_{13}$ cells has been found to be equal to the amount of the hydrolysis products, in tyrosine equivalents, split from the substrate by 1 g cells (d.s.) for 1 minute at 37°C and has been expressed in μM tyrosine/min/g dry cells.

From both immobilized substances samples have been stored at +4°C and +20°C, their activity being daily determined with a view to the establishment of the functional characteristics.

## RESULTS AND DISCUSSION

In the literature of the field, *Sarcina lutea* is not cited as an exoproteinase growing species. Nevertheless, in 1977, Bissel et al. [1] showed that, in the case of *Sarcina sp.*, certain amino acids have an inhibition effect on the proteinase synthesis, without specifying the type of proteinases implied.

The *Sarcina lutea* $PI_{18}$ submerged cultivated, on a rotative stirrer and in a 1% caseine medium, produces exoproteinases whose specific activity varies as a function of the culture time and temperature.

Table 1 shows that the maximum proteinase activity occurs at 48 hours, both in the case of cultures accomplished at 24°C, and at 28°C.

At the same time, an almost double proteinase activity is to be observed with cultures at 24°C, as compared to those at 28°C. By the



*Table 1*

Exoproteinasic activity of the *Sarcina lutea* $PI_{13}$ stain, expressed in micromole tyrosine/ml culture liquid/min.

| T°C / Culture time (hours) | 24°C | | | 28°C | | |
|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 24 | 48 | 72 |
| *Sarcina lutea* $PI_{13}$ | 0.0484 | 0.6452 | 0.4453 | 0.0340 | 0.3969 | 0.1319 |

immobilized *Sarcina lutea* $PI_{18}$ cells, there have been followed the optimum pH and temperature of the proteinase activity, the immobilization capacity, the gel concentration, as well as the yield of the proteinase reactions of the immobilized cells.

### a) OPTIMUM pH

In a first series of experiments, the optimum pH of the proteinase activity of the immobilized *Sarcina lutea* $PI_{18}$ cells has been determined, by using 0.05 M phosphate buffer solutions with different pH (6.0; 6.5; 7.0; 7.4; 8.0 and 8.5);. The experimental results obtained are presented in table 2 and graphically plotted in figure 1.

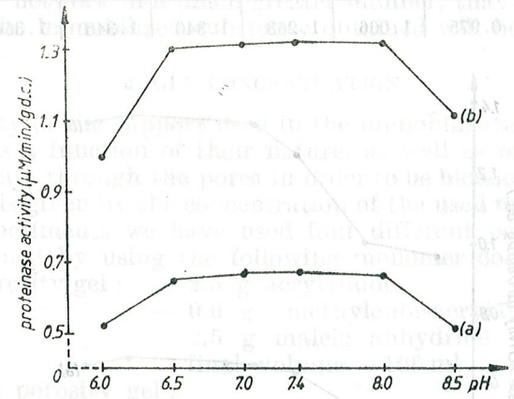


Fig. 1. — Dynamics of the proteinase activity of the *Sarcina lutea* $PI_{18}$ cells immobilized by ion bonding (a) and entrapping (b) as a function of their pH.

*Table 2*

Dynamics of the proteinase activity of the immobilized *Sarcina lutea* $PI_{18}$ cells, as a function of their pH

| Immobilization type | pH of the reaction medium | | | | | |
|---|---|---|---|---|---|---|
| | 6.0 | 6.5 | 7.0 | 7.4 | 8.0 | 8.5 |
| Ionic bonding | 0.525 | 0.650 | 0.667 | 0.670 | 0.662 | 0.510 |
| Entrapping | 1.072 | 1.305 | 1.318 | 1.325 | 1.320 | 1.115 |

As it can be seen from the data presented, the optimum pH of the proteinase activity of the cells immobilized by both methods is of 7.4 (0.670 μM/min/g d.c., in the case of ion bonding immobilization and 1.325 μM/min/g d.c. with the entrapped cells) still having high levels in the 6.5 — 8.0 pH range.

### b) OPTIMUM TEMPERATURE

In order to establish the optimum temperature conditions of the caseinolytic activity of the obtained immobilized substances, there have been performed proteolysis reactions in buffer 0.05 M phosphate solution, pH 7.4, in different thermal conditions (15, 2,0 25, 28, 30, 37 and 40°C), the obtained values being presented in Table 3 and Figure 2.

*Table 3*

Dynamics of the proteinase activity of the *Sarcina lutea* $PI_{18}$ cells as a function of the reaction medium temperature

| Immobilization type | Temperature of the reaction medium (°C) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15 | 20 | 25 | 28 | 30 | 37 | 40 |
| Ionic bonding | 0.423 | 0.518 | 0.640 | 0.656 | 0.660 | 0.675 | 0.667 |
| Entrapping | 0.975 | 1.066 | 1.268 | 1.340 | 1.345 | 1.360 | 1.350 |

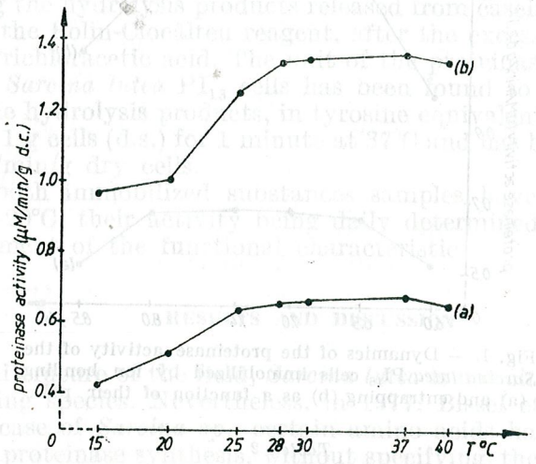


Fig. 2. — Dynamics of the proteinase activity of the *Sarcina lutea* $PI_{18}$ cells immobilized by ion bonding (a) and entrapping (b) as a function of the reaction medium temperature.

Immobilized cells by ionic bonding show an optimum of proteinase activity at 37°C, an insignificant decrease being registered in the 28 — 40°C temperature range. At lower temperatures (15, 20, 25°C) the caseinolytic activity suddenly decreases. A similar situation is to be observed too in the case of the entrapped cells having the same temperature optimum (at 37°C, they show an activity of 1.360 μM/min/g d.c.) the proteinase activity being maintained at higher levels, in a somewhat larger thermal range (25 — 40°C).



### c) IMMOBILIZATION CAPACITY

By determining the amount of dry substance in the influent cellular suspension, in eluent as well as in the washing waters there has been established — for both immobilized substances — the immobilization capacity (the cellular charge). The Ponilex AS-49 anionite, used in the immobilization by ionic bonding, possesses comparatively with the *Sarcina lutea* $PI_{18}$, an immobilization capacity of 1.37 mg dry cells/ml support, while the cellular charge of the entrapped cells is of 15.5 mg dry cells/ml support.

From the quantitative point of view, the immobilization capacity is much inferior in the case of the immobilization by ionic bonding (over 10 times). This can be explained if one takes into account the structural complexity of the microbial cell under study. The cell wall presents a multitude of functional groups which interact — probably — with those of the support during the process of immobilization by ionic bonding.

Nevertheless, in the case of entrapping, microbial cells are included in the copolymer network in a much greater number, that is why the cellular charge of the immobilized substance obtained will be higher.

### d) GEL CONCENTRATION

The porosity of the support used in the immobilization of microbial cells is chosen as a function of their nature, as well as of the substrate that is to penetrate through the pores in order to be biochemically modified. Gel porosity is given by the concentration of the used monomers.

In our experiments we have used four different porosities, which have been obtained by using the following monomer concentrations :

1. High porosity gel : — 2.5 g acrylamide
   — 0.6 g methylenbisacrylamide
   — 2.5 g maleic anhydride
   final volume —100 ml
2. Medium porosity gel :
   — 5 g acrylamide
   — 1.25 g methylenbisacrylamide
   — 3.5 g maleic anhydride
   100 ml
3. Low porosity gel : — 7.5 g acrylamide
   — 1.8 g methylenbisacrylamide
   — 5.25 g maleic anhydride
   final volume —100 ml
4. Very low porosity gel :
   — 10 g acrylamide
   — 2.5 g methylenbisacrylamide
   — 10 g maleic anhydride
   final volume —100 ml

By using with all the four porosities a constant amount of biomass, the proteinase activity of the obtained immobilized substances has been determined (Table 4, figure 3), this activity proving to be maximum in the case of low porosity.

*Table 4*

Dynamics of the proteinase activity of the *Sarcina lutea* $PI_{18}$ cells immobilized by entrapping, as a function of the gel porosity

| Gel porosity | high | medium | low | very low |
|---|---|---|---|---|
| Proteinase activity | 0.785 | 1.020 | 1.220 | 1.055 |

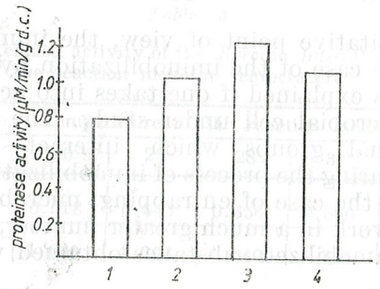


Fig. 3. — Proteinase activity of the *Sarcina lutea* $PI_{18}$ cells immobilized by entrapping, as a function of the support porosity: 1 — high porosity, 2 — medium porosity, 3—low porosity, 4—very low porosity.

e) YIELD OF THE PROTEINASE REACTIONS OF THE IMMOBILIZED CELLS

Due to the interactions appearing between the functional groups of the support and those of the cell constituents, the proteinase activity decreases by immobilization.

With a view to the establishment of the yield of this enzymatic activity, the specific proteolytic activity has been determined, both in the cell suspension and in the immobilized products.

The cell suspension showed an enzymatic activity of 1.935 μM/min/g d.c. while the immobilized substances exhibited in the optimum conditions established by the above-mentioned experiments, the following enzymatic activities: 0.674 μM/min/g d.c. in the case of the ion bonding and 1.350 μM/min/g d.c. with the entrapped cells, which represents 34.83 % and 69.77 %, respectively, of the activity of the free cells.

The more intense activity of the cells immobilized by entrapping can be explained perhaps by the fact that, in the case of ionic bonding, certain supplementary bonds may be formed between the functional groups of the substrate and those of the proteic macromolecules, their quaternary conformation being thus partially modified, a fact that leads to a partial inactivation of the component compounds.



## CONCLUSIONS

1. The *Sarcina lutea* $PI_{18}$, isolated from residual waters, proves to be a good neutral exoproteinase producer. The maximum specific activity (0.6452 micromole/ml/min) is obtained in stirred submerged cultures, after 48 hours, on a 1 % caseine medium, at 24°C.

2. The immobilization capacity is higher in the case of entrapped immobilization (15.5 mg cells d.c./ml support as compared to only 1.37 mg dry cells/ml support in the case of ionic bonding immobilization). The yield of the proteinolytic reaction of the immobilized cells, as compared to the free ones, is also higher with the entrapped cells (69.77 %) comparatively to the ionic bonded cells (34.83 %).

3. The optimum pH and temperature conditions are similar with both the immobilized substances. At 37°C, the cells immobilized by entrapping exhibit a proteinase activity of 0.675 micromole/min/g dry cells.

The proteinase activity is maintained at a high level in the 25 and 40°C temperature range. The optimum pH is of 7.4 for both immobilized substances, the decreases in the proteinase activity being insignificant in the 6.5 — 8.0 pH range.

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*"Al. I. Cuza" University*
*Laboratory of General Microbiology*
*Biological Research Center*
*Iassy, Calea 23 August 20A*

# INHIBITION OF SOME DEOXYRIBOVIRUSES REPLICATION IN TANNIC ACID PRETREATED CELLS. AN ELECTRON-MICROSCOPIC STUDY

GR. MIHĂESCU and D. PAPA

**The tannic acid is a chemical inductor of nucleolar macrosegregation phenomenon in HEp-2 line cells. The cells pretreated with tannic acid and infected with adenovirus 3, herpes simplex 1 or with vaccinia virus reveal no sign of mature progen virion formation, or synthesis of viral components precursors at the ultrastructural level. The inhibition of viral replication is due either to some irreversible cytotoxic effects expressed through cellular or viral DNA replication enzymes inactivation ar to an inhibition of viral DNA replication, due to alkilant effect of tannic acid.**

It is known that tannic acid in sublethal doses is toxic for the experimental animals, having an obvious toxic effect, especially for liver and kidney tissues [1, 2]. Unfortunately, the molecular mechanism of its action is largely unknown. There are nucleolar ultrastructural modifications expressed by a total and particular segregation process of its components known as "macrosegregation", which is different from that caused by other chemicals or biological agents [3]. It seems that tannic acid is an alkylating product which bounds to DNA molecule, preventing the DNA-dependent synthesis of RNA by blocking the molecular site to which RNA-polymerase is bounded, in order to initiate the transcription process. In doing such prevention, tannic acid is an inhibitor not only for transcription process, but also for translation, i.e. for protein synthesis.

This paper makes an analysis of cellular ultrastructural aspects related to infections with some deoxyriboviruses, such as adenovirus 3, herpes simplex 1 and vaccinia virus in tannic acid pretreated cells.

## MATERIALS AND METHODS

The HEp-2 line cells were supplied by the Cell Culture Laboratory of the Virology Institute, which had been grown on Eagle medium, supplemented with 10 % calf serum. When the monolayer was almost complete, the cells were tannic acid treated, in a concentration equivalent to 300 mg/l. At six hours after treatment, the cells were infected with adenovirus 3, herpes simplex 1 and vaccinia virus. At 24 hours after the viral infection, the cells cultures were prefixed with 2 % glutaraldehide and postfixed in 1 % osmium tetraoxide. The ultrathin sections were examined under electronic microscope (Phillips 201).

## RESULTS

The most characteristic aspect of the cells pretreated with tannic acid and infected with deoxyriboviruses, at ultrastructural level is nucleolar segregation as a consequence of redistribution of nucleolar components

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in separated areas, as granules and fibrils, both of them having in their composition, as main component RNA. The tannic acid induces an unusual segregation, structurally expressed as a condensation of granular component in one or several electrondense masses distributed in the area occupied by shrunken fibrillar component (Figs 1, 2). This is a macrosegregation phenomenon. The macrosegregation is induced by other chemicals or biological agents. There are no intermediary aspects in nucleolar segregation. The virus infection is usually followed by nucleolar segregation as a reflection of inhibition of RNA syntheses of the host cell, but the nucleolar abnormalities observed in our case are typical of a macrosegregation phenomenon induced by tannic acid.

The nucleolar macrosegregation is a reversible phenomenon. The profound modifications, perhaps irreversible are expressed by a migration of dense-granular blocks to the nucleolus periphery, a situation that corresponds to the "nucleolar capping" phenomenon (Figs 3, 4). The granular blocks migrate in the nucleoplasm where the dense nuclear bodies (Fig. 5) are initially constituted, then they desintegrate in granular fine subcomponents (Figs 7, 8).

Sometimes the nucleolus undergoes profound lesions : an increased electronic density, compactness and vacuolisation of fibrillar component a progressive loss of granular blocks (Fig. 6). These are irreversible modifications. The nucleoplasm loses an important part of its structural components. As a consequence, wide rarefied areas in the appeor nucleus in which one can see structures resembling nucleosomal structures (Figs 7, 9).

The cytoplasmic architecture undergoes deep modifications. The endoplasmic reticulum, as well as the nuclear envelope undergo a proeminent dilatation, after which they desorganize. Frequently, myelinic structures appear as a sign of intense degeneration processes (Fig. 10).

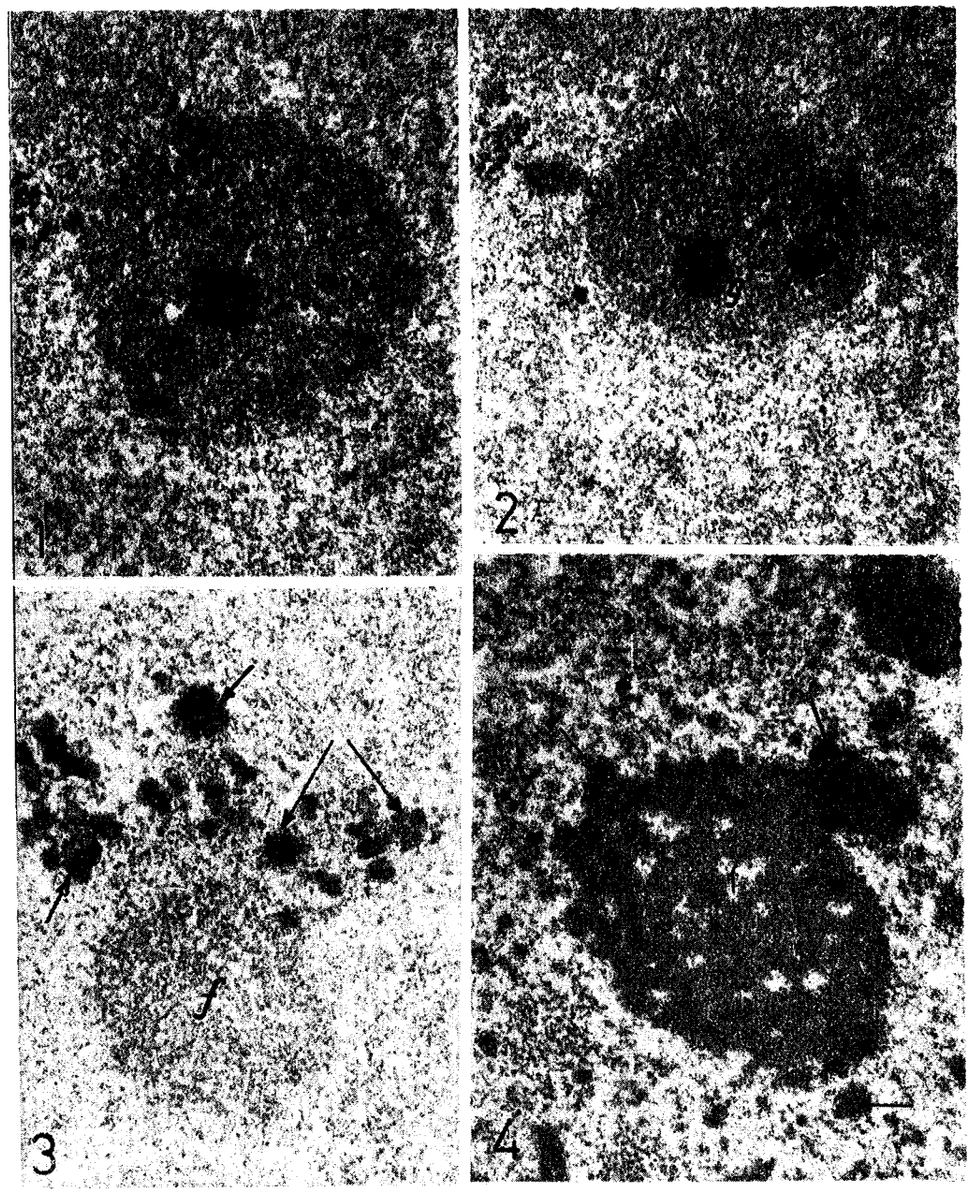
We have never observed the presence of viral particles or structural elements of their precursors. There is not a morphological sign of organization of viral nuclear foci (for the adenovirus 3 and herpes simplex 1, which replicate in the nucleus) or cytoplasmic for vaccinia virus, which replicates in the cytoplasm).

## DISCUSSIONS

The phenomenon of macrosegregation induced by tannic acid, seems to be a morphological expression of nucleolar biochemical alterations. Only antimetabolites which bound to DNA molecule and interfere with its capacity to function as a template for RNA synthesis, produce the nucleolar segregation phenomenon, while chemical agents which inhibit DNA or protein synthesis, do not produce such nucleolar lesions [4].

The blocking of DNA-RNA transcription, is followed by an inhibition of protein synthesis. Tannic acid has also reducing properties, its action being, eventually, an inactivation of some cellular enzymes.

The macrosegregation phenomenon produced by tannic acid is reversible [5]. But intense nucleolar alterations, whose result is a dispersion and a disaggregation of granular blocks, might be irreversible. The appearance of wide empty nucleoplasmic areas, depleted of their content,




Figs 1, 2. — The macrosegregation phenomenon. The two major components, fibrillar (f) and granular (g) are completly separated. The granular component is high electrondense : × 25.800.
Figs 3, 4, 5. — The fragmentation of granular nucleolar component and its dispersion (arrows) in the nucleoplasm. In Fig. 4, the fibrillar component is shrunken : ×39.200, 24.000 and 59.600 respectively.

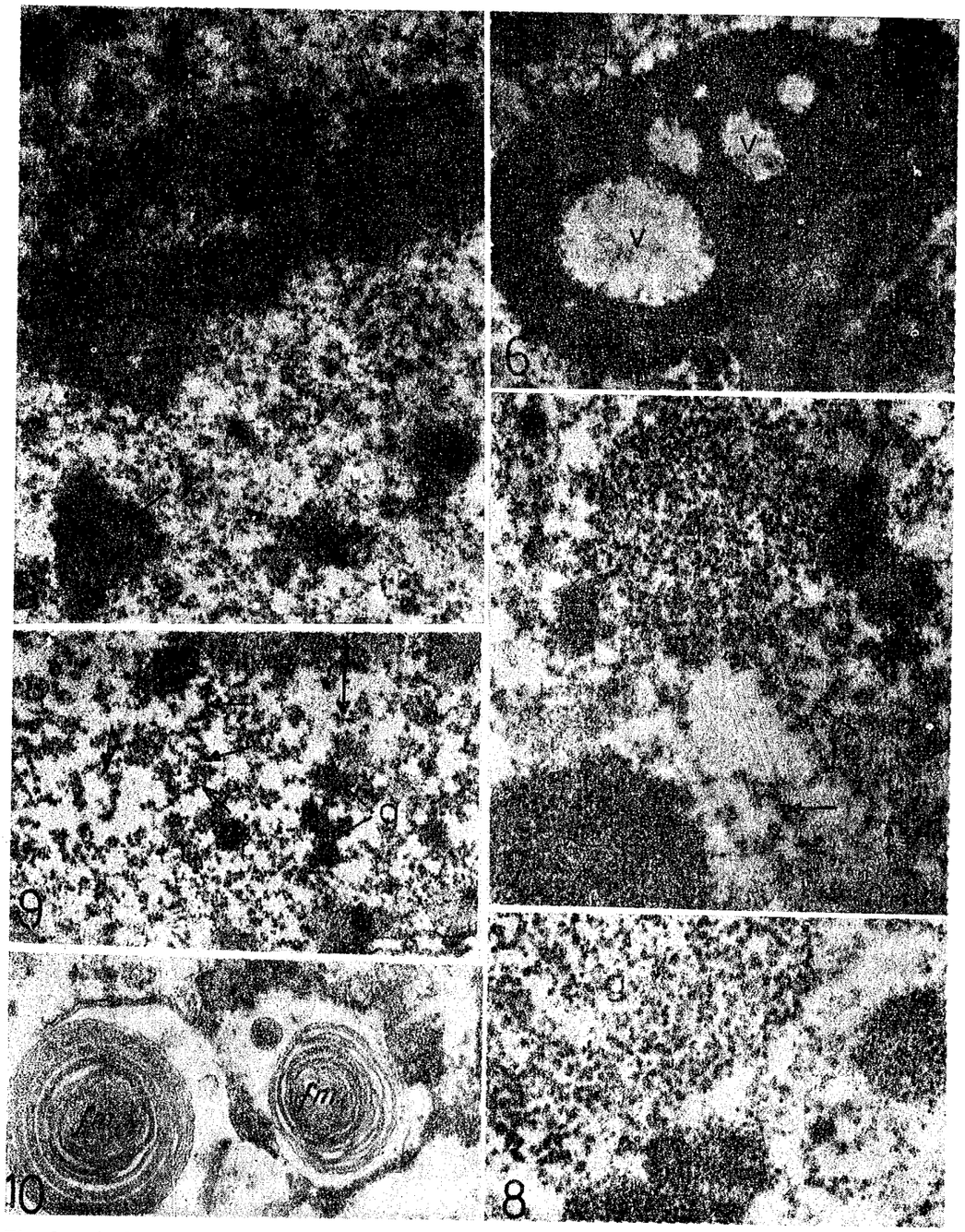



Fig. 6. — A shrunken and vesicular nucleolus (v.) The granular component is high electrodense. Such modifications seem to be irreversible : × 12.600.
Fig. 7. — The desaggregation of granular nucleolar blocks in the nucleoplasm leading to a lax accumulation of granular (g) structures. The arrows indicate nucleosome-like structures : × 25.600.
Fig. 8. — In the rarefied nucleoplasm, the granular nucleolar component is progressively diminished : × 30.000.
Fig. 9. — Nucleosome-like structures are frequently in such rarefied nucleoplasm (arrows) : × 12.600.
Fig. 10. — The presence of myellinic (fm) figures in the cytoplasm is a sign of high degree of cellular alterations : × 19.600.



might be the result of lysis processes induced by tannic acid through an unknown mechanism.

The infection with deoxyriboviruses of cells pretreated with tannic acid is not followed by a detectable appearance, either of progeny virions, or of their precursors. The cause of their absence in quantities morphologically detectable, is completely unknown. Is it possible for this aspect to be due to the impossibility of reading the viral genetic information, and as a consequence of its transcription into a viral mRNA. Viral genetic information transcription is not possible in a cell, where normal cellular genetic information transcription has been prevented. One may ask, what is the biochemical mechanism by which tannic acid acts as an inhibitor of deoxyribovirus replications ? It is either a bounding of this agent to viral DNA or the inhibition is due to its reduction effect on host-cell RNA-polymerase, which becomes inactive. A correct answer to this question is very difficult to give, because the replication inhibition takes place in the case of vaccinia virus, which possesses its proper RNA-polymerase, achieving very early DNA viral transcription, as well as in the case of adenovirus 3 and herpes simplex 1, whose genome is transcribed, by cellular RNA-polymerase II [4].

The possibility of a retardation of the viral replication process, which can start after reversion of tannic acid effects cannot be ruled out. By inhibition of cellular protein synthesis, virus replication remains in an early phase [4]. An exact answer to this question implies a dynamic study of viral replication within different intervals after the treatment of the cells with tannic acid.

There are morphological signs which signify that in some cells the lesions are irreversible. Their different specific reactivity is a reflection of their different sensitivity related to the cell cycle phase.

By its capacity to bound to DNA molecule or to inactivate the transcription enzymes, tannic acid has an effect analogous to viral nucleic acid inhibitors [6]. Due to its toxic and carcinogenic effects on hepatic and renal tissues [1, 2], the tannic acid cannot be used in the in vivo treatment of viral infections.

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*Institute of Biological Sciences*
*Bucharest, Splaiul Independenfei 296*
and
*University of Bucharest*
*Faculty of Biology*
*Bucharest, Splaiul Independenfei 91—95*

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