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The Romanian Academy, Bucharest, organized a conference to mark the 10th anniversary of the foundation of the International Society of Scientists and Peace.

SCIENTISTS AND PEACE

On 4—5 September 1981 the international symposium "Scientists and Peace" developed its proceedings in Bucharest, under the high patronage of the President of the Socialist Republic of Romania, Nicolae Ceaușescu.

The symposium was attended by 68 scientists from 32 countries among whom Nobel Prize winners, presidents of some academies of science, other prestigious scientific personalities, as well as general directors of international organizations.

In the opening session, they read the message of Nicolae Ceaușescu President of the Socialist Republic of Romania, to the participants in the Symposium.

Expressive of the lofty conception of the Secretary-General of the Romanian Communist Party, the President of the Socialist Republic of Romania, concerning the problems of peace and international security, détente and disarmament, cooperation and understanding among peoples in their efforts to build a new economic order in the world, as well as the scientist's responsibility for the solution of national and international issues by placing the most advanced achievements of the contemporary scientific and technical revolution exclusively in the service of the peoples' peaceful development, President Nicolae Ceaușescu's message made an appeal to scientists all over the world to close their ranks in order to fight the hazards which crises, confrontations and war pose to the future of mankind. The President's message was a guideline for the proceedings of the Symposium.

The participants expressed their support for the leading ideas contained in the message of the President of Romania.

The proceedings closed with an Appeal by the participants addressed to the scientists worldwide. Reflecting the basic ideas contained in President Ceaușescu's message, the appeal suggested the establishing of an Action Committee for the organization of the World Congress "Scientists and Peace".

As an extension of this prestigious international reunion was founded the National Romanian Committee "Scientists and Peace" that unanimously elected as president of the Committee and of the Executive Bureau Academician Elena Ceaușescu, D. Chem. Eng., first vice-prime minister of the Government of the Socialist Republic of Romania, President of the National Council for Science and Technology, illustrious political personality and internationally reputed scientist.

The Romanian National Committee "Scientists and Peace" adopted a comprehensive programme of scientific manifestations, meant to illustrate the contribution of Romanian scientists to the efforts of the Romanian people, alongside all people, for safeguarding peace.

The National Romanian Committee "Scientists and Peace" takes action on the international plane for the preparation of the World Congress "Scientists and Peace". The Committee is also represented at the Special Session of the General Assembly of the United Nations Organization devoted to disarmament.

Se 28 mai 1980 în Bucureşti va avea loc o serie de seminarii

seminarii care să analizeze situaţia actuală a dezvoltării mondiale

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MESSAGE

TO THE PARTICIPANTS IN THE INTERNATIONAL SYMPOSIUM "SCIENTISTS AND PEACE" FROM THE PRESIDENT OF THE SOCIALIST REPUBLIC OF ROMANIA, NICOLAE CEAUŞESCU

It gives me particular pleasure to send to you, the participants in the Symposium "Scientists and Peace" which opens today in Bucharest — distinguished figures in contemporary science and technology — cordial greetings and best wishes for the success of the meeting, so that it may give an impetus to the struggle of progressive forces throughout the world for world progress and peace.

The Socialist Republic of Romania attaches the greatest importance to scientific activities; it gives the achievements of science and culture a place among the very foundations of its efforts to construct the new socialist system, in the belief that they are vital factors for progress and civilization.

We are living in the era of the greatest advances in scientific thought that mankind has ever experienced throughout the ages, in the midst of the most awesome technical and scientific revolution, marked by extraordinary discoveries which are constantly changing man's ideas about nature, society and the universe and influencing all aspects of human existence. We see science directly influencing the never-ending change in the conditions of material production, the discovery of the secrets of matter and the increasingly efficient exploitation of our natural wealth and the development of the creative capacity of peoples.

The development of a nation — both in terms of enhancement of the productive forces of society and as regards thinking and mental creativity — is inconceivable without the input of advanced science and technology. One cannot even envisage the future of mankind without the major accomplishments of scientific thought.

However, we must candidly admit that a great many of the major discoveries of scientific research and technological innovation are currently used for the production of highly sophisticated weapons of mass destruction, from atomic weapons down. We are witnessing a particularly alarming emphasis on the arms race, and a major build-up of military arsenals capable of annihilating the entire planet and endangering the very existence of mankind.

We see tension growing, world-wide, as a result of the imperialist policies of domination, force and *diktat*. A strong tendency to consolidate and divide up spheres of influence is evident, and conflicts between States and groups of States are becoming more and more bitter.

At the same time, peoples throughout the world are asserting with increasing vigour their will to live in freedom, to develop in complete

independence, to put an end for ever to colonialism, neo-colonialism and all forms of oppression, to secure the democratic and progressive renewal of society, to ensure the welfare of the great working masses and to introduce a genuinely new set of relationships on the world scene involving full equality between countries, détente, collaboration and peace.

Socialist Romania is doing all in its power to develop its relations with all States irrespective of their social system. We base our relations with all countries on the principles of full equality of rights, profound respect for national independence and sovereignty, non-interference in internal affairs and non-use of force or threat of force. We believe that every effort must be made to stop the deterioration in the world situation, to ensure that all problems arising between States are settled exclusively by negotiation and to revive and pursue policies of détente and peace.

In the serious international situation which now obtains, scientists bear a tremendous responsibility for the present and future of mankind. No one knows better than the scientist or researcher the destructive power of modern weapons and the danger which the continuing arms race poses to civilization, to the security of peoples and to the very survival of humanity.

The choice between a policy of intensifying the arms race and manufacturing new nuclear weapons of mass destruction and a policy of disarmament, détente and peace is today a question of conscience.

There is no middle course !

It is obvious that scientists, who are very well aware of the destructive power of weapons, especially nuclear weapons, cannot but side with the policy of disarmament and peace. They therefore have a greater duty than ever to speak out and do all they can to ensure that the amazing attainments of the human spirit are no longer used in the manufacture of weapons of mass destruction, in preparing for war or to serve the policies of aggression, force and domination. The noblest task of scientists and researchers, in all fields and throughout the world, is to see to it that the entire potential of modern science and technology is devoted to the progress, welfare, freedom and independence of peoples and to the preservation of the supreme human right to life and to peace.

We must work with great determination and resolve for an end to the arms race, for disarmament, especially nuclear disarmament, for a cessation of the deployment and development of medium-range missiles in Europe, against the production of the neutron bomb, for a reduction in military budgets and armed forces, for the final abandonment of the use or threat of force in international life and for the creation of a world without weapons and without wars.

Under-development affects most of the population of the earth; nearly half a billion people suffer from chronic malnutrition. We must therefore establish a new international economic order guaranteeing free access by all peoples, particularly the least advanced, to the amazing achievements of the human spirit — a new international economic order which will guarantee the free flow of knowledge and discoveries and turn science into something that belongs to all mankind.

As the world economic crisis grows worse, science can play a particularly important role in the discovery and development of new sources

of energy and raw materials to be placed at the disposal of people everywhere, making the earth more fertile, increasing its output and solving the great food problem. It has an obligation to contribute to protecting the health of people throughout the world, combating pollution, improving the environment, protecting natural resources and transforming the earth into a verdant garden which can sustain a decent existence for all peoples.

Scientists and specialists in Romania, being profoundly dedicated to the interests of the people, devote all their energies to Romania's economic and social prosperity while at the same time co-operating actively with scientists of other countries in campaigning for progress, for disarmament and for the basic right of all nations to existence, peace and freedom.

Nowadays, the peoples, the masses, throughout the world play an essential role in determining the course of history.

Scientists, vitally involved in the cause of progress and peace, must fight side by side with the peoples for the right to live and work in peace, freely to build their own future without external interference or pressure, to devote their resources and energies to their material and spiritual well-being. Scientists, whatever their philosophical, political and religious views, must close ranks and, together with the peace-loving and anti-imperialistic forces throughout the world, take a stand against the imperialistic policy of domination, against war, and for a world of justice, equality and peace.

It is more important than ever to organize a world-wide front of scientists to act and convey their authoritative views to the United Nations, the Committee on Disarmament and other international bodies, concerning disarmament and the establishment of lasting peace on earth.

We are firmly convinced that united action by the men of science and culture, the workers and the progressive forces of all peoples can end the arms race and bring about a move to general disarmament, and in particular nuclear disarmament.

Let us do all we can to ensure for our children and grandchildren, for our generation and future generations, peace, freedom and happiness in a world without war, a more humane, more just and better world !

In keeping with these sentiments, I am convinced that this important meeting in Bucharest will have a great impact on researchers and scientists throughout the world, and I send you my most cordial wishes for much success and satisfaction in your noble work for the advancement of science, and for the cause of collaboration, peace and the independence of peoples.

NICOLAE CEAUŞESCU
President of the Socialist Republic
of Romania

Bucharest, 3 September 1981

A P P E A L
BY THE PARTICIPANTS IN THE INTERNATIONAL
SYMPOSIUM "SCIENTISTS AND PEACE"

Meeting in Bucharest on 4 and 5 September 1981 for the Symposium "Scientists and Peace" in order to discuss, in a wide-ranging and fruitful dialogue, the fundamental issue of the present day — peace, to which all mankind nobly aspires — we, the scientists from many countries of the world and from all continents, aware of the serious hazards which science and its servants may pose to the halting of the arms race, to continuing progress and to the future of the whole world, urgently appeal to scientists, researchers and intellectuals everywhere, and to all peoples, to join forces and co-operate ever more closely in defence of peace the supreme good of mankind.

The world today is witnessing not only the giant strides of science and technology, as evidenced by awesome discoveries affecting all areas of human existence, but also anachronistic actions which go against the interests of mankind, applying the products of science and technology to destructive ends injurious to the peace and freedom of peoples. Our age is one in which mankind is confronted by highly complex problems, with a new, frenzied arms race, unprecedented growth of military budgets, and the manufacture and development of new means of mass destruction, all of which severely aggravate the international situation, weighing more and more heavily on peoples and increasing the danger of conflagrations which may destroy life everywhere on earth and civilization itself, as it has evolved over the millenia.

Let us, in full awareness of the fact that scientists, faced with the alternative of peace or war, have a duty to defend peace, say a firm NO to war and armaments, this being not only our moral responsibility but essential to the continued existence of all mankind. We call on all scientists, whatever their political, philosophical, religious or other beliefs, to work side by side with the peoples of their countries to halt the deterioration in the international situation, the arms policy, so that we may resume and tirelessly pursue the course towards détente, peace and wide-ranging international collaboration !

Let us act now, before it is too late, now when we have so great a responsibility for the fate of mankind, to end the arms race, to bring about disarmament, especially nuclear disarmament, to create a world without weapons and without wars and to defend the basic right of individuals and peoples — the right to life and to peace.

Let us, as scientists more aware than anyone of the destructive power of modern weapons and the tremendous danger they present to the security of peoples and to the very survival of humanity, join forces more closely and act resolutely against the use of atomic energy for other than

peaceful purposes ! Let us do all we can to ensure that the immense potential of scientific and technical research is not used for weapons production but contributes exclusively to economic development and progress in every country, to the preservation of the finest that the human spirit has accomplished and to the creation of new and important values !

In present circumstances, when there exist numerous economic, social and political problems at the world level, it is our special duty as scientists constantly to increase our contribution to the solution of these problems for the well-being of all nations. Let us use our discoveries to close the great gaps between the rich and poor countries of the world, to eradicate the malnutrition and under-development affecting two thirds of the world population, to eliminate the diseases which continue to claim millions of human lives, and to protect the environment and conserve it for the benefit of future generations ! Let us exert every effort to discover new sources of energy and raw materials, to solve the problems of food, water supply, health, and so forth, on which depend the progress and the future of all mankind ! Let us resolutely oppose any obstacle to the movement of the world's scientific and cultural assets, so that all peoples can derive extensive benefit from the awesome accomplishments of science and technology, so that science may truly become the property of all mankind !

Today, international peace and security provide the most favourable conditions for economic and social progress and for the application of what the human spirit has achieved, the daunting modern technical and scientific revolution, to the benefit of all mankind. Consequently, every effort, every action by scientific and cultural associations, civic organizations and private individuals, or by politicians, Governments and parliaments, that will help to defend and consolidate peace, to promote the cause of peaceful international collaboration based on respect for national independence and sovereignty, equal rights, non-interference in internal affairs and mutual advantage must be appreciated and given determined support, so that the legitimate aspirations of the peoples, of all who are aware of their responsibility for the fate of civilization, may be realized.

We call on scientists, and on their national and international associations, to establish suitable forms of co-operation transcending national, ideological or political differences, to the end that science may be used exclusively in accordance with its humanistic calling.

With this in mind, we have established an International Action Committee to organize scientific activities, to expose the dangers created by the frenzied arms race, particularly the nuclear arms race, to inform public opinion about these dangers and formulate concrete measures to avoid them, and to prepare for a world congress of scientists in the service of peace. We appeal to scientists and intellectuals throughout the world to join the Committee in this noble initiative for peace, to do all in their power to make our views known in the United Nations, in the Committee on Disarmament at Geneva and in all international forums where disarmament, peace and international security and co-operation are discussed.

Let us, in realization of our responsibility to science and to mankind and of the fact that we cannot create an acceptable future without a peace-

ful present, muster our strength of persuasion and the force of our arguments in order to induce the arms enthusiasts to change their approach, in order to influence Governments, parliaments and politicians to promote policies of peace, understanding and collaboration and to abandon entirely the use or threat of force, ensuring that all disputes are settled solely by peaceful means, through negotiation.

Let us do all in our power to ensure that the funds spent on armaments, the enormous military budgets, are used for socio-economic development programmes in each country, for helping the peoples of the developing countries in their struggle for progress, and for creating a more just and better world free from the threat of war !

Let us dedicate ourselves to the noble ideals of peace ; let us do our duty to our own consciences, to our contemporaries, to the supreme commandments of mankind ! Let us show mankind a future commensurate to its most cherished aspirations and its creative abilities, let us prove worthy of all the most precious accomplishments of human civilization down through the centuries !

We are firmly convinced that, if we join forces and intensify our co-operation, science will truly become a weapon for living, enabling all peoples to increase their contributions to the heritage of universal knowledge, so that peace, security and collaboration may triumph on earth !

The participants in the
International Symposium
"Scientists and Peace"

Bucharest, 5 September 1981

BIOLOGIE ET DÉVELOPPEMENT SOCIAL

PAR

MIHAEL FLORESCO

L'une des préoccupations essentielles du Parti Communiste Roumain c'est l'utilisation optimale des ressources naturelles du pays, leur valorisation dans le but d'assurer le développement de l'économie nationale, le progrès et le bien-être, l'amélioration du standard de vie du peuple tout entier. Le Parti a milité contre l'exploitation abusive des ressources, contre leur spoliation par les monopoles capitalistes qui régnaient naguère sans partage sur le territoire du pays. Aujourd'hui, la protection des ressources, le souci de leur utilisation rationnelle, ceci dans le but de transmettre aux générations à venir un cadre naturel de vie autant que possible non altéré par l'intervention de l'homme, complexe, riche et harmonieux, constituent l'un des objectifs essentiels de la politique du parti. Dans le même temps, le parti se préoccupe d'assurer la conservation des beautés et des valeurs naturelles du pays, de promouvoir l'amour et le respect de l'homme et de la nature, du patrimoine du pays. Le Parti a imprimé un équilibre judicieux entre ses activités visant à systématiser l'exploitation élargie des ressources en vue du développement d'une part, et de l'autre d'assurer la conservation et la protection des écosystèmes, des plus précieuses espèces de plantes et d'animaux de la biosphère.

Dans son rapport à la Conférence Nationale du Parti Communiste Roumain, en juillet 1972, le Président Nicolae Ceaușescu, soulignait le fait que l'application du programme de développement du pays requérait la participation active de tous les citoyens. « Etant donné, disait-il, le haut rythme de développement de l'industrie, l'introduction toujours plus accentuée dans la vie sociale des éléments de la civilisation moderne, il convient de souligner l'importance vitale pour la nation de la question de la protection de l'environnement. Il faut prendre des mesures rigoureuses pour combattre les nuisances industrielles, prévenir la pollution de l'eau et de l'air, assurer la conservation des forêts, des lacs, des rivières, des montagnes, des sites classés comme monuments naturels. C'est un devoir d'honneur pour le parti, pour notre peuple tout entier, que de tout faire pour assurer le cadre ambiant favorable à la protection de la santé humaine à la conservation inaltérée des beautés de notre pays, afin de transmettre aux générations à venir les bienfaits que la nature a accordés à la Roumanie »¹.

Conformément aux orientations scientifiques imprimées par le Parti à sa politique d'administration des ressources naturelles, on a développé considérablement l'activité de la recherche dans le domaine de l'écologie.

¹ N. Ceaușescu, *La Roumanie sur la voie de l'édification de la société socialiste développée dans tous les domaines*, VII^e volume, Ed. politică, Bucarest, 1973, p. 511.

Par exemple, on a travaillé à élucider certains problèmes relatifs à la structure et à la fonction des écosystèmes naturels terrestres et aquatiques, ainsi qu'à la gestion rationnelle à long terme et à la conservation des ressources naturelles du pays. Etant donné le caractère limité des ressources terrestres, la dynamique exponentielle du développement de la production matérielle aboutira dans un certain nombre d'années à leur épuisement. D'où la nécessité de les utiliser rationnellement, de prévoir la remise en circulation des matériaux usagés, d'élaborer des technologies pour assurer la mise en valeur des minéraux pauvres en substances utiles, de mettre en valeur les gisements sous-marins, et surtout de passer à l'utilisation des ressources susceptibles de régénération ou inépuisables, parmi lesquelles l'énergie solaire, la force marémotrice, la biomasse agricole et sylvique, l'aquaculture. Dans ce domaine il convient de souligner que les hommes de science ont établi le potentiel bioproducif de certaines zones naturelles du pays, surtout celles des bassins hydrographiques, forêts, prairies, etc., formulé des solutions en vue de l'aménagement judicieux de vastes espaces géographiques, de la lutte contre la pollution, de la remise en circulation des résidus issus des processus industriels. Il faut remarquer l'intérêt tout particulier des études sur les agro-systèmes, dont la problématique est étroitement liée aux tâches fixées en ce qui concerne la valorisation efficiente de toutes les superficies agricoles. De même que les recherches effectuées et qui se sont soldées par des solutions nouvelles et originales appliquées à l'épuration des eaux industrielles résiduelles, avec l'obtention concomitante de la biomasse protéique et énergétique. Il ne faut pas oublier dans cette énumération succincte et incomplète le travail des écologistes, dans la recherche de nouveaux moyens et méthodes pour combattre les parasites dans l'agriculture, favoriser l'aquaculture, fertiliser les sols, sans oublier leur contribution à la connaissance des phénomènes et mécanismes écologiques de base, lesquels conditionnent la réserve de solutions technologiques en vue de la gestion et de la valorisation rationnelle des ressources naturelles du pays dans les années à venir.

Si les mesures prises pour assurer le maintien du cadre naturel favorable à l'équilibre écologique sont d'une importance vitale, il est non moins nécessaire de combattre les calamités naturelles et d'améliorer les rendements des terrains agricoles et sylvicoles. On a donc élaboré un programme spécial pour assurer l'alimentation en eau, par la réalisation de lacs d'accumulation, grands et petits, par un réseau d'irrigation dans les zones sèches, par la protection des terrains inondables, par la lutte contre l'érosion, la désalinisation de certaines terres et l'assèchement des marécages, l'amélioration de la fertilité des sols par une utilisation rationnelle des engrangements chimiques, l'application des produits phytosanitaires sans nuire à l'équilibre écologique, l'accroissement des rendements grâce à la mise en pratique de l'ingénierie génétique en vue d'obtenir des espèces et des hybrides correspondant aux conditions locales pédo-climatiques.

Mais la nature ne cesse également de susciter ses propres manifestations. L'extension des zones désertiques, la sécheresse, les vents destructeurs, les tremblements de terre et les éruptions volcaniques, les épidémies, sont des phénomènes naturels qu'il faut connaître pour les dominer ou au moins les limiter, car ils apportent parfois des changements radicaux dans l'équilibre écologique. Il ne faut pas oublier non plus le

danger que présentent certaines manifestations de l'homme et qui sont le fruit de la lutte des intérêts du capitalisme et de l'impérialisme, du colonialisme et du néo-colonialisme, lesquelles ont engendré de graves déséquilibres dans le niveau de développement des peuples, la sous-alimentation chronique de trois-quarts de la population du globe terrestre, des guerres destructrices, et la menace grave suscitée par les armes nucléaires, chimiques, bactériologiques, les lasers et autres instruments de destruction massive. Les hommes de science de Roumanie et du monde entier doivent empêcher que les grandes découvertes de la physique, de la chimie et de la biologie soient utilisées pour détruire la société et la nature.

Le Conseil National pour la Science et la Technologie a suivi avec une attention toute spéciale le développement de la recherche dans le domaine de l'écologie, qu'il considère sous un double aspect. En premier lieu l'accroissement du rôle et de l'efficience de toute l'activité déployée dans ce domaine en vue de la mise en valeur plus large et plus complète des ressources naturelles du pays, qui représente l'une des composantes de base du processus de développement de la société en Roumanie. Parallèlement, il a poursuivi une politique de conservation des ressources naturelles pour assurer leur utilisation sur une longue période et maintenir l'équilibre écologique.

Nous estimons que dans la période à venir, les recherches dans le domaine de l'écologie doivent être considérablement élargies et approfondies suivant les indications des documents du XII^e Congrès du Parti Communiste Roumain, dans la direction de la connaissance de la structure de la matière, y compris de la matière vivante, des processus biologiques de la nature, ceci afin de pouvoir agir conscientement sur ces processus en vue de la transformation de la nature, de conférer de nouvelles caractéristiques aux plantes et aux animaux, de satisfaire aux besoins croissants de la société. En ce sens, il faudra accorder une plus grande attention aux problèmes particulièrement complexes de l'amélioration des rendements des écosystèmes agricoles, ce qui constitue l'un des objectifs fondamentaux de la nouvelle révolution agraire.

Il faudra aborder avec plus de courage les recherches concernant la base de ressources naturelles, augmenter les réserves et utiliser dans ce but toutes les zones pédo-climatiques du pays, spécialement celles dont le potentiel biologique productif n'a pas été mis en valeur à la mesure des possibilités réelles. Nous accordons une importance toute spéciale aux études écologiques destinées à aboutir à des solutions efficaces pour améliorer la productivité des bassins aquatiques naturels ou aménagés, aptes à fournir à l'économie des protéines de bonne qualité, aussi bien sous la forme classique de farine de poisson que de nouvelles ressources alimentaires, mollusques, crustacés, algues, etc. On entrevoit donc de grandes perspectives dans le domaine de l'aquaculture, laquelle devra fournir des solutions pour l'obtention de produits alimentaires ou à usage énergétique. Dans le même temps il est nécessaire d'intensifier les recherches concernant les écosystèmes forestiers en concordance avec les problèmes qui se posent pour assurer à l'économie les matières premières de remplacement et protéger l'environnement.

La révolution agraire s'exprime par le niveau de mécanisation avancé et l'utilisation généralisée des engrangements chimiques, ainsi que la protection des

terres arables contre les calamités naturelles, l'aménagement de vastes systèmes d'irrigation, l'introduction dans la pratique agricole des grandes conquêtes de la révolution biologique contemporaine. Les sciences agricoles et agrotechniques auront une contribution primordiale dans la révolution agraire car elles sont destinées à mettre en pratique dans l'agriculture les dernières découvertes dans toutes les branches, et les instruments techniques les plus efficaces pour l'exécution des travaux agricoles. Les sciences biologiques et surtout l'ingénierie génétique doivent assurer un accroissement substantiel des récoltes, de la production agro-alimentaire, des cultures et des plantations bioénergétiques, de la biomasse destinée aux processus de production chimique.

Les biotechnologies ont à leur base les processus de la biochimie et de la microbiologie, les biosynthèses de la biologie moléculaire. La biotechnologie a élaboré dès à présent les processus sur lesquels se fondent les installations industrielles pour la production des protéines des monocellulaires, les acides aminés, les enzymes, les antibiotiques, les vitamines. C'est ainsi que l'on a posé les fondements d'une nouvelle branche industrielle, l'industrie biologique. Les usines de Jassy, Curtea de Argeș et Calafat en sont les premiers éléments.

Avec les instruments les plus modernes, depuis le microscope électro-nique jusqu'au laser, la microbiologie a obtenu des résultats remarquables aussi bien dans le domaine de la chirurgie que dans celui des recombinants génétiques *in vitro* et leur insertion dans les bactéries, les levures et les spores. La microchirurgie de la cellule permet la réalisation d'une structure cellulaire qui suscite la perspective d'obtenir des variétés de plantes et des espèces animales à haut rendement et résistant aux maladies. La médecine acquiert par là un puissant instrument de lutte contre les maladies héréditaires.

A cela il convient d'ajouter les réalisations obtenues dans le domaine des manipulations génétiques, des recombinations génétiques *in vitro* qui ont permis de réaliser des souches bactériennes qui produisent l'insuline, l'interféron et les hormones de croissance et qui ouvrent une large application pour de nouvelles perspectives à effets pratiques dans la vie des hommes et de la société.

La microbiologie a posé les bases de l'industrie de la manipulation des microrganismes qui ouvre une nouvelle ère de possibilités pour mettre le microcosme au service de l'homme.

Les recherches fondamentales dans les sciences biologiques : microbiologie, biochimie, biophysique et bionique, ainsi que la découverte des structures qui entrent dans la composition des substances vivantes (biologie moléculaire) ainsi que la découverte des premières formes de vie organisée (qui est l'objet de la biologie cellulaire) devront être élargies. Le Conseil National pour la Science et la Technologie s'efforce de stimuler l'activité des recherches dans ce domaine vital pour la santé publique et l'avancement de la société vers les sommets de la civilisation.

Le Conseil National pour la Science et la Technologie considère que pour développer plus avant les recherches dans le domaine de l'éologie il faut élargir l'intérêt vers l'établissement des possibilités, des voies et méthodes d'accroissement de la biomasse, dans les écosystèmes en régime

naturel aussi bien que cultivés, utilisant dans ce but les dernières conquêtes de la science, y compris les techniques du génie génétique. Il faudra agir dans un large cadre pluridisciplinaire, entraînant les collectivités de spécialistes, biologistes, techniciens, agronomes, chimistes, etc., afin de résoudre les problèmes graves qui se posent aujourd'hui à l'humanité — tels que ceux de l'alimentation en protéines, des énergies de substitution et des matières premières pour l'industrie chimique.

La thématique très diverse de la dernière Conférence d'écologie dénote une large gamme de préoccupations et d'orientations dans la recherche, ce qui ouvre des perspectives favorables à l'ensemble de l'écologie.

Nous exprimons la conviction que les hommes de science, les chercheurs et les ingénieurs prendront toutes les mesures nécessaires pour que les résultats déjà obtenus soient rapidement introduits dans la pratique de l'agriculture, dans l'horticulture et l'élevage, dans la sylviculture et l'aquaculture.

A NEW *PACHYSEIUS* SPECIES (ACARI :
MESOSTIGMATA) AND A NEW ONE
FOR THE ROMANIAN FAUNA

BY

LIBERTINA SOLOMON

The author describes a new species of *Pachyseius*, *P. strandtmanni* n.sp., notifies the presence of *P. humeralis* Berlese, 1910, as a new species in Romania's fauna and gives a key to females of the four known European species of *Pachyseius*.

The genus *Pachyseius* Berlese, 1910 counts a few number of species inhabiting in forest litter, moss, compost, humus, detritus, waterside soil, arable soil and even in caves.

Three European species were described : *Pachyseius humeralis* Berlese, 1910 with the largest spread from west to east (U.S.S.R. : Leningrad, Volga, Gorki regions), *P. angustiventris* Willmann, 1935 in the French Alps and *P. angustus* Hyatt, 1956 in England.

Some ecological investigations undertaken of the Mesostigmatic mites, in different forest ecosystems, afforded the opportunity to find a new species *Pachyseius strandmanni* n.sp. and a new species for the Romanian fauna, *Pachyseius humeralis* Berlese, 1910.

Pachyseius strandtmanni n.sp.

Female. Dorsal side (Fig. 1) : Idiosoma of $676-695\mu \times 438-476\mu$, oval, with slight marked shoulders, is entirely covered with the dorsal shield. This one is well sclerotized, light-brown colored, with a fine hexagonal reticulation which becomes less visible due to the punctiform sculpture, very dense and evident, which marks also the limits of the meshes. It carries 30 pairs of smooth aciculae of different sizes : $i_1, s_1 = 24\mu$, $r_1 = 8\mu$, $i_3 - i_4, z_4, I_1 = 21\mu$, $i_2, s_2 - s_4 = 58-67\mu$, $I_3 - I_5 = 43-48\mu$. The porotaxy and sigillotaxy of the dorsum is shown in the figure.

The tectum (Fig. 2B) is angular with a median top and toothed anterior sides, closer in shape to those of *P. angustiventris*, but broader.

Ventral side (Fig. 3) : The tritosternum is bipartite with laciniae pilose of 108μ and the base of 38μ . The presternal region is provided with two pairs of presternal shields, a bigger one, elongated, with a smaller, oval one on their edges : the latter may be absent. Of the three known species a pair of presternal shields is present only in *P. humeralis*.

All the ventral shields are well sclerotized and evidently punctured so that the reticulation is less visible. On a more profound plane, under the superficial puncture, there is a finer granulation. The sternal shield of $133-138 \mu \times 145-157 \mu$ has the anterior and posterior borders concave, and carries the three pairs of sternal setae : St. St. = $40-50 \mu$. St. = 33μ .

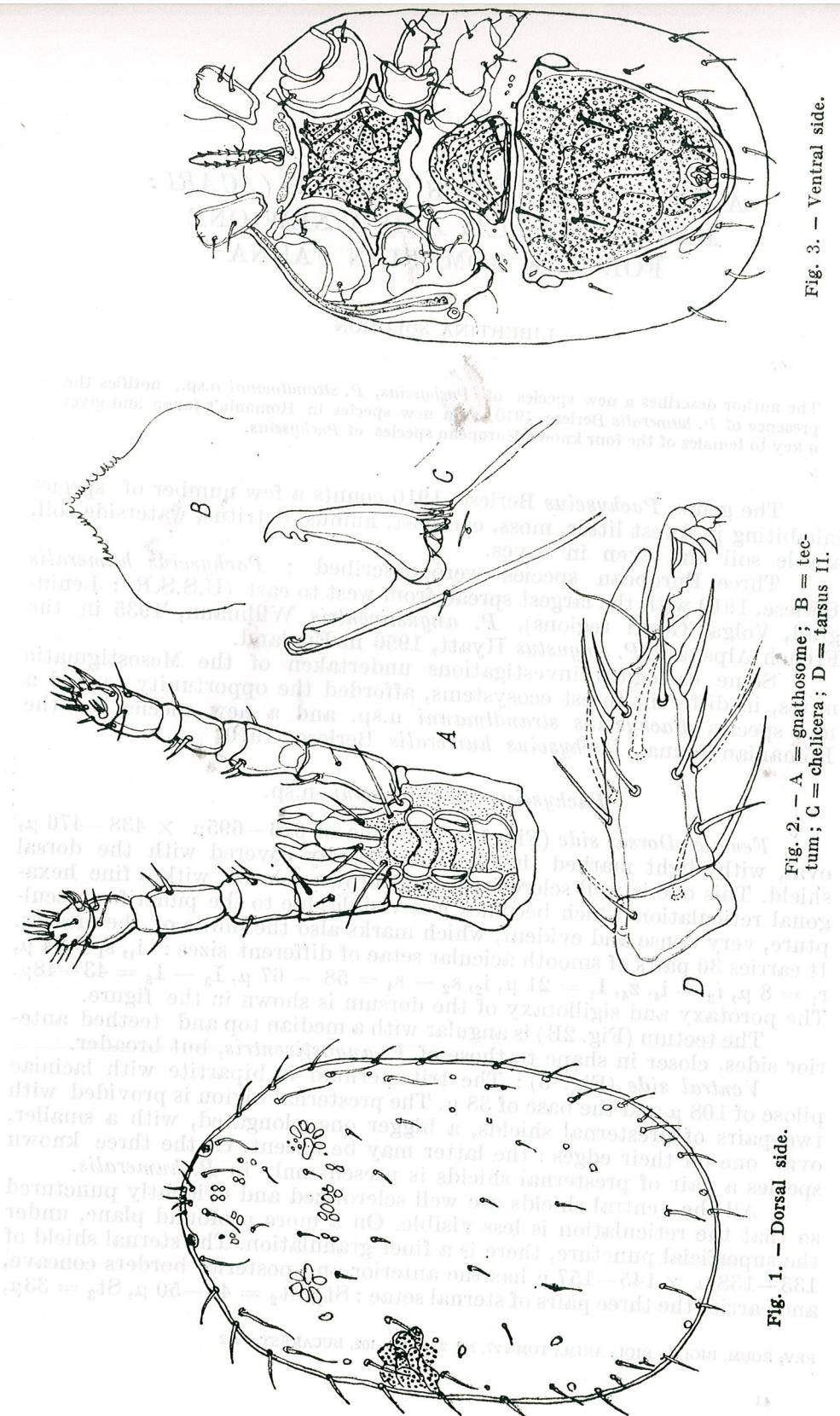


Fig. 3. — Ventral side.

Fig. 2. — A = gnathosome; B = tectum; C = chelicera; D = tarsus II.

Fig. 1. — Dorsal side.

and the two pairs of pores. The two metasternal shields are very small and carry the metasternal pair of setae of $23-29 \mu$. The genital shield, almost half-round, has $105-112 \mu \times 124-133 \mu$ and carries a pair of genital setae of $31-36 \mu$. Under its posterior border there are four sclerotized sticks, covered partly by the posterior genital border. On each side of this border a glandular pore occurs. The endopodal shields are present, those of coxae IV getting a prominence to the genital shield. Ventro-anal shield large, triangular, with the antero-lateral borders slightly concave, is $295 \mu \times 248-262 \mu$. It carries two pairs of preanal setae. Lengths of ventro-anal setae are: $V_1 = 50-52 \mu$, $V_2 = 50-60 \mu$, $V_4 = 33 \mu$ and the post-anal $36-48 \mu$. The paranal setae are above the anal orifice. A curved scleratized band passes under the posterior edge of the ventro-anal shield, going beyond its sides. Four small platelets lie above the antero-lateral borders of the ventro-anal shield. The metapodals of $40 \mu \times 14 \mu$ are oval and joined with the ventro-anal shield, under its anterior corners.

The peritremal shields are well widened at the level of coxae III-IV, free posteriorly, with an orifice near the end of the peritreme; the peritremes extend to the level of the posterior border of coxae I. On the free integument there are 7 pairs of setae of $19-60 \mu$.

Chelicerae three segmented, with segment I = $40-61 \mu$, segment II = $148-155 \mu$, fixed digit monodentate with the tooth immediately under pilus dentilis and a bifid apex, pilus dentilis short, simple; movable digit bidentate of $55-60 \mu$ and a hooked apex. Dorsal seta short, simple, lyrifissure and arthrodial processes well-developed (Fig. 2C).

Hypostome of $169-171 \mu \times 114-131 \mu$, has thin and elongated corniculi of $40-46 \mu$ and the hypostomal processes in the form of simple hyaline lobes. The gnathosomal setae, of 29μ , are absent in ones specimen; the hypostomal setae of: $hy_1 = 29 \mu$, $hy_2 = 59 \mu$, $hy = 12 \mu$. Deutosternum is wide with 6-7 transverse ridges of which the first three are denticulated, and with three sclerotized hollows on each side. Pedipalps, typical of the genus, of 198μ (Fig. 2A)

Tarsus II provided with a stout spur and a spur-like extremity, with acicular setae, some of them thickened. (Fig. 2D). Length of legs: I = $509-524 \mu$, II = $405-410 \mu$, III = $324-343 \mu$, IV = $457-462 \mu$.

Material examined: $2\varphi\varphi$ in the litter of a *Luzulo (silvatica) piceetum* association and 1ϱ in the litter of a *Pinetum mugi piceetosum et cembrosum* association, in the Călimani mountains (East Carpathians) between 1360-2000 m altitude, in July 1981.

Holotype and paratypes in the author's collection.

The new species is dedicated to the American acarologist Russel W. Strandtmann.

Pachyseius humeralis Berlese, 1910 is a new species in the Romanian fauna. There were found $10\varphi\varphi$ in the litter and 1ϱ in manure (6. V. 1976) and 1ϱ in manure (20.VII.1976) in the deciduous forest of Voinești (Iași) at an altitude of 150-402 m.

KEY TO FEMALES OF THE GENUS PACHYSEIUS

(after E. V. Koroleva, modified)

- 1(2) — Ventro-anal shield with 3 pairs of preanal setae. Presternal shields are present. Peritremal shield blunt posteriorly. On the free ventral integument 7 pairs of simple setae. In forest litter, water-side soil, arable soil, moss, compost, humus and in nests of rodents. Spread in Europe *P. humeralis* Berlese, 1910
- 2(1) — Ventro-anal shield with 2 pairs of presternal setae.
- 3(6) — Presternal shields are absent.
- 4(5) — The posterior border of the sternal shield evidently convex. Sternal and ventro-anal shields entirely covered with a network built up of little spots. The ventro-anal shield is widest in its middle part. Between the ventro-anal and metapodal shields there is a pair of setae. In caves. Spread in the French Alps *P. angustiventris* Willmann, 1935
- 5(4) — The posterior border of the sternal shield slightly concave. Sternal and ventro-anal shields without a spotted network. Ventro-anal shield wider in its anterior half. The setae between ventro-anal and metapodal shields are absent. In forest litter, in detritus. Spread in England *P. angustus* Hyatt, 1956
- 6(3) — Presternal shields are present. The posterior border of the sternal shield is concave. All the shields are covered with an evident puncture, making less evident the reticulation. Ventro-anal shield triangular, rather long than wide. The metapodals oval, joined with the ventro-anal shield. Peritremal shields posteriorly widened. On the ventral free integument 7 pairs of setae. In the litter of spruce fir forest and the juniper zone of the Eastern Carpathians. Spread in Romania. *P. strandtmanni* n. sp.

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reducing their electrophoretic mobility to quenching. A. G. G. and G. G. are most scarcely reduced to this extent in the black and red forms.

IS PLANORBARIUS CORNEUS L. A CASE OF POLYMORPHISM?

BY

SERGIO LETELIER and MARGARETA DUMITRESCU

Since there are no morphological differences between *Planorbarius corneus* L. forms, we have tried to demonstrate some molecular differences between the grey, black and red forms by electrophoretic studies of their sarcoplasmatic proteins.

MATERIAL AND METHODS

Each sample was obtained from 21 animals of *P. corneus* after the shell removal, the extraction being made by grinding the material in a tris-borate buffer, pH 8.25. The ratio between the biological material, and the buffer was 1 g/10 ml. The homogenized material obtained was centrifuged at 3 000 r.p.m. The supernatant was used for analysis. Electrophoresis was performed on 10% polyacryl-amide gel beds in tris-borate buffer, pH 8.2.

The electric conditions were 180 V and 0.5 mA/tube.

The migration time was 75 min.

The staining of the proteic fractions was performed with an 1% amido Schwartz solution in distilled water. The excedent stain was removed by immersion of the gel beds in 5% acetic acid solution, for 10 minutes.

DISCUSSIONS

Some previous electrophoretic studies [1] performed on related stagnicola species (*Stagnicola corvus* and *Stagnicola palustris*) of distant geographic origin, contain the same proteins and no evolution could be demonstrated at this level.

Other studies, performed on the extrapallial liquid of different species of the Unionidae family, have proved a relatively great uniformity of the protein composition of this species.

The electrophoretic studies performed by us in the present work, are presented in Fig. 1. From the analysis of this figure it results :

1. The presence of two groups of fractions.

a. A group of fractions with small mobility and intensely stained, in which the different fractions are situated at a small distance from one another.

b. A diffuse group of fractions, less intense stained, with greater mobility, in which the different fractions are at greater distances from one another.

2. The presence of a different number of fractions in the three different forms, nine in the grey form, seven in the black form and seven in the red form, of which three very faintly stained.

3. The presence in the grey and black form of a fraction with great mobility and very intensely stained. In this fraction the red form is completely lacking.

4. Some mobility differences between the probably homologous fractions in the three forms. It results that each one of the three forms, grey, black and red, can be characterized by foot protein electrophoregram. The best characterized is the red form, having the lowest number of fractions.

It is very interesting to note that the general aspect of the extrapallial proteins electrophoregram [2] is similar to that obtained by us in the foot extractions from *P. corneus* in the above mentioned forms.

In connection with this fact we must mention that the extrapallial liquid pH value is 8.15 [2]; and very close to the pH value of our extracelular liquid is formed by the natural "extraction" of some foot proteins occurring under the same pH conditions, as those used by us "in vitro". These data would be important for the knowledge of shell morphogenesis.

Finally, our results indicate that the morpho-physiological differences found between the *P. corneus* forms can be established at the molecular level by electrophoresis. These data confirm the existence of a polymorphism in *P. corneus* from the Danube Delta, although the research should be continued to reach a final conclusion.

Acknowledgements. We wish to express our sincere thanks and gratitude to Prof. Dr. N. Botnariuc for his guidance, encouragement, interest and support throughout this investigation and his criticism of the work.

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THE ERGOSTEROL POTENTIATION EFFECT UPON
THE HYPOLIPEMIANT
AND HYPOCHOLESTEROLEMIANT
ACTION OF CERTAIN POLYENES

REV. ROUM. BIOL. — BIOL. ANIM., TOME 27, NO 2, P. 105–109, BUCAREST, 1982

101

BY
GABRIELA AGRIGOROAEI, ȘT. AGRIGOROAEI, AL. SAUCIU, I. NEACSU,
ELENA CHERA, GEORGETA NĂNESCU

Starting from the fact that ergosterol has by itself some interferences favourable to the general cholesterol metabolism and from the hypothesis that it could intensify the action of nystatins upon cholesterol deposits, the authors evince a very accentuated intensification of the hypolipemiant and hypocholesterolemiant action of the examined agents (nystatin and CM nystatin). The experiments were made upon laboratory animals (Chinchilla rabbits) fed an atherogenic regimen associated with ergosterol. In the animals supercharged with cholesterol, ergosterol tends to maintain ratios close to the normal values between total cholesterol and other lipids, on the one hand, and between cholesterol fractions, on the other hand.

In the search for agents which lead to the increase of the hypocholesterolemiant efficiency of nystatin or CM nystatin [1], we have chosen ergosterol starting from the fact that polyenes achieve their specific fungicide action on the basis of their capacity to preferentially interact with the ergosterol in the fungus membranes [6] — [8]. Other authors have shown that when introducing germinated wheat oil in the food of animals fed an atherogenic regimen the effect of cholesterol is reduced [4], or that the administration of beer yeast in the geriatric treatment has positive effects upon the evolution of atherosclerosis [10].

It is also known, on the other hand, that both germinated wheat oil and beer yeast contain a relatively high amount of ergosterol [3] and that this can act as an inhibitor of cholesterol synthesis in the liver [2], [9], it can reduce the intestinal absorption of cholesterol and can favour its excretion as well [11].

MATERIAL AND METHODS

By proposing ourselves to observe if the association of ergosterol with cholesterol during an atherogenic regimen favours the hypocholesterolemiant and hypolipemiant action of nystatin and CM nystatin when subsequently applying a treatment with these antibiotics, experiments were performed on two groups of six animals each (Chinchilla rabbits). Each animal had a body weight around 2 kgs. The animals in the first group received a usual atherogenic regimen (based on cholesterol), and those in the second were simultaneously given cholesterol and ergosterol. Cholesterol was administered in a mixed regimen (various amounts at

different stages): two weeks of heavy regimen (1.55 g/kg b.wt./day), another two weeks of light regimen (0.355 g cholesterol/kg b.wt./day), and then another two weeks of medium regimen (0.765 g cholesterol/kg b.wt./day). The cholesterol source was dry ground yolk with a determined concentration. The amount of yolk was fed into two equal portions per day and its ingestion was strictly controlled.

Ergosterol was administered orally only to the second group, every day (0.5 mg ergosterol/kg b.wt./day) for six weeks during the atherogenic regimen, under the form of beer yeast (0.25 g beer yeast/kg b.wt./day).

After the interruption of the atherogenic regimen each group was divided into two smaller ones. In each case one of the smaller groups was treated with nystatin (0.666 mg/kg b.wt./day in 1 ml 1,2-propylene glycol) and the other one with CM nystatin (25 mg/kg b.wt./day in 1 ml saline).

The effects of the treatment were observed by way of periodically determining the total serum lipids, total cholesterol and its fractions in the serum by photocolorimetric methods [5], [12].

RESULTS

A. TOTAL SERUM LIPIDS

a. In the group to which only cholesterol was administered, total serum lipids increased during the atherogenic regimen up to a value by 9.98 times higher (3143.70 mg %) than the initial one (Fig. 1, I-A). After the interruption of the atherogenic regimen, and at the administration of polyenes a decrease of serum lipids takes place in the course of the following four weeks. The decrease reaches a value by 7.60 times higher

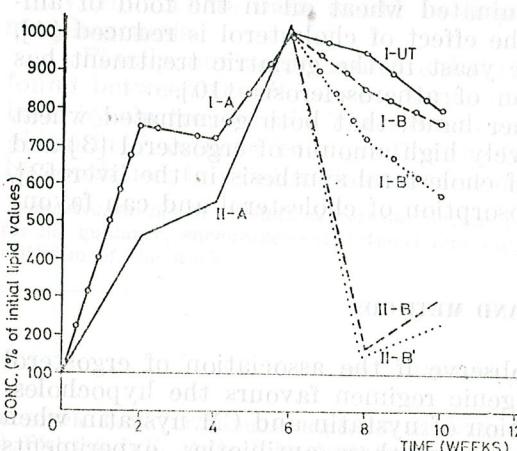


Fig. 1.—The variation of total serum lipids in animals subjected to an atherogenic regimen with cholesterol (I—A) or cholesterol and ergosterol (II—A), and treated after that with CM nystatin (B) or nystatin (B').
UT = untreated animals.

(2394.0 mg %) than the initial one in the case of CM nystatin treatment (Fig. 1, I-B) and by 5.70 times higher (1795.50 mg %) than the initial one in the case of nystatin (Fig. 1, I-B').

b. A somewhat different evolution of total serum lipids was noticed in the group which had simultaneously received ergosterol and cholesterol. During the heavy and light regimens the serum lipids increase to a smaller extent than in the control animals, but during the medium regi-

men the difference is totally recovered by a rapid increase which raises the concentration of lipids to a value (3816.31 mg %) by 10.01 times higher than the initial one (Fig. 1, II-A). After the interruption of the atherogenic regimen and when administering nystatin and CM nystatin respectively, a surprisingly rapid decrease of the serum lipids concentration is to be observed. After two weeks, their concentration reaches values by 1.52 times higher (Fig. 1, II-B') and respectively by 1.62 times higher (Fig. 1, II-B) than the initial one (i.e., 579.5 mg % and 617.63 mg %) and with slight subsequent increases.

B. TOTAL SERUM CHOLESTEROL

a. In the group subjected to the atherogenic regimen without ergosterol, total serum cholesterol reaches eventually a value by 19.05 times higher (2771.78 mg %) than the initial one (Fig. 2, I-A). During the following four weeks its concentration decreases up to a value by 12.36 times higher (1798.85 mg %) than the initial one in the case of CM nystatin treatment (Fig. 2, I-B), and by 8.92 times higher (1298.14 mg %) than the initial one in the case of nystatin (Fig. 2, I-B').

b. In the case of the atherogenic regimen associated with ergosterol, total serum cholesterol increases less and slower than in control animals, reaching, after six weeks, a value only by 16.09 times higher (2288.80 mg %) than the normal one (Fig. 2, II-A).

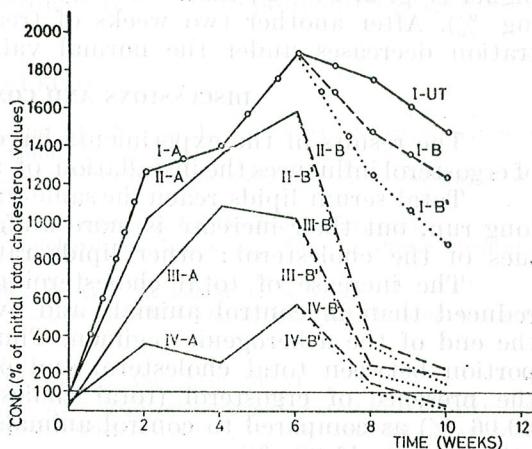


Fig. 2.—The variation of serum cholesterol in animals subjected to an atherogenic regimen with cholesterol (I—A) or cholesterol and ergosterol (II—IV—A), and treated after that with CM nystatin (B) or nystatin (B').
I and II = total cholesterol, III = esterified cholesterol, IV = free cholesterol, UT = untreated animals.

After the interruption of the atherogenic treatment and the application of nystatin and CM nystatin treatment, a sudden decrease towards small values in concentration was observed for cholesterol, too, in the first two weeks. Cholesterol reaches a concentration only by 3.5 times higher (Fig. 2, II-B') and by 3.73 times higher (Fig. 2, II-B) than the initial one (i.e., 498.88 mg % and 530.59 mg % respectively). The decrease in concentration continues after that, too.

C. ESTERIFIED SERUM CHOLESTEROL

The increase of total cholesterol concentration during the atherogenic regimen associated with ergosterol is due to esterified cholesterol. However, a slow decrease of the esterified cholesterol is noticeable during the medium regimen. Consequently, at the end of the atherogenic regimen its value becomes only by 21.26 times higher (1465.24 mg %) than the initial value (Fig. 2, III-A).

When subsequently applying nystatin and CM nystatin treatment, esterified cholesterol decreases very rapidly in concentration, reaching after two weeks a value only by 4.69 times higher (Fig. 2, III-B') and respectively by 5 times higher (Fig. 2, III-B) than the initial one (i.e., 323.23 mg % and 347.70 mg %). In the following two weeks of treatment esterified cholesterol concentration decreases steadily but slower than before.

D. FREE SERUM CHOLESTEROL

The association of the ergosterol to the atherogenic regimen influences the increase of free serum cholesterol concentration very strongly. After six weeks of regimen, free serum cholesterol concentration reaches a value only by 11.23 times higher (823.50 mg %) than the initial one (Fig. 2, IV-A).

When nystatin and CM nystatin treatment is applied a rapid decrease of free cholesterol concentration is noticeable and its value becomes only by 2.4 times higher (Fig. 2, IV-B') and respectively by 2.5 times higher (Fig. 2, IV-B) than the initial one (i.e., 175.0 mg % and 183.80 mg %). After another two weeks of treatment, free cholesterol concentration decreases under the normal value.

DISCUSSIONS AND CONCLUSIONS

The results of the experiments have shown that the administration of ergosterol influences the installation of atherosclerosis to a great extent.

Total serum lipids reach the same value as in control animals in the long run, but their increase is more uniform and this leads to other values of the cholesterol : other lipids ratio.

The increase of total cholesterol concentration is generally more reduced than in control animals and even more slowed down towards the end of the atherogenic regimen. Thus, in the last phase the disproportion between total cholesterol and other lipids greatly improves in the presence of ergosterol (total cholesterol 59.94 % and other lipids 40.06 %) as compared to control animals (total cholesterol 88.12 % and other lipids 11.88 %).

On the other hand, the ratios between cholesterol fractions vary in a totally different manner as compared to the atherogenic regimen without ergosterol.

In control animals the increase in cholesterol fractions concentration develops in such a way that during the entire atherogenic treatment period and even after its interruption the EC/FC ratio decreases.

Ergosterol determines the EC/FC ratio, that increases very much during the first two stages (up to 4.18), to record a substantial improvement until the end of the atherogenic regimen (1.77).

In this way, the experiment animals treated as such are found to be in a better condition in comparison to those to which ergosterol has not been administered; both ratios total cholesterol : other lipids and respectively esterified cholesterol : free cholesterol have values closer to the normal ones.

A subsequent application of nystatin and CM nystatin treatment has as a direct effect a rapid decrease of both lipids and cholesterol in the blood, with some slight differences to the advantage of nystatin.

Acting in these conditions nystatin and CM nystatin determine a very rapid decrease of all lipidic fractions in the blood. They accelerate the elimination of cholesterol from the body — this being probably coupled with an accentuated reduction of its assimilation through the enterohepatic circuit — and massively mobilize esterified cholesterol in the deposits.

Hepatic synthesis being inhibited both by the atherogenic regimen and ergosterol, it seems that the important mobilization of esterified cholesterol in the deposits is accompanied by its de-esterification and the elimination of free cholesterol. It is possible that the mobilization of cholesterol in the deposits be due to the intensification of nystatin and CM nystatin action as a result of the presence of vegetal sterol in the organism.

It is clear that ergosterol creates a complex of conditions leading to a great intensification of nystatin and CM nystatin hypocholesterolemic and hypolipemiant action.

These studies open new possibilities for the efficient use of the studied polyenes as hypocholesterolemic and hypolipemiant agents.

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ot binol mi ducere boala de la rata, care este o boala foarte comună și deosebit de agresivă. În cadrul unei observații de la Institutul de Cercetări Biologice din Iași, în perioada 1978-1980, s-a constat că în rata de 60 de răți cu boala de la rata, doar 10% au suferit de boala de la rata. Această boala este cauzată de un virus care se transmite prin contactul sexual sau prin mamară. În cadrul unei observații de la Institutul de Cercetări Biologice din Iași, în perioada 1978-1980, s-a constat că în rata de 60 de răți cu boala de la rata, doar 10% au suferit de boala de la rata. Această boala este cauzată de un virus care se transmite prin contactul sexual sau prin mamară.

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DEFIZITÄRE BEFRUCHTUNG BEI RATTEN IN KUPFERMANGEL

VON

E. BORDÁS, SUSANA NAGY, SILVIA GÁBOR und V. V. PAPILIAN

The fecundity index was determined in a group of 60 copper-deprived rats—males and females—kept on milk diet and mated as follows: females fed normal diet containing 130 µg copper/day + copper-deprived males (only 3.0 µg copper/day), respectively copper deficient females mated with males with normal copper intake. No impairment of the reproductive function due to the copper deficiency was observed in females mated with males kept on normal diet. Ovaries were histologically unaltered, disclosing an increase of the Cu and Zn amount. In copper-deprived males, mated with control females, the reproductive function was completely abolished, reading the zero value. Morphological investigations on the testicles evinced a necrosis of the superficial germinal layer and the fall of the Cu and Fe values.

In unseren früheren Untersuchungen zeigten die auf Milchdiät (mit Kupfermangel) umgestellten Tiere Entwicklungsverzögerungen, mikrozytäre, hypochrome Anämie mit Leukopenie, Hypokupferämie und Natalitätsenkung [1], Zeichen die für den herabgesetzten Kupferbeitrag charakteristisch sind [2] — [4].

MATERIAL UND METHODE

Die Untersuchungen wurden an 60 weiblichen und männlichen Weißratten, mit einem initialen Körpergewicht von 56 g durchgeführt.

Die Diät mit Kupfermangel wurde durch Milchdiät (Griessbrei), mit einem täglichen Beitrag von 3,0 µg Kupfer/Tier realisiert. Die Untersuchungstiere wurden in folgenden Gruppen verteilt: I. Kontrolltiere mit normaler Ernährung, 40 Tiere, 20 Weibchen und 20 Männchen; II. Tiere die einer Diät mit Kupfermangel unterzogen wurden, 20 Weibchen und 20 Männchen. Nach 50 Wochen wurden die Tiere wie folgt zur Begattung gelassen: a) 10 Kontrollweibchen mit 10 Kontrollmännchen; b) 10 Weibchen mit Kupfermangel mit 10 Kontrollmännchen und c) 10 Männchen mit Kupfermangel mit 10 Kontrollweibchen.

Im Rahmen der Reproduktionsprobe wurde die Anzahl der trächtigen Weibchen, aus welcher der Fertilitätsindex berechnet wurde, verfolgt. Nach der Tötung wurden die Vermehrungsorgane im Formal fixiert, in Paraffin eingeschlossen und mit Häatoxylin-Eosin gefärbt. Die Bioelemente Kupfer, Eisen und Zink aus diesen Organen wurden mit Hilfe des Atomabsorption-Spektrophotometers Perkin-Elmer 300 bestimmt. Die Ergebnisse wurden in Mikrogramm/Gramm Trockensubstanz ausgedrückt.

ERGEBNISSE UND DISKUSSIONEN

Die Ergebnisse hinsichtlich der Reproduktionsproben sind in der Tabelle 1 enthalten. So wie aus der Tabelle hervorgeht, zeigte der Reproduktionsindex der Gruppe der Kontrolltiere a) sowie der Gruppe der Kontrollmännchen, die mit Weibchen mit Kupfermangel begattet wurden b) ähnliche Werte (100 %, bzw. 80 %) gegenüber dem Wert 0, der bei der Gruppe der Kontrollweibchen die mit Männchen mit Kupfermangel begattet wurden, registriert war.

Tabelle 1

Der Reproduktionsindex nach der gekreuzten Begattung der Tiere mit den Kupfermangel mit den Kontrolltieren

GRUPPE	TIERANZAHL		BEGATTUNG DER TIERE		DER REPRODUKTIONINDEX %
	Männchen	Weibchen	Kontrolle	mit Kupfermangel	
a.	10	10	Weibchen Männchen	—	100
b.	10	10	Männchen Weibchen	Weibchen Männchen	80
c.	10	10	Weibchen Männchen	—	0

Tabelle 2

Kupfer, Zink und Eisen in den Organen (Eierstock, Hode) der Tiere mit Kupfermangel

GRUPPE	Kupfer $\mu\text{g/g}$		Zink $\mu\text{g/g}$		Eisen $\mu\text{g/g}$	
	Eierstock	Hode	Eierstock	Hode	Eierstock	Hode
I. Kontrolltier mit Normalnahrung	2,50	14,04	528,16	391,22	358,33	394,22
II. Ernährung mit Kupfermangel	8,61 ($p < 0,01$)	9,15 ($p < 0,01$)	1369,16 ($p < 0,01$)	477,92 ($p < 0,05$)	40,76 ($p < 0,01$)	43,21 ($p < 0,01$)

Bei der biologischen Untersuchung zeigten die Hoden der Tiere mit Kupfermangel Veränderungen im Herd, die unregelmässig dispergiert waren, wobei ein grosser Teil der seminalen Tubuli integer waren. In den geschädigten Tubuli war das Germinalepithel zusammengeschrumpft, und oft, besonders bei Sertoli-Zellen nur auf den tiefen Schichten reduziert. In diesen Tubuli wurden auch nekrotische Veränderungen der oberflächlichen Germinalschicht mit dem Ausfall der Zellen in das Lumen festgestellt (Abb. 1,2). Die Eierstöcke der Weibchen mit Kupfermangel wiesen einen normalen Aspekt, ohne Veränderungen, auf (Abb. 3).

Die spektralphotometrischen Bestimmungen hoben erhöhte Kupfer- und Zinkkonzentrationen und verringerte Eisenkonzentrationen in den

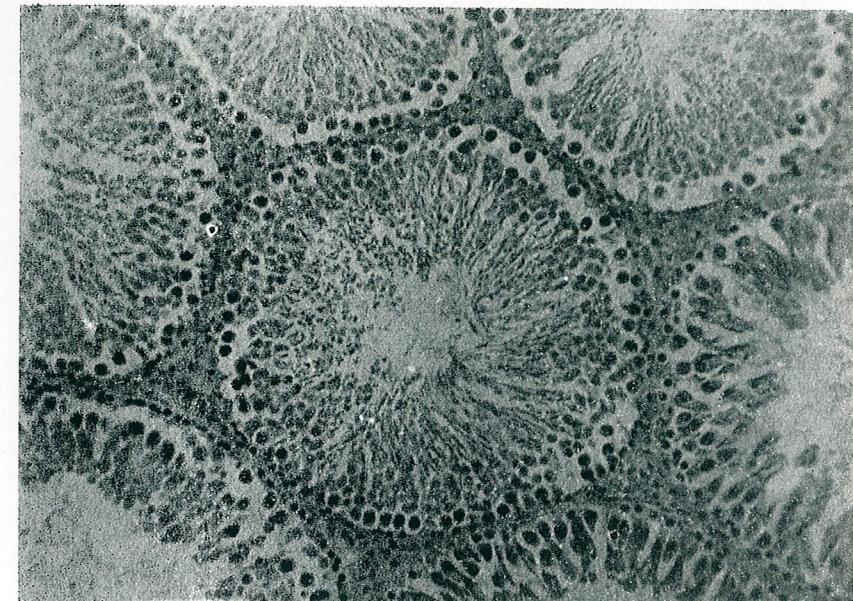


Abb. 1.— Rattenhoden, nichtbehandeltes Kontrolltier, Färbung mit Hämatoxylin-Eosin, $\times 100$.

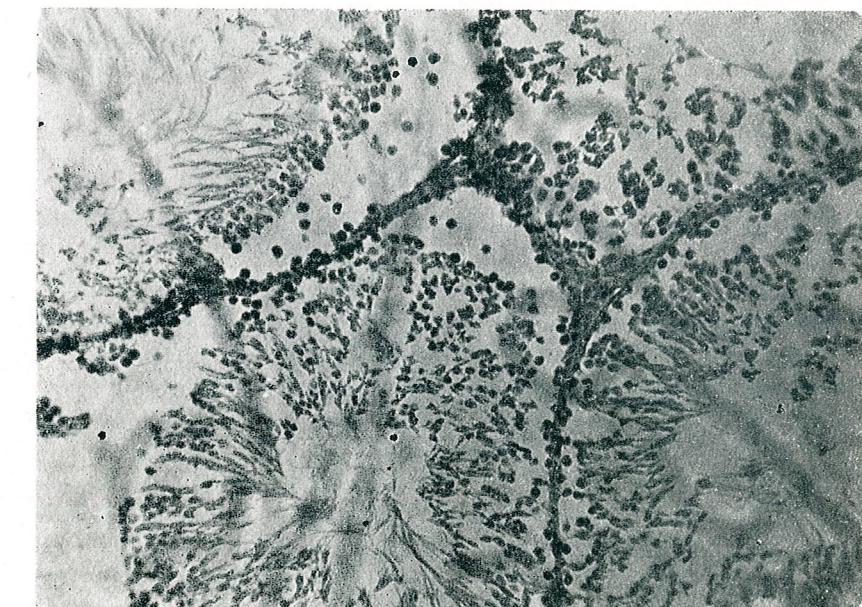


Abb. 2.— Hoden einer Ratte mit Kupfermangel, Färbung mit Hämatoxylin-Eosin, $\times 100$.



Abb. 3.—Eierstöcke einer Ratte mit Kupfermangel, ohne Veränderungen, Färbung mit Hämatoxylin-Eosin, $\times 100$.

Eierstöcken der Weibchen mit Kupfermangel hervor. In den Hoden der Männchen wurden herabgesetzte Kupfer- und Eisenkonzentrationen und erhöhte Zinkkonzentration — die aber nicht die in den Eierstöcken gefundenen Werte erreichten — gegenüber den bei der Kontrollgruppe, mit normaler Ernährung, beobachteten Werten, nachgewiesen.

Die erhaltenen Ergebnisse heben die Auswirkung des Kupfermangels auf die männlichen Tiere hervor, was durch die Milchdiät, welche lange Zeit (50 Wochen) verabreicht wurde, bestimmt wird. Die Auswirkung zeichnet sich durch die Senkung des Reproduktionsindexes nach der Begattung mit Kontrollweibchen, durch die Schädigungen in der Höhe des Germinalepithels aus den Hoden und durch die Herabsetzung der Bioelemente Kupfer und Zink in diesen Organen aus. Bei den Weibchen zeigte der Kupfermangel keine Auswirkungen auf die Natalität, im Falle der Begattung mit männlichen Tieren mit normaler Diät. Der Reproduktionsindex stellte ähnliche Werte wie bei der unbehandelten Kontrollgruppe vor. Histologisch wiesen die Eierstöcke einen normalen Aspekt, mit erhöhten Kupfer- und Zink-Konzentrationen, parallel mit der Eisenherabsetzung, auf.

Die verminderte Natalität der mit den Männchen mit Kupfermangel begatteten Weibchen zusammen mit den beobachteten Schädigungen, sowie die Senkung der Bioelemente in den Hoden und jener Erhöhung in den histologisch unveränderten Eierstöcken, wobei es bekannt ist, dass diese Elemente den aktiven Teil einer Serie von Enzymen bilden [2], [4], sprechen für eine defizitäre Befruchtung.

SCHLUSSFOLGERUNGEN

Die herabgesetzte Natalität der Ratten mit Kupfermangel kann auf eine defizitäre Befruchtung zurückgeführt werden.

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EFFECT OF WHOLE BODY NEUTRON IRRADIATION ON HEPATIC COLLAGEN IN RAT

BY

C. VLĂDESCU and MIOABA CÎBSTEANU

The irradiation of animals with neutron leads to a conformational modification of the hepatic soluble collagen molecule so that, by electrophoresis, five bands are separated like in the thermal-degraded collagen of control. Thirty days after irradiation the electrophoretic patterns of salt and acid soluble collagen are the same as that of nondegraded controls. The hydroxyproline concentration in the hepatic collagen of irradiated animals proves that neutron radiations lead to important modification at this level in the first 24 hours.

1. INTRODUCTION

The whole body irradiation effect on the collagen metabolism in different organs has pointed out the appearance of important qualitative and quantitative modifications. Most of these experiments made use of X and γ radiations and were performed "in vitro".

"In vitro" irradiation of the collagen leads to a decrease of solubility accompanied by aggregation simultaneously with molecular fragmentation [1], [12].

The collagen fibers irradiation "in vitro" determines the formation of thermo-molecular crosslinks between molecules of orientated protein chains in addition to protein chain scission [3].

A study of the effect of γ irradiation on the subunits of thermally degraded neutral salt soluble and acid soluble collagen solutions "in vitro" revealed the depolymerization of dimeric sub-units and fission of peptide bonds, yielding crystalline irradiation-resistant portions of the molecule incapable of associating with the native structure [8].

An increase of urinary hydroxyproline (Hyp) excretion was mentioned in accidentally irradiated persons [9], [11]. Also, an increased excretion of pyrrole-2-carboxylic acid (specific metabolite of Hyp) was mentioned after a total exposure of rats to 750 r [13].

In our laboratory it was established that the whole body irradiation leads to an Hyp excretion depending on the radiation dose and time. Differences were observed also between acute and prolonged irradiation. Similar effects were observed concerning hepatic collagen [17].

2. MATERIAL AND METHODS

Wistar rats, groups of 6 animals of 10–12 months old and 150 g weight. The animals were irradiated with 150, 200, 300 and 600 r fast neutrons, and sacrificed after one, two, three, five and thirty days.

Neutron source: beryllium target bombarded with deuterons accelerated in a 13.5 MeV cyclotron. The neutron flow corresponding to 1A accelerated deuteron current was: $\Phi = 1.63 \cdot 10^8$ n/cm².sec. The dosimetric equivalent of the neutron flow, 1r/sec corresponds to a flow of $2 \cdot 10^8$ n/cm².sec. The dose flow was

$$\frac{dD}{dt} = \frac{1.63 \cdot 10^8}{2 \cdot 10^8} (\text{r/sec}) = 0.81 \text{ r/sec}$$

In the condition in which the deuteron current intensity is the same the variation of the dose will be given by the time variation of exposure (in our case 6 min for a dose of 600 r, 3 min for a dose of 300 r, 2 min for a dose of 200 r and 1.5 min for a dose of 150 r neutrons).

The disk-electrophoresis method as modified by Reisfeld et al. [15] was used for the neutron irradiation effect on collagen macromolecules, utilizing a 7% polyacrylamide gel.

In each tube 50 μ l collagen solution were added.

Preparation and determination of hepatic collagen: the fractionation of hepatic collagen was achieved by the method of Hyrayama [10].

The quantitative determination of hydroxyproline as a measure of collagen metabolism in the liver was achieved by the method of Barbarino et al. [5].

3. RESULTS

In Figs 1, 2 and 3 are represented the electrophoretic diagrams of hepatic salt and acid soluble collagen from controls and irradiated animals. In the electrophoregrams of collagen extracted from controls two bands appear: one representing the high polymers (hp) near the start and the other corresponding to the nondegraded collagen (ndc). The whole body irradiation of rats with 600 r neutrons led, in our conditions, to a degradation of the collagen molecule similarly to its thermal degradation (Fig. 1).

Both salt and acid soluble collagen from irradiated animals leads to the same electrophoretic fractions as those obtained after "in vitro" thermal degradation of collagen extracted from controls, respectively α_2 , α_1 , β_{12} , β_{11} , γ_{112} , and a last fraction near the start with a great molecular weight (high polymers, hp). The process takes place 24–48 hours after irradiation. When the animals were sacrificed 72 hrs subsequently, the electrophoretic bands were diffuse and could not be identified.

The electrophoretic mobility of salt soluble collagen fractions from irradiated animals is greater than that of collagen fractions obtained from the thermal degraded collagen of controls.

There is no increase of electrophoretic mobility for acid soluble collagen from irradiated rats.

The salt soluble collagen from animals irradiated with 300 r neutrons leads to electrophoretic diagrams in which appear only the bands corresponding to β_{12} , β_{11} , γ_{112} , fragments and the start band (high polymers).

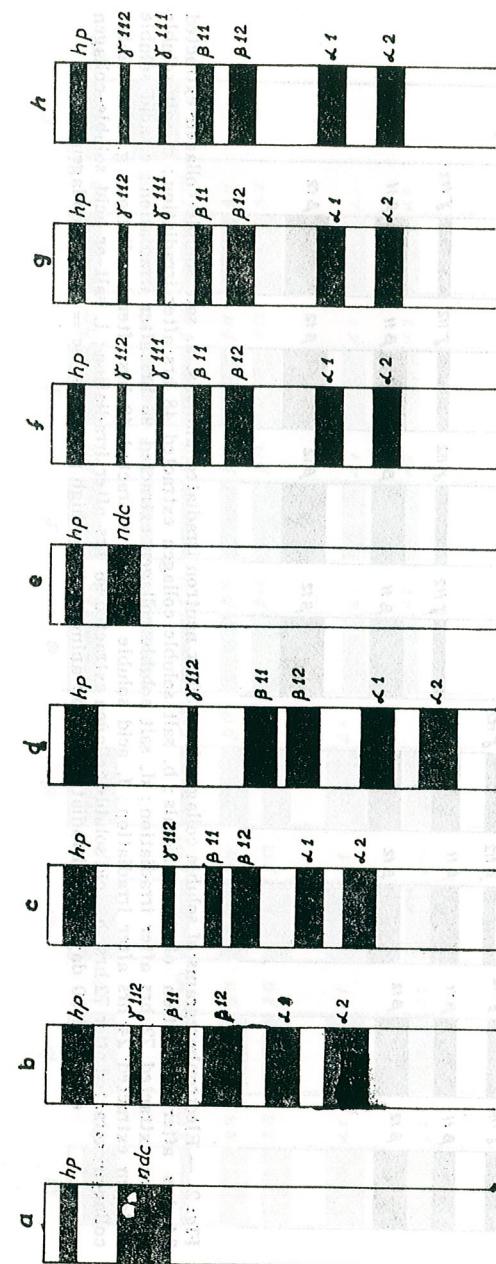


Fig. 1. — Electrophoregrams of control collagen and soluble collagen from 600 r neutron irradiated animals. a. salt soluble collagen from control; b. thermal-degraded salt soluble collagen extracted 24 hrs after irradiation of the animals; c. salt soluble collagen extracted 48 hrs after irradiation; d. acid soluble collagen from control; e. thermal-degraded acid soluble collagen from control; f. thermal-degraded acid soluble collagen extracted 24 hrs after irradiation of the animals; g. acid soluble collagen extracted 48 hrs after irradiation. hp = high polymers; ndc = nondegraded collagen.

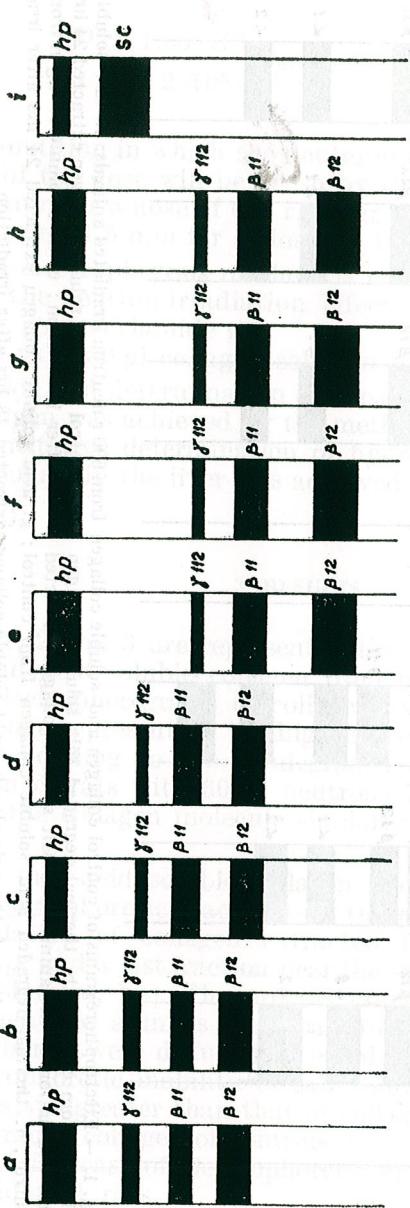


Fig. 2. — Electrophoregrams of soluble collagen from 300 r neutron irradiated animals. a, salt soluble collagen extracted 24 hrs after irradiation of the animals; b, salt soluble collagen extracted 48 hrs after irradiation; c, salt soluble collagen extracted 72 hrs after irradiation; d, salt soluble collagen extracted 96 hrs after irradiation; e, acid soluble collagen extracted 24 hrs after irradiation; f, acid soluble collagen extracted after 48 hrs after irradiation; g, acid soluble collagen extracted after 72 hrs; h, acid soluble collagen extracted 96 hrs after irradiation; i, salt or acid soluble collagen extracted 30 days after irradiation of the animals. hg = high polymers; sc = soluble collagen.

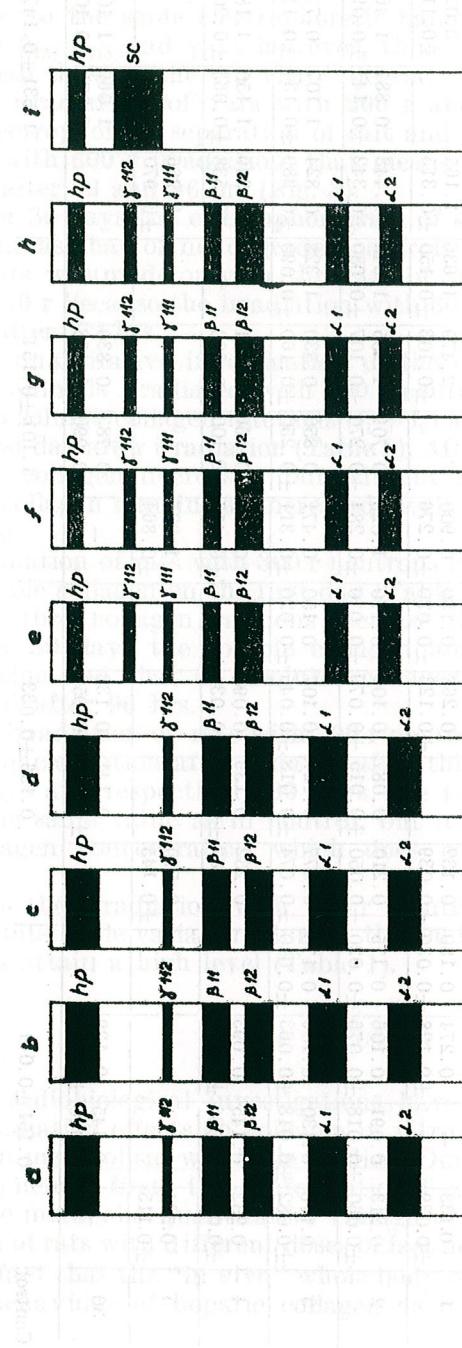


Fig. 3.—Electrophoregrams of soluble collagen from animals irradiated with 200 r or 150 r neutrons. a. salt soluble collagen extracted 24 hrs after irradiation of the animals; b. salt soluble collagen extracted 48 hrs after irradiation; c. salt soluble collagen extracted 72 hrs after irradiation of the animals; d. salt soluble collagen extracted 96 hrs after irradiation of the animals; e. acid soluble collagen extracted 24 hrs after irradiation; f. acid soluble collagen extracted 48 hrs after irradiation; g. acid soluble collagen extracted 72 hrs after irradiation; h. acid soluble collagen extracted 96 hrs after irradiation; i. salt or acid soluble collagen extracted 30 days after irradiation of the animals.
hp = high polymers; sc = soluble collagen.

Table 1
Amount of hepatic collagen (mg/g dry tissue) in neutron irradiated rats

Time (Days)	F ₁ (Salt soluble)			Soluble collagen (acid soluble)			Insoluble collagen (F ₃)			Total collagen		
				Dose (r)			Dose (r)			Dose (r)		
	150	200	300	600	150	200	300	600	150	200	300	600
1	0.133 ± 0.039	— ± 0.128	0.274 ± 0.030	0.180 ± 0.039	0.132 ± 0.123	— ± 0.020	0.268 ± 0.020	0.180 ± 0.020	0.900 ± 0.276	— ± 0.103	2.479 ± 0.150	1.636 ± 0.315
2	0.213 ± 0.060	0.091 ± 0.018	0.106 ± 0.076	0.175 ± 0.020	0.210 ± 0.050	0.087 ± 0.015	0.103 ± 0.079	0.170 ± 0.010	1.025 ± 0.284	0.709 ± 0.270	1.255 ± 0.107	1.343 ± 0.080
3	0.176 ± 0.082	0.130 ± 0.018	0.109 ± 0.063	0.170 ± 0.010	0.177 ± 0.074	0.127 ± 0.018	0.107 ± 0.045	0.150 ± 0.020	0.471 ± 0.311	0.850 ± 0.250	0.530 ± 0.160	1.328 ± 0.090
4	0.232 ± 0.082	0.145 ± 0.036	0.093 ± 0.050	— ± 0.050	0.232 ± 0.082	0.139 ± 0.028	0.090 ± 0.035	— ± 0.028	0.885 ± 0.456	0.866 ± 0.100	1.001 ± 0.300	1.349 ± 0.538
5	0.569 ± 0.115	— ± 0.040	— ± 0.040	— ± 0.056	— ± 0.044	— ± 0.044	— ± 0.052	— ± 0.044	— ± 0.064	— ± 0.064	— ± 0.064	— ± 0.064
30	— ± 0.040	0.145 ± 0.040	0.139 ± 0.040	— ± 0.056	— ± 0.044	— ± 0.044	0.133 ± 0.052	— ± 0.044	— ± 0.090	0.783 ± 0.158	0.830 ± 0.158	— ± 0.070
Control	0.151 ± 0.041	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040	— ± 0.040
									1.101 ± 0.154			1.531 ± 0.147

The description is the same at 24, 48, 72 and 96 hrs but after 72 and 96 hrs the electrophoretic mobility of the fragments is greater than that of the corresponding bands of thermal-degraded collagen (Fig. 2).

Acid soluble collagen from the same animals led, in our working conditions, to the same electrophoretic bands corresponding to the same fragments β_{12} , β_{11} , and γ_{112} ; however, their electrophoretic mobilities are greater than those of the "in vitro" degraded controls (Fig. 2).

The irradiation of rats with 200 r and 150 r neutrons leads to a similar electrophoretic separation of salt and acid soluble collagen as that obtained with 600 r irradiation, that means five bands, after 24 hrs and 48 hrs as after 72 and 96 hrs (Fig. 3).

After 30 days the electrophoregram of salt and acid soluble collagen is the same as that of nondegraded controls (Figs 2 and 3). This sort of experiments was made only in the case of animals irradiated with 300, 200 and 150 r because the irradiation with 600 r leads to the death of all animals after 72 hrs.

The quantitative investigation of the collagen metabolism by Hyp dosing of animals irradiated with 600 r neutrons pointed out an increase of the two soluble collagen fractions ($f_1 + f_2$) and of the insoluble collagen (f_3) the first day after irradiation (Table 1). After 2 and 3 days, the amount of soluble collagen decreases, but without attaining control value. The insoluble collagen remains at increased levels during the first 3 days after irradiation.

Irradiation of rats with 300 r neutrons leads to an increase of soluble and insoluble collagen on the first day (Table 1). In comparison with these values, the three collagen fractions decrease after 2, 3 and 4 days.

After 30 days the soluble collagen amount increases close to the control value and that of insoluble collagen remains close to the value determined after 96 hrs.

The irradiation of rats with 200 r neutrons leads to a decrease of the collagen concentration after 2 days; after this, an increase occurs so that after 4 days and respectively 30 days, the soluble collagen concentration reaches the same value as in control, but it is not enough for the insoluble collagen concentration which does not reach the control value (Table 1).

After the irradiation with 150 r neutron the hepatic soluble collagen exhibits little variation during the 96 hrs; after 6 days, however, the values attain a high level (Table 1).

4. DISCUSSION

The radiobiological investigations have pointed out especially the X and γ radiation effects on collagen in vitro. In fact, the radiation effect on collagen metabolism was little studied. Our investigations have in view two phenomena: first, the conformational qualitative modifications and second, the metabolic quantitative change of hepatic collagen after total irradiation of rats with different doses of fast neutrons.

The fact that the "in vivo" whole body irradiation leads to an electrophoretic behaviour of hepatic collagen as if it were thermally degraded

leads us to the conclusion that under neutron influence a conformational transition takes place.

It can be noted that with 300 r neutron irradiation the molecular fragmentation leads only to β and γ components (electrophoretically, α_1 and α_2 components can not be obtained).

The increase of the electrophoretic mobility of some bands in some cases of irradiated animals leads to the hypothesis that the molecular weight of fragments, supposed to be similar with that of the corresponding controls, is in fact different.

It is probable that in this case there occur changes of rigidity, restriction of collagen trihelix flexibility imposed by imino groups and therefore by molecular crosslinking changes that are similar, yet different from those which occur in thermal degradation process [4].

Our results concerning the Hyp concentration in the hepatic collagen of irradiated animals prove that neutron radiations lead to important modifications at this level in the first 24 hrs. After this interval the body begins to reestablish its anabolism-catabolism balance for the hepatic collagen, especially when a small dose is employed (for example a dose of 150 r neutrons). The increase of insoluble collagen fraction when the rats were irradiated with 600 r neutrons is in accord with the other researches [4] [7] which mention that the amount of collagen in irradiated lung begins to increase half a year after exposure of a hemithorax.

The comparison of results concerning the conformational qualitative and metabolic-quantitative changes leads to the conclusion that the modification observed is the result of earlier changes in collagen properties [14].

Although it is a similarity between the aging of collagen and the effect of irradiation, Anderson [2] pointed out that this conclusion is valid only when the hexozamine-collagen ratio is a factor of aging. On the other hand, Sandberg and Doull [16] have noted that the viscosity of skin salt collagen solutions decrease with age, and this decrease is not intensified by chronic irradiation.

Our results are in agreement with those obtained by other researchers regarding disorder effects [6]; this appears to be the effect of the disruption of a number of hydrogen bonds after the covalent bonds have been broken.

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DYNAMICS OF -SH GROUPS IN THE THYMUS OF OJ ASCITIC CARCINOMAS BEARING RATS

BY

V. TOMA and N. FABIAN

The intraperitoneal transplantation to Wistar male rats (100 ± 10 g) of 1 ml (32,000 cells) of OJ ascitic carcinoma (OJA) induced a rapid thymus involution with significant imbalance of the —SH groups, assayed by an ampero-argentometric method. The dynamics of thymic —SH groups reflects rapidly and faithfully the development of neoplasia and this test proves to be more sensitive than the ponderal or histological ones. The role of immunological and endocrine thymus is discussed in connection with the oncogenesis.

It is well known that the thymus may be considered the master gland of the "cell mediated immunity" (CMI) of the hosts, including the tumor immunity [3], [4], [15]. Thus, the pre-thymic stem cells can be activated by polypeptide hormones of the gland into T immunocompetent lymphocytes [1], [5]. For this purpose, the histochemical and metabolic processes in the thymus during carcinogenesis may reflect the basic steps of the organism immune balance, i.e. the multiple biological activity of this immuno-endocrine gland.

In previous papers [18], [19], we pointed out that in the experimental tumours development there are considerable modifications of the thymus nucleic acids, proteins or glycogen content. The aim of the present study is to investigate the effect of OJ ascitic carcinomas (OJA) on the thymus -SH groups dynamics; these thiolic groups have an essential role in protein and nucleic acid metabolic processes, both in the normal or pathological cell division [1], [10], [21], [22].

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 100 ± 10 g subjected to intraperitoneal transplantation with 1 ml ascitic fluid of OJA containing 32,000 cells/ml [11]. Eight to twelve animals were sacrificed by decapitation 1, 3, 7, 9, 11 and 17 days after tumour inoculation, together with control rats. All animals were necropsied and the thymus weighed (TW) on a torsion balance. The determination of the free-total (FT), proteinic (P) and non-proteinic (NP) -SH groups, was performed by an ampero-argentometric method [13]. In cases of strong involution, the thymi of 2–3 animals were used for each determination of the -SH groups. The results were expressed in µM/g fresh thymic tissue, and Student's test was used in the statistical analysis of the data.

Table 1
Quantitative value of the thymic — SH groups: free total (FT), proteinic (P), nonproteinic (NP), P/NP % ratio of rats inoculated with ascitic carcinomas

	Control	1 day	3 days	7 days	9 days	11 days	17 days
FT $\mu\text{M/g}$	10.67 ± 0.19 (10)	11.06 ± 0.10 (11)	10.3 ± 0.07 (12)	8.57 ± 0.12 (10)	8.43 ± 0.13 (8)	7.89 ± 0.11 (9)	8.06 ± 0.09 (8)
$\pm \%$	+3.70 <0.10 >0.05	-2.72 >0.20 >0.10	-19.70 <0.20 >0.10	-21 <0.01	-21 <0.01	-26.10 <0.01	-24.50 <0.01
P $\mu\text{M/g}$	9.46 ± 0.15 (10)	9.83 ± 0.10 (11)	9.06 ± 0.18 (12)	7.41 ± 0.10 (10)	7.52 ± 0.12 (8)	7.05 ± 0.10 (9)	6.46 ± 0.08 (8)
$\pm \%$	+4 <0.10 >0.05	-4.20 <0.20 >0.10	-21.70 <0.20 >0.10	-20.50 <0.01	-20.50 <0.01	-25.50 <0.01	-31.70 <0.01
NP $\mu\text{M/g}$	1.21 ± 0.04 (10)	1.23 ± 0.01 (11)	1.32 ± 0.02 (12)	1.16 ± 0.02 (10)	0.91 ± 0.01 (8)	0.84 ± 0.02 (9)	1.60 ± 0.01 (8)
$\pm \%$	+2 <0.5	+10 <0.20 >0.10	-4.10 <0.50 >0.20	-4.10 <0.50 >0.20	-24.80 <0.01	-30.6 <0.01	+32.2 <0.01
P/NP $\%$	7.81 ± 0.13 (11)	7.99 ± 0.03 (11)	6.86 ± 0.06 (12)	6.39 ± 0.06 (10)	8.26 ± 0.07 (8)	8.39 ± 0.10 (9)	4.04 ± 0 (8)
$\pm \%$	+2.30 <0.5	-12.20 <0.01	-18.20 <0.01	-18.20 <0.01	+5.80 <0.02	+7.40 <0.02	-48.40 <0.01
TW (mg)	208.3 ± 12 (8)	226.1 ± 6.7 (8)	25.2 ± 17.7 (8)	-	182.7 ± 13.8 (8)	-	75.6 ± 11.7 (9)
$\pm \%$	+8 -	+8 -	+20 -	-	-13 -	-	-64 <0.001

n = values are given as means of animals; SE = standard error; n = number of animals; ± % = percentage differences against the control animals are given.

RESULTS

1. THYMUS WEIGHT WITH RESPECT TO THE DEVELOPMENT OF MALIGNOMAS

Three days after OJA transplantation in the abdominal cavity of rats the ascitic fluid became apparent; with its progressive growth the TW decreased in the same manner. On the 17th day, when the absolute massive death of tumour bearing animals was observed, the value of the TW represented -64 % ($p < 0.001$) of that of controls.

2. THE DYNAMICS OF THYMIC — SH GROUPS

On the 3rd day of oncogenesis the increase of NP—SH groups (+10 %) at the significance limit was detectable, while the —SH groups ratio P/NP % decreased by 12 % ($p < 0.01$). From the 7th day until the 11th, the FT and NP—SH groups were at statistically significant lower levels against the controls thymic —SH groups. Sometimes the P/NP % ratio remains at a higher level.

In the exacerbated phase of cancer after 17 days of the OJA inoculation appears a new increase by 32 % of NP—SH groups ($p < 0.001$), simultaneously with a significant decrease of the P/NP %—SH groups ratio (-48 %; $p < 0.01$).

DISCUSSION

Our data show that the dynamics of thymic —SH groups reflects rapidly and faithfully the phases of development and progression of the neoplasia, respectively the degree of the antitumour potency of the hosts. This test proves to be more sensitive than the ponderal and the histological ones [8], [14]. Thus the OJA with a strong aggressivity and reduced survival time of the inoculated animals induces a dramatic variation of the thymic —SG groups content, even in the first days of tumour growth. The keystone moments of OJ carcinogenesis seem to be the 3rd day when the NP—SH groups of the thymus present a sudden rise, equivalent to a deficient alarm and adaptation phase, and the 17th day in the final stage of rats survival, respectively in the exhaustion stage under action of the tumoral stressor. In fact, the increase of the thymic NP—SH groups and its subsequent depletion, together with the fact that the FT and P—SH substances decreased continuously, mirror the involution of the gland induced by the glucocorticoid hormones with immunosuppressive property [17]. Also a constant involution accompanied by a decrease of thymic FT—SH groups was observed in aged rats [12], this ontogenetical moment characterizing a severe deficiency of the immunobiological potency of the organism against the cancer development [3], [4]. In accordance with Simu et al. [14], the thymus involution might be correlated with the depressed immunity of advanced stages of malignant diseases. The precocious thymus involution and depression of CMI in the OJA instance seems to be due to the adrenal's hormonal hypersecretions, triggered by the tumoral stimulus, as well as by some tumour metabolites [7].

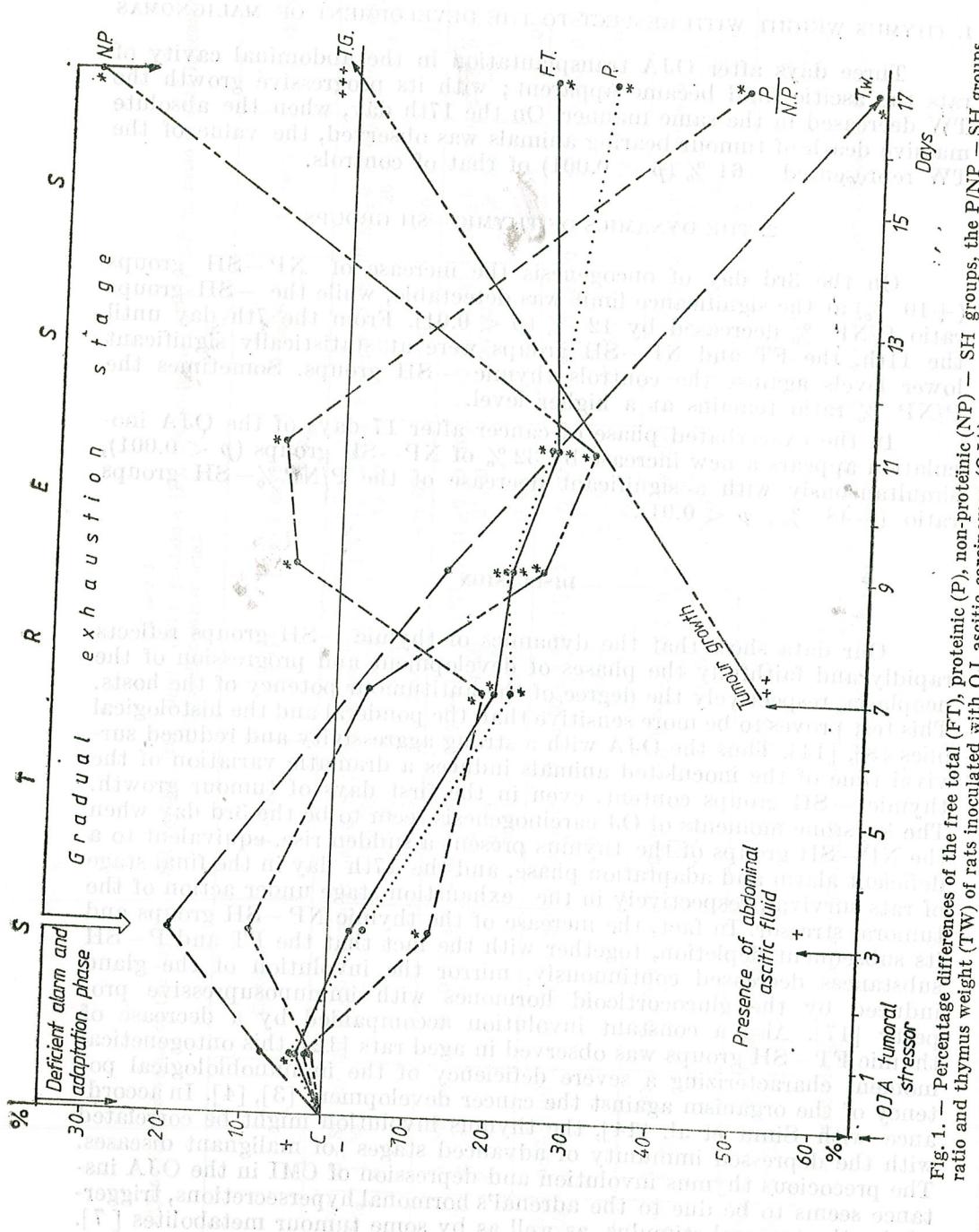


Fig. 1. — Percentage differences of the free total (FT), proteinic (P), non-proteinic (NP) — SH groups, the P/NP — SH groups ratio and thymus weight (TW) of rats inoculated with OJ ascitic carcinomas (OJA), as compared with control animals (C).

The disturbance of the SH substances in the thymus of OJA bearing rats can indicate a deviation of the nucleic acids synthesis [16], [22]. Also in the function of the T cell system under the action of malignant tumours, the RNA may serve as an "informational code" in the transfer of the immunoresponsiveness of the T killer cells [6].

On the other hand, after Oeriu and Oeriu [10], the thiolic groups are involved in a variety of chemical reactions, by their function as structural components of enzymes, coenzymatic vitamins and protein hormones. In this sense the deviation of thymic —SH groups in aging or tumour bearing rats may be followed by a deficiency in the hormonal activity of the gland, simultaneously stopping the differentiation of T precursor cells into fully immunocompetent lymphocytes. The assertion might be harmonized with Metcalf's [9] opinion that: "In part at least, the thymic influence on host immune responses to antigenic cancer cells is mediated via humoral factor".

CONCLUSIONS

Our data point out that the dynamics of the thymic —SH groups reflects the degree of thymic involution of the OJ ascitic carcinomas bearing rats. The test proves that the Key moments of OJ carcinogenesis seem to be the 3rd and the 17th day after the intraperitoneal transplantation of the tumour.

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antibiotice A 6.7 în cinci, astăzi cincisprezece, 10% și la doar trei din 42.36 și 40% cind au obținut o dezvoltare excepțională, cu o creștere de celule mai mare decât în control, și chiar totuși astăzi "stările biologice" nu se pot spune că sunt bune. Cu alte cuvinte, "in vitro" testele de citostatică demonstrează că aceste două noi preparații au un efect de inhibiție puternic asupra creșterii celulelor, care este deosebit de intens și durată, comparativ cu cea obținută cu antibioticul clasic, quinacrine hidroclorură, care este totuși mult mai scăzută și deosebit de scurtă. În același timp, din punct de vedere al creșterii celulelor, ambele noi preparații au un efect similar, chiar și la doar trei sau patru zile de incubație, deși sunt obținute din bacterii cu proprietăți degradante și destruoare ale celulelor.

CONCLuzIOn

În concluzie, rezultatul obținut în ceea ce privește citostaticitatea noilor antibiotice A 6.7 și A 42.36 este deosebit de interesant, întrucât în ceea ce privește citostaticitatea bacterienelor, până în prezent, nu există niciun alt antibiotic care să obțină astfel de rezultate.

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În continuare, în cadrul acestui articol, vor fi menționate rezultatele obținute cu ajutorul unor metode de citostatică.

"IN VITRO" CYTOSTATIC ACTIVITY INVESTIGATION OF SOME NEW BIOSYNTHETIC ANTIBIOTIC PREPARATIONS

P. ROTINBERG, A. SAUCIUC*, ECATERINA DUCA, SMARANDA KELEMEN, CSÖNGE BRANDSCH și GEORGETA NANEȘCU*

The cytostatic action of antibiotic preparations A 6.7 and A 42.36 on HeLa cell culture development has been investigated. The decrease of protein concentration observed at different time intervals in the development of drug-treated cultures reflects an inhibitory action of the studied antibiotics on cell culture growth. The protein dynamics, which characterizes the development of HeLa cell cultures, as well as the dose-effect response obtained in this study indicate these two new antibiotic preparations as potential cytostatic drugs.

Until recently, the importance of antibiotic substances has been exclusively related to the antimicrobial activity based on their specific effect on Prokaryotes. However, the area of investigation of their action has broadened lately, a bulk of reports revealing some as yet unknown actions of these biosynthetic substances on Eukaryotic cells or even on mammalian organism. This opens new vistas for their applicability [1].

One of the most interesting effects is linked to the antitumoral action of some secondary metabolites elaborated by microorganisms [8], [15] due either to their cytostatic action [2], [3], [4], [6], [10] or to the potentiation of the immune system [1], [7], [14], [16].

Searching for new natural products with antitumoral activity represents at present a major concern in cancer treatment when chemotherapy is still a priority choice. On this line, investigation is continued by a research group from the Biological Research Centre, Iași and from the Centre for Antibiotic Research, Iași.

This paper presents the results of our investigations of the cytostatic activity of some new biosynthetic antibiotic preparations, isolated by a team from the Centre for Antibiotic Research, Iași, in preliminary "in vitro" screening tests, evaluated by the total protein dynamics during the development of HeLa cell cultures incubated with antibiotic preparations coded A 6.7 and A 42.36.

MATERIAL AND METHODS

Due to their rapid growth and reproducibility of the results, the tests for the identification and quantification of the cytostatic action of the isolated secondary metabolites A 6.7 and A 42.36 were performed on HeLa cell cultures.

The culture tubes containing culture medium IC-65 were inoculated with 1×10^5 cells. After 24 h, when the culture formed a monolayer and was in the logarithmic stage of development, the medium was changed with a medium containing either 0.5, 1 or 1.5 mg/ml of the studied drugs. After 48 and 72 h of development the culture medium was discarded from both control and drug-treated culture tubes. The cell layer was washed with TFS and total protein concentration was determined according to a modification of Oyama and Eagle's method [9]. For each concentration and time-interval five cell cultures were processed.

The results were evaluated statistically by Student's "t" test.

RESULTS AND DISCUSSION

The experimental data obtained in the investigation of the action of A 42.36 preparation on HeLa cell culture development as compared to controls are shown in Fig. 1.

It is evident that the protein level of 48 h cultures incubated 24 h with the drug are equal to the control values, regardless of the drug concentration, the antibiotic having no negative effect on the evolution of 24 h-treated cultures. However, protein concentrations determined in cultures of 72 h, incubated for 48 hours with the A 42.36 drug are significantly lower than those of the controls ($p < 0.002$ and $p < 0.001$). The protein values progressively decreased with the increment of drug concentration in the medium, revealing an inhibition in the development of treated cultures by 10, 30 and 40 per cent, respectively, that is a dose-effect response.

The other antibiotic preparation tested by us "in vitro" was the isolate A. 6.7, the experimental results being illustrated in Fig. 2.

The cytostatic action of this drug was evident after 24 h of incubation, that is 48 h in culture development, the decrease in protein concentration being significant for drug concentrations of 1 and 1.5 mg/ml ($p < 0.002$), which induced an inhibition of approximately 23 per cent compared to the control cultures.

After 48 h of incubation (72 h of development) with the preparation A. 6.7, a significant decrease ($p < 0.02$ and $p < 0.001$) in protein concentration was observed for all the doses used in the experiment.

The inhibition of HeLa cell development from 25 to 50 per cent, induced by the antibiotic according to the increment of drug concentration in the medium also reveals a dose-effect relationship.

HeLa and KB cell cultures were included by many Institutions around the world in the chemotherapeutic screening programmes for discovery of new therapeutic antitumor agents. Cell cultures have been used successfully as a prescreening test for both natural fermentation and synthetic products. A good correlation between cytotoxic and antitumor activity has been generally found [5], [12], [13].

This screening test offers direct indications on the cytotoxic or cytostatic actions, provides, in short time-intervals, indications on the biological activity of a large variety of compounds and requires only

small amounts of natural or semisynthetic products with potential antitumoral activity.

However, the "in vitro" tests on cell cultures, although reproducible, represent only a preliminary step of initial selection followed by "in vivo" test on experimental tumor systems for those agents which exhibited a potential cytostatic or cytotoxic action "in vitro".

Our experimental data, showing an inhibitory action on HeLa cell development and dose-effect relationship, indicate the antibiotic preparations A 6.7 and A 42.36 as agents with potential cytostatic action. However, these findings impose "in vivo" investigations on animals bearing several tumoral systems in order to ascertain their antitumoral action and also to investigate their molecular mechanisms of action.

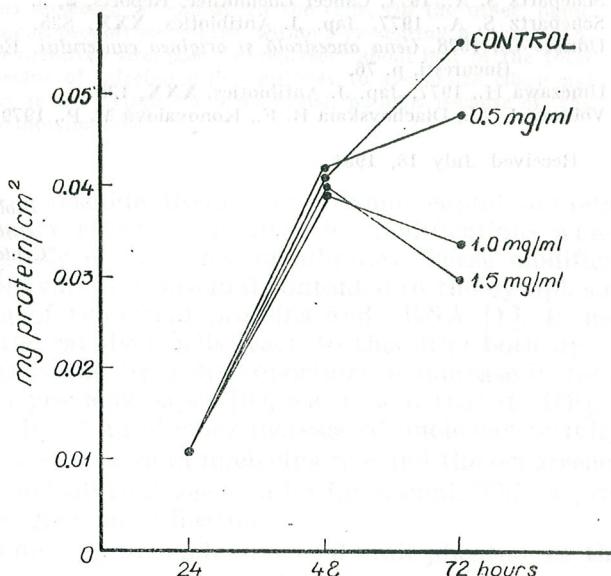


Fig. 1. — Protein content of HeLa cell cultures incubated with different concentrations of A 42.36 antibiotic isolate.

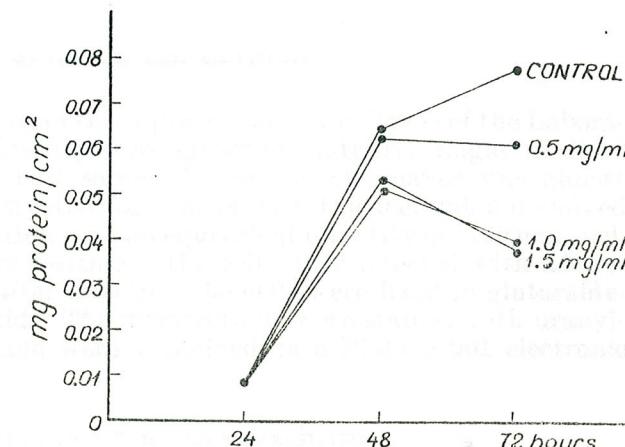


Fig. 2. — Protein content of HeLa cell cultures incubated with different concentrations of A. 6.7 antibiotic isolate.

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ULTRASTRUCTURAL STUDY OF ADENOVIRUS 3 REPLICATION IN THIOACETAMIDE-TREATED HEP-2 LINE CELLS

BY

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In the thioacetamide-treated HEp-2 line cells, infected with adenovirus 3 at 24 hours after infection early inclusions appeared in almost all the cells. On the other hand, virions are present in a very small proportion of cells, without presenting a characteristic crystalline disposition. The defective viral particles represent about half of the total number of virions. The nucleolus of infected cells continues to synthesize ribosomal precursors, but their transport to the cytoplasm is disturbed, perhaps because of the nuclear envelope permeability modification.

It is known that thioacetamide disturbs the ribonucleoproteic metabolism because of its primary effect represented by modifications which it induces in the permeability of lysosomal membranes. These modifications lead to a release of enzymatic lysosomal content into the cytoplasm, followed by a degradation of ribosomal proteins and rRNA [1]. It has been shown that *in vivo* the rat liver cells react to this drug both by an increase of albumin synthesis and by a disproportionate increase in total mRNA synthesis [2]. In a previous paper [9], we showed that in HEp-2 line cells, thioacetamide induced an obvious increase of nucleolar activity which is reflected both in the increase in nucleolus size and the occurrence of a constellation of mininucleoli that seem to be functional. This aspect is typical of the ribosomal gene amplification.

The present paper is dealing with adenovirus 3 multiplication in the cells treated with thioacetamide which drastically modifies the ribonucleoproteic metabolism.

MATERIAL AND METHODS

The HEp-2 line cells from the collection of cell cultures of the Laboratory of the Institute of Virology were grown on nutritive Eagle medium supplemented with 10% calf serum. When the monolayer was almost completed the cells were treated for 3 days with thioacetamide dissolved in physiological saline solution with an equivalent quantity of 150 mg. pel. At 10 hours after the last treatment the cells were infected with adenovirus 3, and at 24 hours after infection the cells were fixed in glutaraldehyde and osmium tetroxide. The ultrasections were stained with uranyl-acetate and lead citrate, and were examined in a Phillips 201 electronic microscope.

RESULTS

The HEp-2 line cells treated with thioacetamide and subsequently infected with adenovirus 3, presented an obvious densification and aggregation of heterochromatin which appears as blocks with preferential peripheral location, sometimes being diffusely dispersed in the nucleus (Fig. 1). The nucleolus exhibits segregation resulting its granular and fibrillar components. The granular component is voluminous, dense compact, while the fibrillar one is quantitatively more reduced (Fig. 2). The granular component undergoes a fragmentation process and the resulting fragments are released and migrate into the nucleoplasm (Fig. 3). In the nucleus there are numerous small, dense, structures which represent mininucleoli resulting from the nucleolus following thioacetamide treatment [9].

After infection with adenovirus 3 the mininucleoli become very dense, compacted and perhaps nonfunctional, because they also exhibit the segregation phenomenon (Fig. 3). Most of the cells present in their nucleus distinct areas with a fine fibrogranular appearance, sometimes very well delimited. It has been shown, by isolation and chemical analysis [11], that these inclusions contain viral DNA as well as viral mRNA and viral protein. They correspond to early inclusions which appear after infection with adenoviruses. In the thioacetamide-treated cells, at 24 hours after infection with adenovirus 3 these inclusions occupy almost the whole nuclear volume (Fig. 3, 4).

There is a second category of structures with a dense, prominently granular texture which do not appear so frequently. They seem to originate from the nucleolus because they have close space relationships, sometimes relations of continuity with the nucleolus (Fig. 3). Perhaps these formations represent the product of nucleolar activity, i.e., ribosomal precursors. They also appear in the vicinity of the nuclear envelope (Fig. 5b), and even in the cytoplasm (Fig. 5c). The assumption that these granular components are the products of nucleolar activity relies on the nucleolar hyperfunction induced by thioacetamide, hyperfunction which continues for a while even after viral infection. The cells treated with thioacetamide, but which are not infected with adenovirus 3, lack such structures [9], because in these uninfected cells, the transport of ribosomal precursors into the cytoplasm takes place normally, i.e., in a continuous flow. On the other hand, in the infected cells this transport is impaired, perhaps because of alterations in the nuclear envelope permeability as a consequence of viral infection. There is a retardation in the transfer of ribosomal precursors into the cytoplasm where they are aggregating in large blocks. Even when ribosomal precursors have reached the cytoplasm (Fig. 6), they remain in an aggregated compact state, maybe nonfunctional, although apparently there is a structural continuity between the granular component of nucleolar origin and cytoplasmic ribosomes.

There is another interesting aspect in these infected cells following thioacetamide treatment: despite the fact that the majority of the cells present early viral inclusion, virions appeared only in a small proportion of these cells. On the other hand, in the untreated cells, at 24 hours

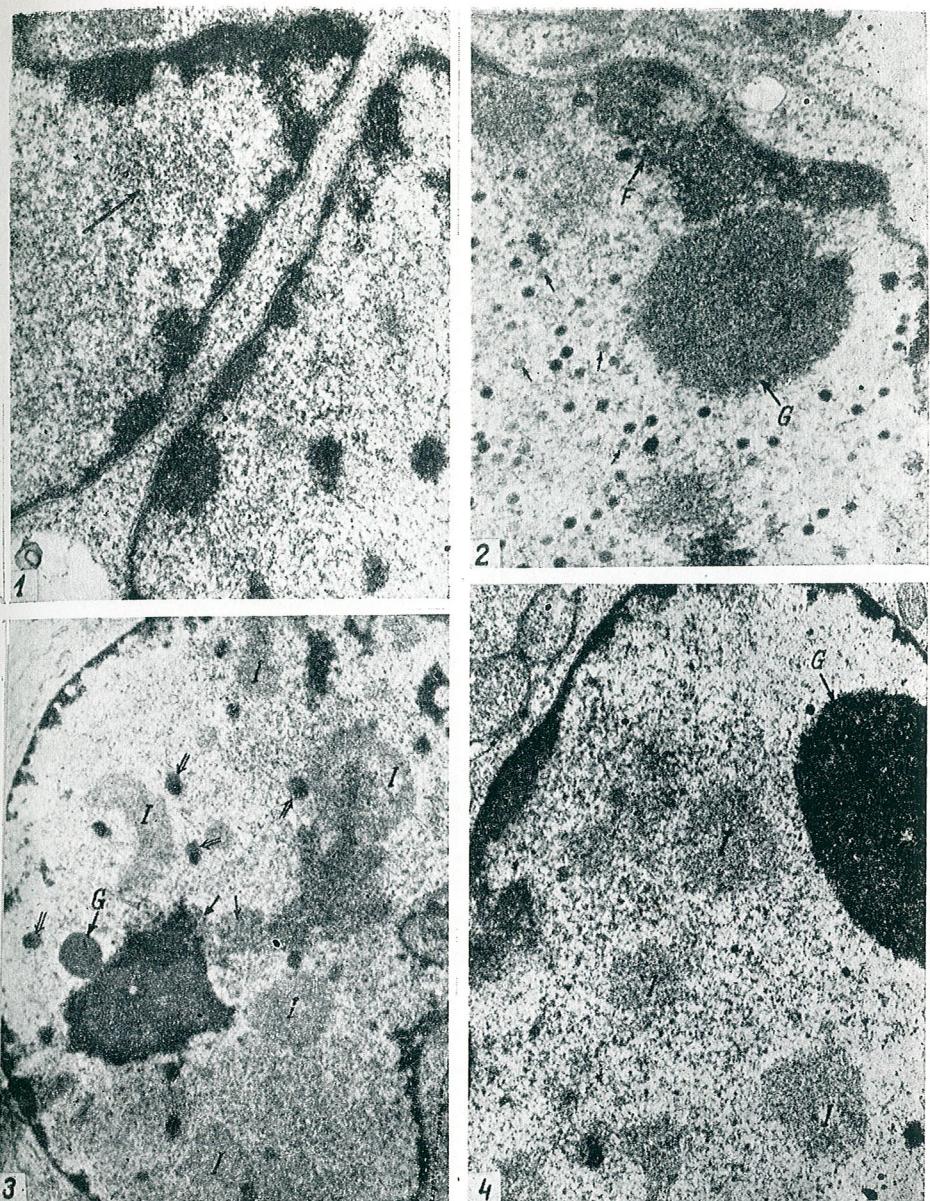


Fig. 1. — The dense heterochromatic blocks are preferentially located at the nucleus periphery and rarely in the nucleoplasm. The arrow indicates the presence of interchromatin granules. $\times 32,250$.

Fig. 2. — The nucleolus is segregated into granular (G) and fibrillar (F) components. Numerous viral particles are defective (arrows). $\times 39,200$.

Fig. 3. — Dense segregated nucleolus. Grossly granular structures (arrows) appear to have continuity with the nucleolus. In the nucleus there are numerous early viral inclusions (I) and dense segregated bodies—mininucleoli (double arrows), $\times 12,600$.

Fig. 4. — The persistence of granular nucleolar component (G). Numerous early viral inclusions can be observed (I). $\times 29,400$.

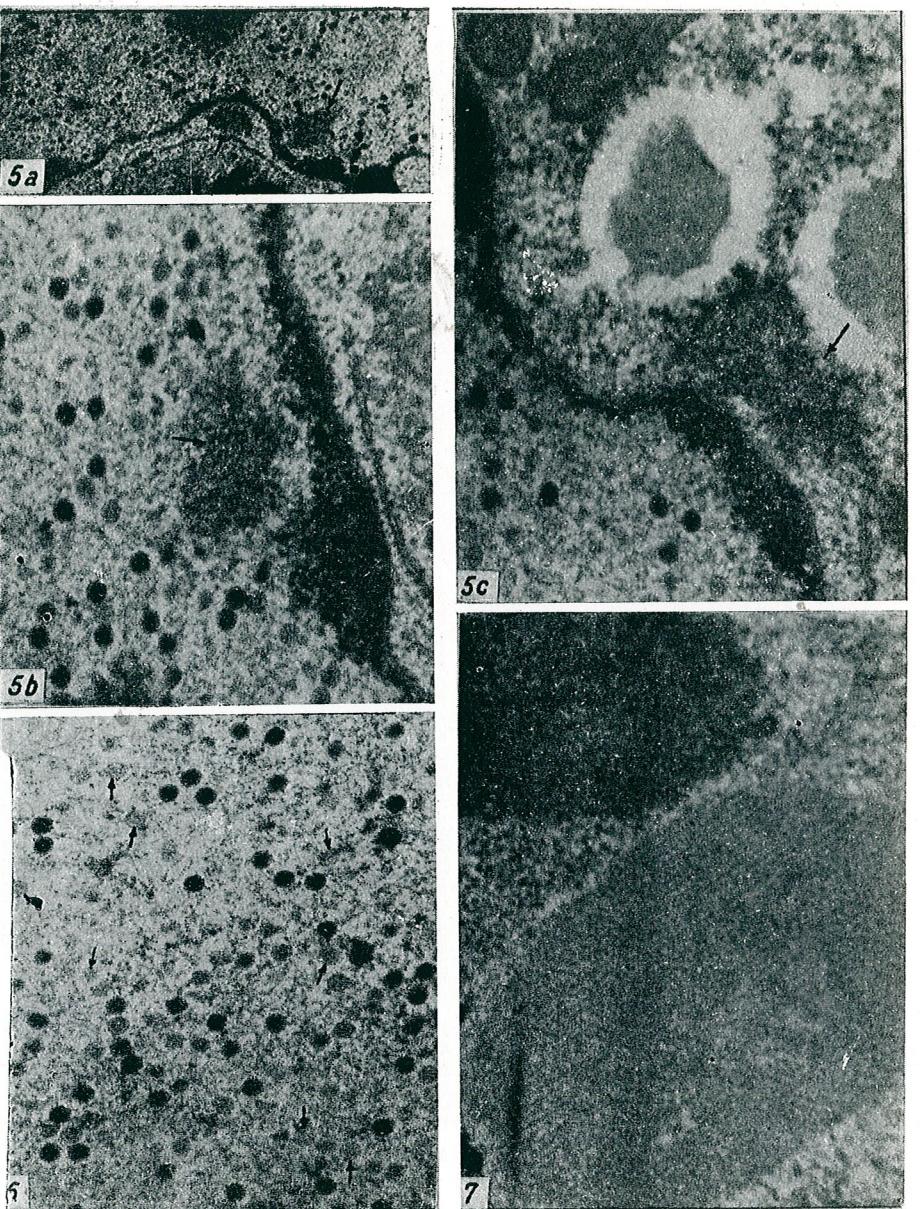


Fig. 5a. — The granular structures with gross texture in the nucleus and cytoplasm (arrows).
x 14,500.

Fig. 5b. — A detail of the image from 5a. A granular structure (arrow) in the vicinity of nuclear envelope. x 88,500.

Fig. 5c. — A detail of the image from 5a. A granular structure (arrow) in the cytoplasm that seems to be continued with the cytoplasmic ribosomes. x 88,500.

Fig. 6. — Viral particles are rare and often defective (arrows). x 59,200.

Fig. 7. — The proteic paracrystalline inclusion in the nucleus. x 58,000.

after infection, the virions are present in almost every cell [8], and this aspect is an indication of the delay of viral multiplication cycle in the cells treated with thioacetamide. In such cells the quantity of the virions is drastically diminished. The appearance of defective virions with reduced frequency is a common aspect in normal conditions, but the replication of adenovirus 3 in the cells pretreated with thioacetamide leads to the appearance of a large number of defective virions which represents almost half of the total number of virions and which do not acquire the crystalline disposition characteristic of replication in untreated cells. Sometimes, proteic crystalline inclusions can be encountered (Fig. 7).

DISCUSSIONS

The thioacetamide treatment leads to nucleolar hyperactivity mainly expressed by a compensatory intensification of ribosomal precursor synthesis. This type of intensification leads to the nucleolus hypertrophy and the appearance of a constellation of mininucleoli which seem to be functional, because they are surrounded by granules [9].

As a consequence of infection with adenovirus 3 in the thioacetamide treated cells, there appears an abundance of inclusions, which are supposed to be of an early type [7, 15]. Similar inclusions, or with a different consistency, have also been observed for other adenoviruses [3], [6], [14]. The diversity of these inclusions seems to be dependent both on the type of infecting virus and the cell substrate, but the specific conditions for every experiment also account for this diversity. The abundance of early viral inclusions encountered at 24 hours after infection can be interpreted in terms of an efficient net delay in viral replication cycle. This very reduced quantity of virions reflects a defective viral assembly process, perhaps due to an absence or to a quantitative insufficient synthesis of some capsid polypeptide or of some of viral core proteins [13]. The hexones are still excessively synthesized and this assumption is based on the observations that crystalline inclusions are represented even by this hexonic capsomeres (4, 5).

The fragmentation of the nucleolar fibrillar component leads to the appearance of dense nuclear bodies which have also been encountered after infection with other viruses [12]. We have found that at least a part of these dense nuclear bodies originate from mininucleoli that appeared after a thioacetamide treatment. This assumption is based on the observation that the dense nuclear bodies exhibit a segregation process.

The granular structures, with a thick texture that appear to be released from the nucleolus, probably represent ribosomal precursors whose synthesis is continued even after the infection [10]. The absence of such structures in the thioacetamide-treated cells, but uninfected, suggests the possibility of a postinfection synthesis of some nonfunctional ribosomal precursors. The lack of functionality of the ribosomal precursors is suggested by their aggregation in large blocks which disturbs their transfer to the cytoplasm.

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THE ROLE OF THE SEXUAL PHEROMONE IN THE REPRODUCTION OF *MAMESTRA BRASSICAE* L. (LEPIDOPTERA, NOCTUIDAE)

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By using the antennectomy method, the role of the male and female sexual pheromone in the reproduction of *Mamestra brassicae* was studied. In the antennectomized males, where the perception capacity for the sexual pheromone is decreased, the mating capacity is also decreased. Completely antennectomized males coupled with females in a very small number and without transfer of spermatophores in the bursa copulatrix of the females. The completely antennectomized females normally mated with the males, which proves that the male sexual pheromone does not influence the reproduction in this species. The antennectomy of males also causes a delay in the onset of the mating behaviour.

In insects, the reproduction is a complex biological activity which comprises three phases: courtship, mating and oviposition. Each phase, in turn, has several physiological and behavioural sequences, succeeding in a certain chronological order and conditioning one another [5]. Certain external stimuli are involved in the development of the reproduction phases and in the onset of some behavioural manifestations characteristic of mating and oviposition, among which a very important role is played by the chemical stimuli of the pheromones group. The investigations of different insect species showed that in most species the female sexual pheromone has a determining role in the mating behaviour [2], [3], [9], [11]. The female sexual pheromone is useful for the male orientation towards the female and, at the same time, it determines in males a physiological and behavioural state adequate for the mating process. There are species of Lepidoptera where the males secrete a certain sexual pheromone with an aphrodisiac role [1], [4], [5], [6], [10], in the absence of which the female does not mate, as it is the case in *Heliothis virescens* [6], *Plodia interpunctella* [5], *Danaus gilippus* and *D. chrysippus* [10]. It has been observed that in another species, mating takes place even in the absence of the male pheromone, as in *Trichoplusia ni* [4], *Danaus plexippus* [10], *Cadra cautella* [5].

Our investigations tried to establish the role played by the male and female sexual pheromone in the reproduction of *Mamestra brassicae*, a principal pest for cabbage cultures.

MATERIAL AND METHOD

Adults of *M. brassicae*, used in the experiments, came from a population of larvae grown under laboratory conditions in a semi-natural

medium. Both the larvae and the adults were kept at +25°C, and humidity over 70 %. The pupae were separated according to sex and put in different cages. After the emergence, the adults were kept — until the experiment — in 7 l. glass vessels and fed with a 10 % honey solution. In our investigations we used the antennectomy method, since the chemoreceptors sensilla for the sexual pheromone are located on the antennae [8], [12]. The amputation of the antennae was done 24 hours before the experiment.

Only healthy individuals, manifesting a vigorous flight, were selected for the experiments.

We estimated the role of the sexual pheromone in the reproduction of the species according to the number of matings of the antennectomized individuals and to the number of the females laying fertile eggs, which indicated that the matings were normal.

We established five variants: normal females with males having one completely amputated antenna, normal females with males having both antennae half-amputated, normal females and males with 3/4 of the antennae amputated, normal females and males with both antennae completely amputated and females having both antennae completely amputated coupled with normal males.

For each variant we have assayed 5 groups, each of them composed of 10 pairs, which means 50 pairs for each variant. The control variant consisting also of 50 pairs was assayed under the same conditions.

Each pair was put separately in glass vessels (20 cm diameter/ 10 cm height). On the bottom we put paper strips. The observations were made daily for each pair and the butterflies were kept and fed until their death.

RESULTS AND DISCUSSIONS

In our investigations we have tried to establish the role played by the sexual pheromone in the reproduction of *M. brassicae*, where both the male [2] and female [7] pheromone were described. In *M. brassicae* the antenna is of a filiform type, similar in the two sexes. The antenna flagellum is composed of a variable number of segments, between 70 and 75. On the antennae of both sexes there are sensilla trichodea and basiconica, about 9,000 in females and 11,000 in males. The greatest number of sensilla are on the segments, 5–45 in both sexes [12]. Other types of sensilla were also identified, but it was found that in Lepidoptera the trichodea ones are very important for the perception of the sexual pheromone [8]. In establishing the variants we considered the structure of the antenna as an olfactory organ, namely the distribution of the sensilla on the antenna flagellum. We aimed at establishing to what extent the reduction of the sensilla number, through the partial or total amputation of the antenna, determines a decrease of the mating capacity in males. We also observed the rate of normal matings as compared to the total number of matings for one variant. We considered a mating to be normal when the

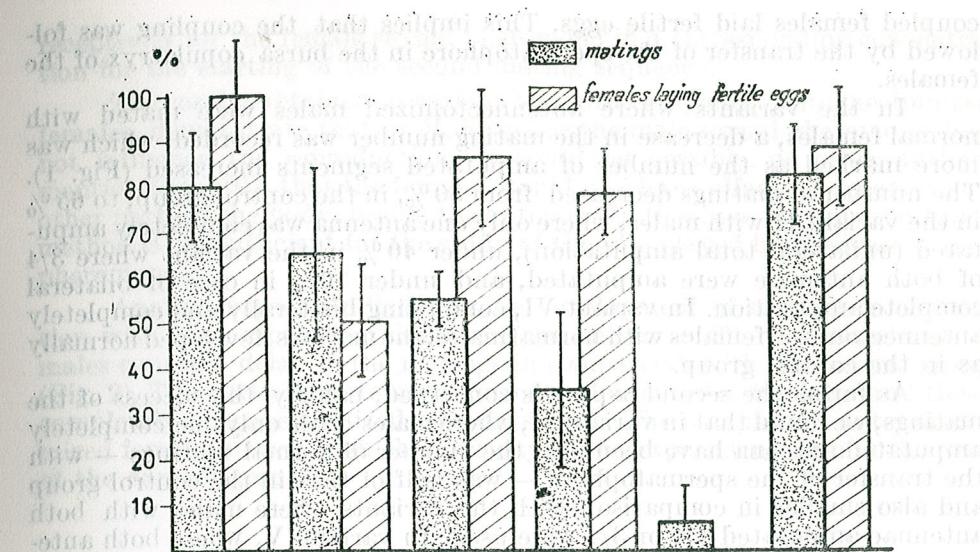


Fig. 1. — Matings in the variants assayed by antennectomy and the percentage of females that laid fertile eggs, related to the number of coupled females, for each variant: I = control group; II = males with one antenna completely amputated; III = males with both antennae amputated 1/2; IV = males with both antennae amputated 3/4; V = males with both antennae completely amputated; VI = females with antennae completely amputated.

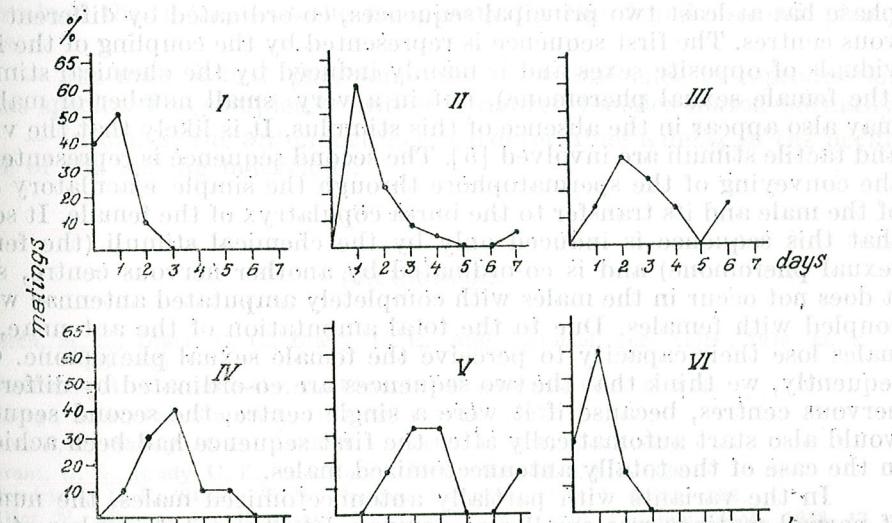


Fig. 2. — The effect of antennectomy on the onset of the mating behaviour in *Mamestra brassicae*.

coupled females laid fertile eggs. This implies that the coupling was followed by the transfer of the spermatophore in the bursa copulatrix of the females.

In the variants where antennectomized males were mated with normal females, a decrease in the mating number was recorded, which was more marked as the number of amputated segments increased (Fig. 1). The number of matings decreased from 80 %, in the control group, to 65 % in the variant II with males, where only one antenna was completely amputated (unilateral total amputation), under 40 % in the variant where 3/4 of both antennae were amputated, and under 10 % in case of bilateral complete amputation. In variant VI, comprising bilaterally and completely antennectomized females with normal males, the matings developed normally as in the control group.

As far as the second aspect is concerned, namely the success of the matings, we found that in variant II, where males with only one completely amputated antenna have been put, the number of normal matings — with the transfer of the spermatophore — was half of that in the control group and also smaller in comparison with the variants where males with both antennae amputated 1/2 or 3/4 were used. In variant V, where both antennae of the males were completely amputated, no coupling was normal. All the 6 females coupled with antennectomized males laid sterile eggs. In turn, in the variant with antennectomized females, the matings developed normally in a proportion of almost 90 %.

The results obtained lead to the following conclusions. The female sexual pheromone is the principal stimulus involved in the reproduction of *M. brassicae*. When the female sexual pheromone is absent, very few males mate with the females and no mating is normal, since it is only the coupling which takes place, without the transfer of the spermatophore in the bursa copulatrix of the females. Hence, we infer that the mating phase has at least two principal sequences, co-ordinated by different nervous centres. The first sequence is represented by the coupling of the individuals of opposite sexes and is mainly induced by the chemical stimulus (the female sexual pheromone), but in a very small number of males it may also appear in the absence of this stimulus. It is likely that the visual and tactile stimuli are involved [5]. The second sequence is represented by the conveying of the spermatophore through the simple ejaculatory duct of the male and its transfer to the bursa copulatrix of the female. It seems that this sequence is induced only by the chemical stimuli (the female sexual pheromone) and is co-ordinated by another nervous centre, since it does not occur in the males with completely amputated antennae which coupled with females. Due to the total amputation of the antennae, the males lose their capacity to perceive the female sexual pheromone. Consequently, we think that the two sequences are co-ordinated by difference nervous centres, because if it were a single centre, the second sequence would also start automatically after the first sequence had been achieved in the case of the totally antennectomized males.

In the variants with partially antennectomized males, the number of normal matings was smaller as compared to the total number of matings per variant, while in the control group the normal matings were 100 %. The reduction in the number of normal matings, in the case of partially antennectomized males, can be explained by the decrease of their

receptivity for the female sexual pheromone in a corresponding concentration for the starting of the second mating sequence.

The results obtained in variant VI, with completely antennectomized females, demonstrate that in *M. brassicae* the male sexual pheromone does not influence the reproduction. It could be possible, as other authors mention [5], [10], that the females would also have olfactory receptors on other parts of the body, beside the antenna. In this case the antennectomy method is not sufficient for the study of the role played by the male sexual pheromone.

Another aspect studied was the development of matings in correlation with the amputation degree of the antennae. The antennectomy of males caused a delay in the matings in comparison with the control group (Fig. 2). The partially antennectomized males mated a day later and those completely antennectomized two days later. The completely antennectomized females mated from the first day of the experiment even in the case of the control group.

CONCLUSIONS

— The female sexual pheromone has a determining role in the normal development of the mating phase in *M. brassicae*. The completely antennectomized males, unable to perceive the female pheromone, mated in a very low number. These matings were abnormal; only the coupling took place, without the transfer of the spermatophores in the bursa copulatrix of the females. In the partially antennectomized males, the number of the matings decreased proportionally to the increase in the number of the antennal segments amputated. The amputation of the antennal segments determined the decrease of the receptivity for the female sexual pheromone.

— The male sexual pheromone does not influence to reproduction in this species. The completely antennectomized females mated normally.

— In males, the antennectomy also determines a delay in the occurrence of the mating behaviour.

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The specific sex-attractant was employed to control the plum fruit moth, *Grapholitha funebrana* Tr., by communication disruption between sexes and mass trapping methods. To obtain the communication disruption the synthetic sex-attractant was evaporated from widely spaced dispensers at a daily rate of 506 mg per hectare (7 g per season); the mass trapping was accomplished by means of 50 sticky traps per hectare. Both methods were effective in the five years of experiments; the attack frequency diminished constantly. Consequently, in 1980 it achieved 1.22% and 2.43% (first and second generation of the pest) for the disruption method, and 1.12% and 3.14% for the mass trapping, while in the untreated check plot it was 23.46% and 38.02 respectively.

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ESSAIS DE LUTTE CONTRE LE CARPOCAPSE
DES PRUNES, *GRAPHOLITHA FUNEBRANA* TR.,
PAR L'EMPLOI DE L'ATTRACTIF SEXUEL
SYNTHÉTIQUE

PAR

JUSTIN GHIZDAVU

The specific sex-attractant was employed to control the plum fruit moth, *Grapholitha funebrana* Tr., by communication disruption between sexes and mass trapping methods. To obtain the communication disruption the synthetic sex-attractant was evaporated from widely spaced dispensers at a daily rate of 506 mg per hectare (7 g per season); the mass trapping was accomplished by means of 50 sticky traps per hectare. Both methods were effective in the five years of experiments; the attack frequency diminished constantly. Consequently, in 1980 it achieved 1.22% and 2.43% (first and second generation of the pest) for the disruption method, and 1.12% and 3.14% for the mass trapping, while in the untreated check plot it was 23.46% and 38.02 respectively.

La lutte contre les insectes nuisibles à l'aide des phéromones sexuelles a comme but la réduction du nombre des femelles fécondées dans l'habitat, but qui peut se réaliser par deux voies : l'interruption de la communication phéromonale normale entre les partenaires sexuels et le piégeage intensif des mâles.

La méthode de lutte par l'interruption de la communication entre les partenaires sexuels, nommée aussi lutte par confusion ou par désorientation des mâles, préconisée en 1963 par Babson [2] et par Wright [22], consiste dans l'imprégnation de l'atmosphère avec les molécules de la phéromone sexuelle spécifique de telle façon que les mâles soient désorientés et incapables de localiser les femelles émettant leur phéromone.

La méthode du piégeage intensif des mâles, envisagée et mise au point théoriquement en 1966 par Knippling et McGuire [11], vise à capturer la totalité, ou, au moins, le plus des mâles possible avant qu'ils puissent féconder les femelles.

Pendant la dernière décennie les deux méthodes ont fait l'objet de nombreuses recherches de lutte contre plusieurs espèces d'insectes nuisibles, les plus proches de nos préoccupations étant celles concernant *Laspeyresia pomonella* L. [4], [5], [12], [15], *Argyrotaenia velutinana* Walk. [17], [20], *Grapholitha molesta* Busck. [18], *Adoxophyes orana* F.v.R. [14] et *Paralobesia viteana* Clem. [20], [21].

La découverte de Granges et Baggioini [10] que les mâles du carpocapse des prunes, *Grapholitha funebrana* Tr., sont fortement attirés par les appâts contenant l'acétate de *cis*-8-dodecenyle (*cis*-8-DDA) synthétique, le composant majeur de la phéromone sexuelle de la tordeuse orientale du pêcher, *Grapholitha molesta* Busck., [16], a ouvert de nouvelles et intéressantes possibilités pour surveiller ces populations et pour combattre ce dangereux ravageur du prunier.

En se basant sur des essais préliminaires de piégeage entrepris en 1975 et sur d'autres informations disponibles [1], [7] les expériences qui font l'objet du présent article ont été effectuées durant cinq ans (1976-1980) dans le but de combattre le carpocapse des prunes par l'emploi exclusif de l'attractif sexuel synthétique, comme alternative à l'actuelle lutte chimique polluante et responsable de graves dérèglements bio-coenotiques.

MATÉRIEL ET MÉTHODES

L'attractif synthétique spécifique*, contenant 97% *cis*-8-DDA et 3% *trans*-8-DDA [3], [6], a été employé dans la lutte contre le ravageur par les deux méthodes présentées ci-dessus.

Les expérimentations ont été réalisées dans le verger didactique de l'Institut Agronomique de Cluj-Napoca sur une parcelle isolée d'environ 3 500 m², contenant 160 pruniers (fig. 1), représentant un reste d'une plantation plus étendue, défrichée il y a 15 ans et replantée avec des pommiers. A cause de sa petite étendue, de son relief accidenté et de la diversité des variétés de pruniers présentes, pendant les années antérieures au début des expériences, les traitements phytosanitaires ont été appliqués assez superficiellement, et par conséquent la fréquence de l'attaque du ravageur sur les fruits (taux des fruits véreux) enregistrée dans la parcelle a varié de 15 à 35 pour cent, la population du ravageur étant uniformément répartie sur sa superficie.

En 1976 sur la parcelle on distinguait cinq sections ; trois (notées A, C et E sur la figure 2) contenaient des pruniers en pleine production et deux (notées B et D) contenaient de jeunes arbres plantés en 1975. Cette situation nous a déterminé d'adopter les suivantes structures des expériences :

- Nous a déterminé à deux types d'variantes suivant les expériences : En 1976 pendant la période 15 avril-15 juillet, deux variantes : 1 — Capture intensive des mâles (sections A, B, C, D, fig. 2). 2 — Témoin « non traité » ne recevant aucun traitement contre le geur (section E).

En 1976 à partir de 15 juillet et pendant les années 1977—1980, trois variantes :

- Confusion (section A, fig. 2).
 - Capture intensive des mâles (sections B, C, D, fig. 2).
 - Témoin non traité (section E).

Pour assurer un bon isolement spatial entre les sections A, C et E à partir de 1978 les sections B et D ont reçu quatre traitements chimiques contre le ravageur, appliqués selon les indications de la station locale de prévision et d'avertissements.

La confusion a été réalisée par la technique des diffuseurs distancés [1], [8], [13], [18], [19]. Les diffuseurs ont été suspendus au milieu de la couronne de chaque deuxième prunier (distribution en échiquier), sur la superficie d'environ 1 000 m² de la section A étant emplacés 23 diffuseurs (fig. 2), ce qui correspond à une densité de 230 diffuseurs par hectare. Un

* Produit par le Laboratoire de Produits Naturels de l'Institut de Chimie de Cluj-Napoca, Roumanie.

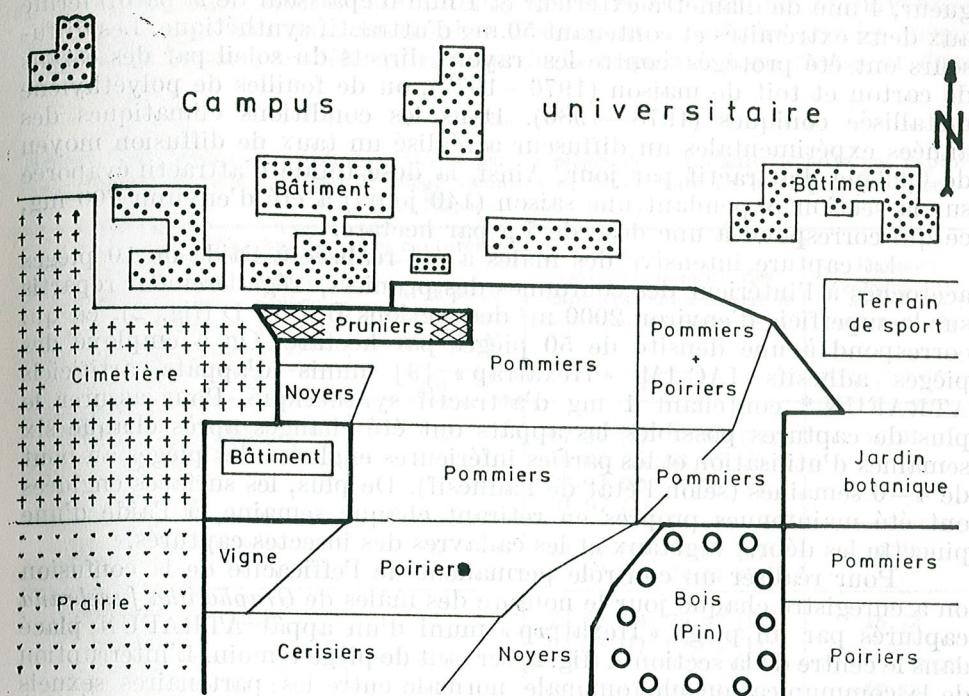


Fig. 1

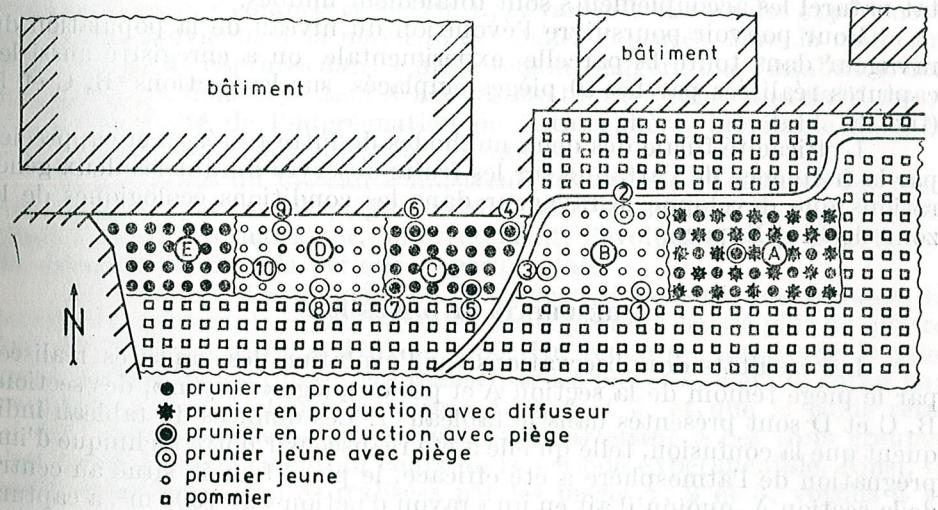


Fig. 2

diffuseur a été constitué d'un petit tube de polyéthylène (6 cm de longueur, 4 mm de diamètre extérieur et 1 mm d'épaisseur de la paroi) fermé aux deux extrémités et contenant 50 mg d'attractif synthétique. Les diffuseurs ont été protégés contre les rayons directs du soleil par des écrans de carton et toit de maison (1976-1977), ou de feuilles de polyéthylène métallisé coniques (1978-1980). Dans les conditions climatiques des années expérimentales un diffuseur a réalisé un taux de diffusion moyen de 0,22 mg d'attractif par jour. Ainsi, la dose totale d'attractif évaporée sur la section A pendant une saison (140 jours) a été d'environ 700 mg, ce qui correspond à une dose de 7 g par hectare.

La capture intensive des mâles a été réalisée à l'aide de 10 pièges accrochés à l'intérieur des couronnes des pruniers, régulièrement répartis sur la superficie d'environ 2000 m² des sections B, C et D (fig. 2), ce qui correspond à une densité de 50 pièges par hectare. On a employé des pièges adhésifs IAC-1M « Hexatrap » [9] munis d'appâts artificiels ATRAFUN * contenant 1 mg d'attractif synthétique. Pour assurer le plus de captures possibles les appâts ont été changés après chaque six semaines d'utilisation et les parties inférieures engluées des pièges au bout de 4-6 semaines (selon l'état de l'adhésif). De plus, les surfaces engluées ont été maintenues propres en retirant chaque semaine, à l'aide d'une pincette les débris végétaux et les cadavres des insectes capturés.

Pour réaliser un contrôle permanent de l'efficacité de la confusion on a enregistré chaque jour le nombre des mâles de *Grapholitha funebrana* capturés par un piège « Hexatrap » muni d'un appât ATRAFUN placé dans le centre de la section A (fig. 2) servant de piège témoin. L'interruption de la communication phéromonale normale entre les partenaires sexuels de la communication phéromonale normale entre les partenaires sexuels a été considérée efficace aussi longtemps qu'on n'a pas enregistré des captures, en appréciant qu'un mâle qui ne peut pas trouver un appât équivaut à plusieurs centaines de femelles est suffisamment désorienté pour être capable de localiser une seule femelle, et par conséquent dans l'habitat naturel les accouplements sont totalement inhibés.

Pour pouvoir poursuivre l'évolution du niveau de la population du ravageur dans toute la parcelle expérimentale on a enregistré aussi les captures réalisées par les 10 pièges emplacés sur les sections B, C et D (fig. 2).

L'efficacité finale des deux méthodes de lutte testées a été appréciée par la fréquence de l'attaque sur les fruits observés durant les deux générations que développe le ravageur dans les conditions écologiques de la zone de Cluj-Napoca.

RÉSULTATS ET DISCUSSIONS

Les résultats des observations sur l'évolution des captures réalisées par le piège témoin de la section A et par un piège « moyen », des sections B, C et D sont présentés dans le tableau 1. Les données du tableau indiquent que la confusion, telle qu'elle a été réalisée par notre technique d'imprégnation de l'atmosphère a été efficace, le piège témoin situé au centre de la section A, quoiqu'il ait eu un « rayon d'action » de 1000 m², a capturé

* Produits et commercialisés par l'Institut de Chimie Cluj-Napoca.

11,97-19,66 fois moins de mâles qu'un piège « moyen » des sections B, C et D, ayant un « rayon d'action » cinq fois inférieur (200 m² de moyenne).

La baisse continue du nombre des captures réalisées par les pièges indique la baisse parallèle du niveau de la population du ravageur dans

Tableau 1

Evolution du nombre des captures réalisées par les pièges de la variante de lutte par capture intensive des mâles, et par le piège « témoin » de la variante de lutte par confusion (Cluj-Napoca, 1976-1980)

Année	Nombre des mâles capturés		Date et nombre des captures réalisées par le piège « témoin » (parcelle A)	Rapport colonne (2) colonne (3)
	(1)	(2) en moyenne par un piège dans les parcelles B, C et D		
(2)	(3)	(4)	(5)	
1976	203,9	12	17.07 (2) : 18.07 (1); 20.07 (2); 21.07 (3); 22.07 (1); 24.07 (2); 31.07 (1)	16,91
1977	98,3	5	14.07 (1); 18.07 (2); 21.07 (1); 24.07 (1)	19,66
1978	62,9	4	26.07 (1); 29.07 (1); 03.08 (1); 07.08 (1)	15,72
1979	55,3	4	10.07 (1); 12.07 (1); 17.07 (1); 18.07 (1)	13,82
1980	47,9	4	27.07 (1); 29.07 (1); 01.08 (1); 03.08 (1)	11,97

toute la parcelle, y compris la section E, la variante témoin des expériences de laquelle proviennent la majorité des mâles capturés. Mais le maintien d'un nombre de captures dans le piège témoin suggère que l'inhibition des accouplements dans la section A n'a pas été totale à cause du relatif manque d'uniformité de l'imprégnation de l'atmosphère, inévitable dans le cas de la technique des diffuseurs distancés.

Les données du tableau 2 illustrent l'évolution du nombre des captures réalisées par les dix pièges installés dans les sections B, C et D (piègeage intensif des mâles) et, indirectement, l'évolution de la population du ravageur pendant la période expérimentale.

De l'analyse des données de ce tableau il s'ensuit que pendant la période expérimentale le nombre total des captures a baissé plus de quatre fois, de 2039 en 1976 à 479 en 1980. On remarque aussi que le plus grand nombre de captures a été réalisé par les pièges 8, 9 et 10, suivis par les pièges 1, 2 et 3 et par les pièges 4, 5, 6 et 7, et que le poids relatif des captures réalisées par les pièges appartenant à ces trois groupes s'est modifié sensiblement au cours de la période expérimentale. Ainsi, le poids relatif des captures réalisées par les pièges 1, 2 et 3, voisins à la section A (confusion) a baissé relativement peu, de 22,7 % (7,56 % par piège) en 1976 à 18,16 % (6,05 % par piège) en 1980, celui des captures

Tableau 2
Evolution du nombre des captures réalisées par les pièges de la variante de lutte par capture intensive des mâles, parcelles B, C et D
(Chuj-Napoca, 1976-1980)

Pièges	Captures réalisées pendant les années										Total des captures réalisées pendant les années 1976 - 1980	
	1976		1977		1978		1979		1980			
	Nom- bre	%	Nombre	%	Nombre	%	Nombre	%	Nombre	%		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	168	8,23	90	9,15	50	7,95	37	6,69	31	6,47	376	7,70
2	163	8,00	81	8,25	56	8,90	51	9,23	34	7,10	385	8,22
3	132	6,47	73	7,42	21	3,34	26	4,70	22	4,59	274	5,85
$\Sigma(1 + 2 + 3)$	463	22,70	244	24,82	127	20,19	114	20,62	87	18,16	1035	22,19
$\bar{M}(1 + 2 + 3)$	154,3	7,56	81,3	8,27	42,3	6,37	38	6,78	29	6,05	345	7,36
4	141	6,92	66	6,72	28	4,45	18	3,26	16	3,34	269	5,75
5	152	7,45	42	4,27	23	3,65	16	2,89	13	2,71	246	5,25
6	169	8,28	57	5,80	24	3,81	19	3,43	16	3,34	285	6,09
7	168	8,23	75	7,63	29	4,61	29	5,24	25	5,22	326	6,96
$\Sigma(4 + 5 + 6 + 7)$	630	30,88	240	24,42	104	16,52	82	14,82	70	14,61	1026	24,05
$\bar{M}(4 + 5 + 6 + 7)$	157,5	7,72	60	6,10	26	4,13	20,5	3,70	17,5	3,65	256,5	6,01
8	244	11,98	126	12,82	106	16,86	102	18,44	95	19,83	673	14,37
9	303	14,86	179	18,20	135	21,46	112	20,27	103	21,51	832	17,76
10	399	19,58	194	19,74	157	24,97	143	25,85	124	25,89	1017	21,26
$\Sigma(8 + 9 + 10)$	946	46,42	499	50,76	398	63,29	357	64,56	322	67,23	2522	53,85
$\bar{M}(8 + 9 + 10)$	315,3	15,47	327,6	16,92	132,6	21,09	119	21,55	107,3	22,41	840,6	17,95
Total gén.	2039	100,00	983	100,00	629	100,00	553	100,00	479	100,00	4683	100,00

réalisées par les pièges 4, 5, 6 et 7 a baissé sensiblement, de 30,88% (7,72% par piège) en 1976 à 14,61% (3,65% par piège) en 1980, tandis que celui des captures réalisées par les pièges 8, 9 et 10, voisins à la section E (témoin non traité), s'est agrandi de 46,42% (15,47% par piège) en 1976 à 67,23% (22,41% par piège) en 1980.

Ces constatations suggèrent que le niveau de la population du ravageur a baissé continuellement sur toute la superficie de la parcelle expérimentale, y compris dans la section E, mais que cette section a constitué un réservoir et une source permanente de mâles qui ont immigré dans les autres sections et qui se sont fait capturer surtout dans les pièges 8, 9 et 10. Une autre source, moins importante, de mâles a été représentée par la section A de laquelle ils ont immigré dans la section B et ont été capturés par les pièges 1, 2 et 3.

L'évolution de la fréquence de l'attaque du ravageur enregistrée dans les sections A, C et E, représentant les trois variantes expérimentales est illustrée par les données du tableau 3.

De l'analyse de ces données il s'ensuit que les deux méthodes de lutte testées ont été efficaces, leur efficacité s'améliorant constamment au fur et à mesure que le niveau de la population du ravageur baissait. Ainsi, on peut constater que chez la variante de lutte par piégeage intensif la fréquence de l'attaque enregistrée au cours de la première génération du ravageur a baissé de 3,86% (en 1976) à 1,12% (en 1980) et de 11,30% (en 1976) à 3,14% (en 1980) au cours de la deuxième génération. Chez la variante de lutte par confusion la fréquence de l'attaque enregistrée durant la première génération a baissé de 3,45% (en 1977) à 1,22% (en 1980) et de 14,30% (en 1976) à 2,37% durant la deuxième génération. En même temps la fréquence de l'attaque chez le témoin non traité s'est située à des niveaux élevés, étant toutefois en baisse légère, de 32,60% à 23,46% au cours de la première génération et de 54,80% à 38,02 au cours de la deuxième, cette baisse peu importante confirmant la baisse générale du niveau de la population du ravageur (voir aussi les données des tableaux 1 et 2).

La hausse continue de l'efficacité des deux méthodes de lutte est illustrée par les données de la colonne 7 contenant les valeurs des rapports entre les fréquences de l'attaque enregistrés chez les témoins non traités et chez les variantes de lutte testées, au cours de la même génération du ravageur $\left(\frac{F\% - V_3}{F\% - V_x}\right)$. Ainsi, dans le cas du piégeage intensif les valeurs

de ces rapports calculés pour la première génération du ravageur ont augmenté de 8,44 (en 1976) à 20,92 (en 1980) et de 4,84 (en 1976) à 12,10 (en 1980) pour la deuxième génération, tandis que dans le cas de la confusion elles ont été en hausse de 7,73 (en 1977) à 19,21 (en 1980) pour la première et de 3,83 (en 1976) à 16,04 (en 1980) pour la deuxième génération.

On remarque toutefois que la baisse de la fréquence de l'attaque observée dans le cas des deux méthodes de lutte expérimentées manifeste la tendance de s'arrêter et ce serait intéressant de déterminer s'il y a encore des possibilités d'accroître davantage leurs performances et de préciser leurs limites supérieures.

Les valeurs des rapports contenus dans la colonne 7 du tableau 3 indiquent aussi que l'efficacité des méthodes de lutte testées a été supérieure dans le cas de la première génération du ravageur. Cela s'explique par le fait que les nouvelles méthodes de lutte agissent en empêchant

Tableau 3

Efficacité des deux méthodes de lutte testées exprimée par la fréquence de l'attaque du ravageur sur les fruits pendant les années 1976—1980 (Cluj-Napoca)

Année	Génération	Variante expérimentale (parcelle)	Fruits analysés	Fruits attaqués	Fréquence de l'attaque (F%)	F% — tém.
			(4)	(5)		F% — V _x
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1976	I	capture (A + C) témoin (E)	880 500	34 163	3,86 32,60	8,44 —
	II	désorientation (A) capture (C) témoin (E)	880 460 500	126 52 274	14,30 11,30 54,80	3,83 4,84 —
1977	I	désorientation (A) capture (C) témoin (E)	1043 915 539	36 27 144	3,45 2,95 26,71	7,73 9,05 —
	II	désorientation (A) capture (C) témoin (E)	916 775 530	53 49 234	5,78 6,32 44,15	7,63 6,98 —
1978	I	désorientation (A) capture (C) témoin (E)	1000 1000 1000	32 21 342	3,20 2,10 34,20	10,68 16,28 —
	II	désorientation (A) capture (C) témoin (E)	100 1000 1000	41 51 401	4,10 5,10 40,10	9,78 7,86 —
1979	I	désorientation (A) capture (C) témoin (E)	1128 835 623	16 9 142	1,41 1,07 22,79	16,16 21,20 —
	II	désorientation (A) capture (C) témoin (E)	634 765 821	23 31 386	3,62 4,05 47,01	12,98 11,60 —
1980	I	désorientation (A) capture (C) témoin (E)	1312 1250 439	16 14 103	1,22 1,12 23,46	19,21 20,92 —
	II	désorientation (A) capture (C) témoin (E)	699 604 497	17 19 189	2,43 3,14 38,02	16,04 12,10 —

l'activité copulatrice des adultes, et celle-ci est plus affectée quand la densité des adultes dans l'habitat est faible, or c'est le cas des adultes hibernants, fondateurs de la première génération. On observe aussi que

dans le cas de la première génération c'était l'efficacité du piégeage intensif qui a été supérieure, tandis qu'au cours de la deuxième génération c'était celle de la méthode de lutte par confusion. Cela nous montre que l'efficacité de la méthode de lutte par confusion est moins dépendante de la densité des adultes dans l'habitat que la méthode de lutte par piégeage intensif.

Nos résultats, obtenus après cinq ans d'expérimentations coïncident avec ceux obtenus en Suisse par Arn et al. [1] dans des conditions naturelles et techniques différentes. Dans ce contexte nous remarquons que dans les conditions climatiques de la zone de Cluj-Napoca, caractérisée par des températures modérées pendant l'été et par un calme atmosphérique, on a obtenu une désorientation efficace des mâles en évaporant pendant une saison entière, seulement 7,0 grammes d'attractif synthétique par hectare, dose nettement inférieure à celles appliquées par les chercheurs Suisses.

Les résultats obtenus prouvent que les deux méthodes testées peuvent être considérées comme méthodes opérationnelles de lutte contre le carpocapse des prunes, *Grapholitha funebrana* Tr., soit indépendamment, soit comme éléments de la lutte intégrée, leur efficacité et leur économie étant plus importantes dans les vergers à faible densité des populations du ravageur, bien isolés d'autres vergers de prunier et situés dans des zones caractérisées par des températures modérées et par des régimes éoliens peu actifs.

Même si à présent, dans certaines circonstances, les nouvelles méthodes de lutte contre le carpocapse des prunes peuvent être encore dépassées du point de vue technique et économique par la lutte chimique, notre conviction est qu'elles s'imposeront dans la pratique grâce à leur nette supériorité écologique.

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1912–1982

Le professeur V. Preda, membre de l'Academie de la République Socialiste de Roumanie est décédé à Cluj-Napoca, le 17 avril 1982, à la suite d'une brève maladie. Il était né le 20 novembre 1912 à Bucarest.

Docteur ès sciences médicales, il avait également reçu une bonne formation dans le domaine des sciences naturelles, ce qui lui avait permis de réaliser de nombreuses recherches dans différents domaines de la biologie.

Son activité didactique et scientifique s'est déroulée à la Faculté de Médecine de Cluj (devenue après 1948 Institut médico-pharmaceutique de cette ville). Il y avait parcouru tous les degrés d'une belle carrière universitaire, en commençant par celui d'assistant à la Chaire d'Anatomie et en finissant par celui de professeur titulaire de Biologie médicale, de Doyen et de Vice-Recteur. En 1951–1958 il avait également donné les cours de Biologie générale à la Faculté des Sciences Naturelles de l'Université.

Après la fondation du Centre de recherches biologiques de Cluj (1957) il y dirigea d'abord le Secteur de Morphologie et de Cytologie expérimentale et assuma ensuite (après la retraite du prof. E. Pop) la direction de cette institution qu'il garda jusqu'à sa mort. Il fut également Secrétaire de la Filiale de Cluj de l'Académie et son Président depuis 1977.

Attrié d'abord par les problèmes anthropologiques, promus par la Société roumaine d'Anthropologie de Cluj (fondée en 1933) dont il fut membre, V. Preda avait entrepris des recherches sur l'os épactal, sur les corrélations des indices crâniens et faciaux chez les schizophrènes et les maniaques, sur les caractères anthropométriques de la tête chez les oligophréniques, sur l'application de la théorie des facteurs en Anthropologie, sur l'évolution du type anthropologique en rapport avec l'âge, sur certains aspects des groupes sanguins. Il avait publié également deux travaux de synthèse (sur le „Paysage anthropologique de la Roumanie” et sur „l'Expérience anthropologique et le déterminisme des manifestations humaines”) ainsi qu'un manuel „Traité élémentaire d'Anthropologie” (1947).

Mais bientôt les charges didactiques l'obligerent à aborder un autre domaine de recherches, celui de la Biologie générale et de la Biologie médicale. D'ailleurs il avait entre temps publié 3 volumes de synthèse dans ce domaine : Biologie théorique (1944), Problèmes modernes de Biologie (1946) et en collaboration avec son maître, Prof. V. Papilian, un manuel d'Embryologie (1946).

Ses recherches de Biologie furent orientées vers les aspects morpho-physiologiques de l'embryologie des vertébrés.

Avec les élèves et collaborateurs qu'il avait formés, il consacra ses travaux (la plupart d'ordre expérimental) aux problèmes concernant la régénération des tissus et des organes des vertébrés, certains aspects de la culture des tissus, le déterminisme du sexe et surtout l'embryologie causale.

Ses expériences concernent l'influence du système nerveux sur la régénération, ainsi que le rôle qui y jouent certaines vitamines (C, K, D₂, B₁₂, PP). En précisant certains facteurs biochimiques, enzymatiques et nerveux, il a largement utilisé ces données dans un travail de synthèse intitulé „La régénération des tissus et des organes”, publié en collaboration avec Octaviana Crăciun (1976).

Une autre caractéristique des êtres vivants qui attira son attention fut celle du déterminisme de la différenciation sexuelle d'abord chez les plantes, et ensuite chez les vertébrés aboutissant à un autre travail de synthèse „Détermination et différenciation sexuelle chez les vertébrés” (1968).

Mais le principal domaine d'investigation de V. Preda et de ses collaborateurs reste celui de l'embryologie causale. Il entreprit l'étude de l'action de différents facteurs du milieu (température, luminosité, humidité, air ionisé) ainsi que du système nerveux sur l'évolution des caractères biochimiques de l'embryon de poulet, du têtard, des larves du ver à soie et des poissons. Grâce aux résultats obtenus en cette matière, il publia un important volume de synthèse : „Biochimie du développement embryonnaire des vertébrés” (1969).

Dans d'autres travaux d'ordre expérimental, une place importante fut accordée à l'influence du régime alimentaire sur la structure microscopique et l'histochimie du tube digestif (surtout chez le rat) et d'autre part sur la cytogénétique des formations tumorales.

Membre de nombreuses organisations scientifiques de Roumanie qu'il contribua à animer, il le fut également des Sociétés scientifiques internationales et étrangères : Association internationale des hommes de science, Association des anatomistes polonais, Société internationale de Biologie cellulaire, Académie des sciences de New York.

Il fut membre de nombreux Comités de rédaction des revues scientifiques roumaines, dont celles d'Anthropologie, de Biologie animale, de Morphologie et d'Embryologie de l'Académie de la République Socialiste de Roumanie, de la revue de Morphologie normale et pathologique de l'Union des Sociétés des Sciences Médicales, ainsi que des périodiques de biologie et de médecine de Cluj-Napoca.

La perte de V. Preda laisse un grand vide, mais l'Ecole scientifique qu'il a fondée compte déjà bien des spécialistes de valeur qui sauront continuer son œuvre.

Le professeur Vasile Gh. Radu, né le 26 juin 1903 à Pîrgărești, département de Bacău. Il acheva ses études à l'Université de Iași, Section des sciences naturelles et obtint une thèse en morphologie animale sous la direction du réputé professeur Paul Bujor qui, appréciant ses qualités remarquables, le fait nommer préparateur à son laboratoire (1926). Il travaille pendant une année en France, dans les laboratoires d'histologie de Christian Champy (Faculté de médecine) et Maurice Parat (Faculté des sciences), où il manifeste un vif intérêt aux problèmes cytologiques.

De retour au pays, il est promu maître de conférences à l'Université de Iași et professeur titulaire, successeur du professeur Ioan A. Scriban, à la chaire de zoologie de l'Université de Cluj (1939), où il déploie une riche activité jusqu'à sa retraite (1973), en continuant ses recherches jusqu'à la fin de sa vie.

Les cours de zoologie donnés par Vasile Gh. Radu embrassaient des aspects très variés : phylodynamiques, adaptatifs, écologiques, etc. et se faisaient remarquer par l'actualité de ses conceptions. Son activité didactique est marquée par l'élaboration des deux volumes de son cours de zoologie des invertébrés qui constituent la principale source d'information en roumain dans ce domaine.

Dans son activité de recherche il débute par des travaux cytologiques parachevés dans le laboratoire du professeur D. Voinov, qui seront proposés pour le prix de l'Académie (1930). Il a également effectué des études cytologiques concernant les glandes surrénales dans la série des invertébrés, les trachées des insectes, les glandes tégumentaires et les cellules glandulaires du conduit différent chez les isopodes terrestres. Dans le domaine de l'anatomie comparée il entreprend d'importants travaux sur le système artériel trachéo-laryngien chez les sauriens et sur le système nerveux des tétrapodes. Ses études portant sur l'estomac chez les isopodes terrestres, mettent en évidence des modifications adaptatives et évolutives, ainsi que des caractères morphologiques valables pour leur taxonomie. Ses recherches systématiques et faunistiques sur les isopodes terrestres de Roumanie ont fait l'objet de plus de 60 articles.

Sur un ensemble de plus de 160 travaux publiés, son nom est lié à la fondation d'un groupe pour l'étude de la faune du sol, dans le cadre du Centre de recherches biologiques de Cluj-Napoca. V. Gh. Radu a effectué en collaboration des recherches sur le rôle de la faune dans la conservation de la fertilité des sols, dans la modification des biocénoses édaphiques sous l'influence des activités humaines, dans le but d'élucider le rapport entre le sol et sa propre faune.



Le professeur VASILE GH. RADU

1903—1982

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Au professeur V. Gh. Radu revient aussi le mérite d'avoir organisé un laboratoire de microscopie électronique, afin de former des spécialistes à l'époque où cette technique moderne se frayait chemin en Roumanie.

Il a assumé de hautes fonctions dont nous mentionnons celles de : doyen de la Faculté de Biologie-Géographie de l'Université de Cluj-Napoca (1947-1951); directeur de l'Institut de Spéléologie (1947-1952); président de la section de Zoologie de la Société des Sciences naturelles et de Géographie - filiale de Cluj-Napoca - (1948-1973) et de la Société des Sciences biologiques, filiale de Cluj-Napoca (1967-1973); chef de la section de systématique morphologique et écologie animale du Centre de recherches biologiques de Cluj-Napoca (1951-1973); membre du comité de rédaction de plusieurs périodiques, dont « Studii și cercetări de biologie - Seria biologie animală », « Revue Roumaine de Biologie, Série de Biologie Animale », « Pedobiologia », « Studia Universitatis Babeș-Bolyai » et de la série « La faune de la République Socialiste de Roumanie ».

Sa prestigieuse activité scientifique et didactique lui valut de hauts titres scientifiques, notamment : membre correspondant de l'Académie des Sciences de Roumanie (1935), membre correspondant de l'Académie de la République Socialiste de Roumanie (1948), « Homme de science émerite » (1970), ainsi que 9 ordres et médailles.

V. Gh. Radu demeure une figure éminente de l'histoire de la biologie roumaine.

Nicolae Tomescu

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AVIS AUX AUTEURS

La « Revue roumaine de biologie — Série de biologie animale » publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie animale : taxonomie, morphologie, physiologie, génétique, écologie, etc. Les sommaires des revues sont complétés aussi par d'autres rubriques, comme : 1. *La vie scientifique*, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. 2. *Comptes rendus* des livres de spécialité parus en Roumanie.

Les auteurs sont priés d'envoyer les articles, notes et comptes rendus dactylographiés à double interligne (31 lignes par page) en deux exemplaires.

La bibliographie, les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes exécutés à l'encre de Chine noire sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes.

La répétition des mêmes données dans le texte, les tableaux et dans les graphiques sera évitée. Les références bibliographiques citées par ordre alphabétique comporteront le nom de l'auteur, l'initiale du prénom, l'année, le titre de la revue, abrégé conformément aux usances internationales, le tome, le numéro, la page.

Les travaux seront accompagnés d'un court résumé de 10 lignes aux maximum, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages (y compris les tableaux, la bibliographie et l'explication des figures).

La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.