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THE USE OF IONOPHORES AND CHANNEL FORMERS IN THE STUDY OF MEMBRANE BIOENERGETIC PHENOMENA

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SUMMARY. — After a short general presentation of the most important properties of the ionophores and channel formers, including an attempt to definition and classification, the paper deals with practical aspects of the use of selected ionophores in the study of membrane phenomena, such as the distribution of ions across model and natural membranes, the calculation of the internal concentration of certain ions, the creation, destruction and interconversion of ion gradients and membrane potentials in liposomes, reconstituted systems, organelles and cells, as well as the associated bioenergetic phenomena. Specific examples are selected from the field of oxidative and photosynthetic phosphorylation and Ca^{2+} involvement in various metabolic processes.

1. Definition, Classification and General Properties

Ionophores are substances that are able to transport ions across artificial and natural membranes by a process of recycling between the two compartments separated by the membrane, whereas channel formers achieve their transport function through the formation of an immobile pore or channel across the membrane. Ionophores are able to accomodate specific ions and make complexes that can penetrate through lipid membranes. In many instances, both the complexed and uncomplexed forms are lipid-soluble, even though their solubility may be different. Channel formers are molecules which can combine with each other or with certain membrane lipids and thus create aggregates sufficiently long to span the membrane or, at least, its hydrophobic portion. The hydrophilic interior of these aggregates represents an aqueous channel that allows the relatively free diffusion of certain ions. Since, regardless of their mechanism, both categories are able to translocate ions across membranes, we shall sometimes refer to them as ion translocators.

It is worth mentioning that important conclusions about the properties of these ion translocators were drawn essentially from experiments on artificial bilayer lipid membranes, the first such studies being performed by Mueller and Rudin in 1967 [21]. However, observations on natural membranes had been made much earlier. For example, in 1944, Hotchkiss (cited in [6]) reported an alteration of the membrane permeability by certain antibiotics, although the true era of ionophores could be considered as having been initiated in 1964 by the observation of Moore and Pressman [20] regarding the valinomy-

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cin-mediated K^+ uptake in mitochondria. These and later observations, which indicated a similar behaviour of ion translocators in both natural and artificial lipid membranes, made it clear that such substances were operating in the lipid environment of the membrane and had nothing to do with the membrane proteins. Since the hydrocarbon region of the membrane possesses a low dielectric constant, the energy required to introduce an ion from the aqueous phase into the membrane is several times the thermal energy of the ion. As a first approximation, the work required to solubilize an ion into the membrane lipid is described by the Born equation, which takes into account the dielectric constant of the membrane and of the surrounding media and the radius and charge of the ion (see, for example, [12]). In these terms, the problem of ion permeation mediated by ionophores and channel formers can be considered as a large decrease in the transfer work due to the screening of the ion charge by the encapsulating molecules and to the presence of hydrophobic residues on the exterior of such substances. Specific examples of this kind will be given when discussing different types of ionophores.

Most of the ion translocators known so far have been isolated from microorganisms or obtained synthetically and belong to the group of antibiotics. Ionophores are usually divided into two classes: neutral and carboxilic [6].

Neutral ionophores include substances such as valinomycin, macrotetrolides, eniantins, polyethers, etc., all possessing a cyclic molecule. Crystalline complexes of these compounds with alkali metal ions have been isolated and studied by X-ray crystallography. In each case, the ion is sequestered in a polar core, whereas the exterior of the complex is hydrophobic. Specifically, the formation of the ion-ionophore complex involves 6 of the oxygen groups of the carrier in the case of valinomycin and eniantins, 8 for macrotetrolides and 10 for crown polyethers. With the exception of these last substances the stoichiometry of the complexes is 1:1.

An interesting correlation between the structure of the ionophores and their ability to translocate cations is observed with nonactin and eniantin (Fig. 1 a and b, respectively). The K^+-nonactin complex resembles a ball, while the K^+-eniantin complex is a disklike structure. Although the cation lies in the center of the complex in both cases, in the first case its electric charge is better screened and this explains the greater ability of the nonactin for ion translocation [21]. Another correlation which deserves mentioning is that between the hydrophobicity of two crown polyethers and their ability to translocate ions. Thus, the crown polyether XXXII which possesses two t-butyl groups that are absent in the crown polyether XXXI is about 1000 times more effective in mediating ion transport. This property is also in agreement with the partition coefficient of the ion-ionophore complex between water and hexane [15]. These examples illustrate how the Born energy required to translocate an ion can be reduced by complexation with an appropriate ionophore.

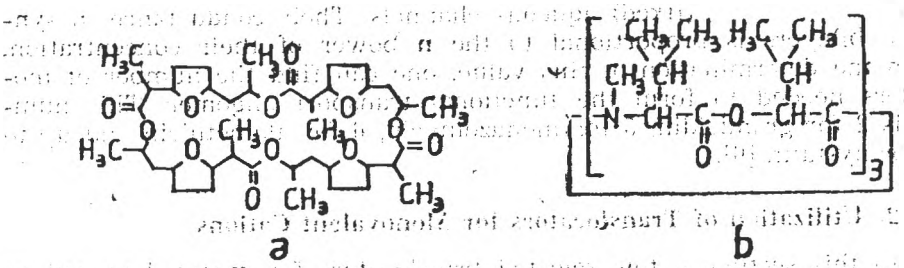


Fig. 1. *The chemical formulae of nonactin* (a) *and enniantin A* (b).

Carboxilic ionophores (nigericin, monensin, dianemycin, X—537A, A—23187), in contrast to the neutral ionophores, are noncyclic molecules. They contain a carboxilic group that is dissociated at a pH around 7 [23] and a variable number of oxygen groups. A—23187 contains, in addition, some nitrogen groups, which apparently confer specificity for the divalent cations Ca^{2+} and Mg^{2+}, as we shall see later. X-ray analysis and infrared studies indicate that upon complexation these ionophores adopt a cyclic configuration, a fact which favours the penetration of the complex through the lipids.

A special group of ion translocators is formed by the so-called *uncoupling agents*. They can be found among both the ionophores and the channel formers. The uncoupling ionophores are usually weak acids which, by reversible dissociation, can carry protons across membranes *(i.e.*, they are *protonophores)*. Since the high energy intermediate in the coupling of oxidation and phosphorylation (as well as in the photophosphorylation) is a proton electrochemical gradient or protonmotive force (in the terminology of Mitchell [17]), these agents uncouple oxidation from phosphorylation by dissipating the proton gradient. Among such uncouplers we mention: DNP (2,4-dinitrophenol), FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone), CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), TTFB (tetrachloro-2-trifluoromethylbenzimidazole), and 1799 (bis [hexafluoroacetonyl]-acetone). The carboxilic ionophores nigericin and especially X—537A may also act as uncouplers at high concentrations. Channel formers or combinations of certain ionophores can also serve as uncouplers, although their mechanism of action is different from that of the classical uncouplers (weak acids).

Channel formers, by permitting a relatively indiscriminate passage of ions (monovalent cations, including protons), are energy dissipators. They dissipate electrochemical gradients of protons and other ions being thus very effective uncouplers. Examples of best known channel formers are gramicidin, alamethicin, monazomycin and different polyene antibiotics (such as the antifungal antibiotic nystatin). Unlike ionophores, which diminish their activity as the temperature decreases, and even stop upon „freezing" of the membrane, the activity of channel formers is very little affected by temperature [11]. This behaviour results from their mechanism of transport. As mentioned before, they are not (mo-

vable) carriers but (fixed) aqueous channels. Their conductance in synthetic bilayers is proportional to the n power of their concentration. From the determination of this value, one can find the number of monomers needed to form the functional transport oligomer. This number is 2 for gramicidin, 5 for monazomycin, 6 for alamethicin and up to 12 for nystatin [6].

2. Utilization of Translocators for Monovalent Cations

In this section, a few selected translocators for monovalent cations will be treated in more detail, with special emphasis on their practical utilization as tools in the study of membrane bioenergetic phenomena.

Valinomycin is probably one of the best known and most widely used ionophores in studies of cellular bioenergetics. It is a cyclic dodecapeptide which has a high selectivity for K^+, facilitating its transfer through lipid membranes. It can also transfer other monovalent cations, the ratio of the selectivity between K^+ and Na^+ (the other physiological cation present in great amounts in living systems) being somewhere between 500 and 1000. Its affinity is higher only for Rb^+, which is not usually encountered in biological systems. For protons, it is very low. The chemical formula and a schematic representation of the K^+-valinomycin complex as well as its mode of action are depicted in Fig. 2 a,

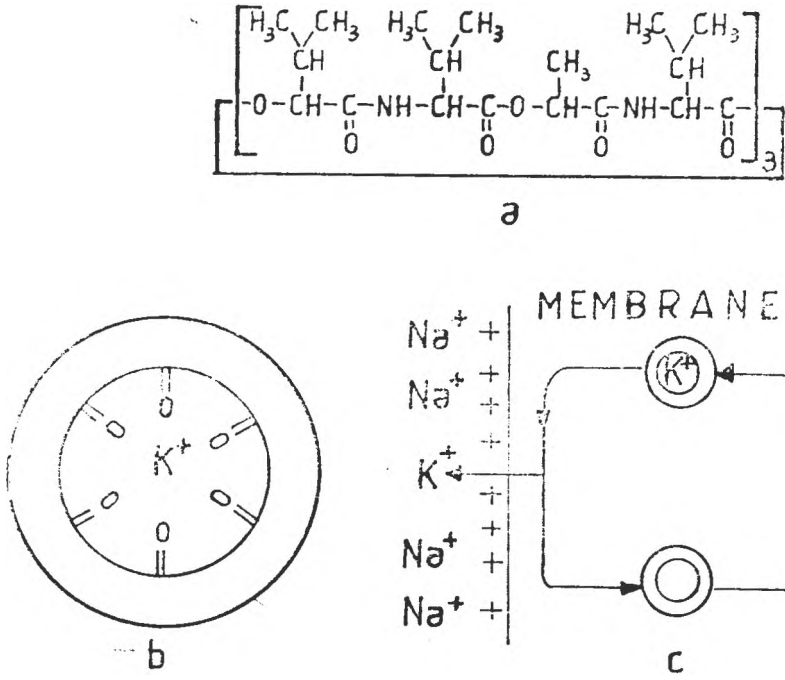


Fig. 2. *The chemical formula of valinomycin* (a) *and a schematic representation of* K^+*-valinomycin complex* (b) *and its mechanism* (c).

b and c, respectively. As can be seen, valinomycin is an electrogenic carrier, *i.e.*, it moves electric charges from the compartment with a high K^+ concentration to the one with a low K^+ concentration, creating a membrane potential difference. Even for a system of small unilamellar liposomes (average diameter around 500 nm), whose relative surface (*i.e.*, the ratio of surface area to volume) is very large in comparison with that of a cell population of similar total internal volume, the charge that must be translocated to create an appreciable membrane potential difference is relatively small (see, for example, [29]). More exactly, the quantity of ions moved from one compartment to another until the electrochemical equilibrium is attained is much less than 1%. This permits us to apply the Nernst equation for the calculation of the potential difference created by the limited diffusion of K^+ ions in the presence of valinomycin (Eq. (1)).

$$\Delta\psi = \frac{RT}{F} \ln \frac{K_2^+}{K_1^+} \tag{1}$$

where R, T and F have their usual thermodynamic meaning and K_2^+ and K_1^+ represent the concentrations of the respective ion in the two compartments. At a temperature around 30°C, if the natural logarithm is replaced by the usual (ten-based) logarithm and the value of $\Delta\psi$ is expressed in millivolts, then Eq. (1) becomes

$$\Delta\psi \text{ (mV)} = 60 \log (K_2^+/K_1^+) \tag{2}$$

which is a very practical formula for calculating potential differences in both artificial and natural systems. It also allows us to calculate one of the concentrations if $\Delta\psi$ and the other concentration are known. For example, in a system where we can measure $\Delta\psi$ and the external concentration (be it K_2^+), we can easily calculate the internal concentration (K_1^+) according to one of the following relations (resulting from the exponentiation of Eq. (1) and (2), respectively):

$$K_1^+ = K_2^+ \cdot e^{-(F\Delta\psi/RT)} \text{ or } K_1^+ = K_2^+ \cdot 10^{-(\Delta\psi/60)} \tag{3}$$

However, valinomycin can be used not only for developing membrane potential differences, but also for dissipating a pre-existing membrane potential, created by the diffusion or active pumping of another ion (Na^+ or H^+, for example), if K^+ (or Rb^+) is present in the system. In this case, the K^+-valinomycin complex, which, irrespective of the screening effect, still bears a net positive charge, is moved by the potential difference to the negatively charged compartment. The result is the dissipation of the potential difference, with the preservation of the chemical gradients originally present (if exchange mechanisms are not operating). But, if a permeant anion is now added to the system this can be accumulated along with further valinomycin-mediated K^+ entrance (Fig. 3). In the absence of valinomycin this could not happen because the uptake of the permeant anion would set a membrane potential which would limit its own entry. The result is a massive uptake of K^+ and permeant

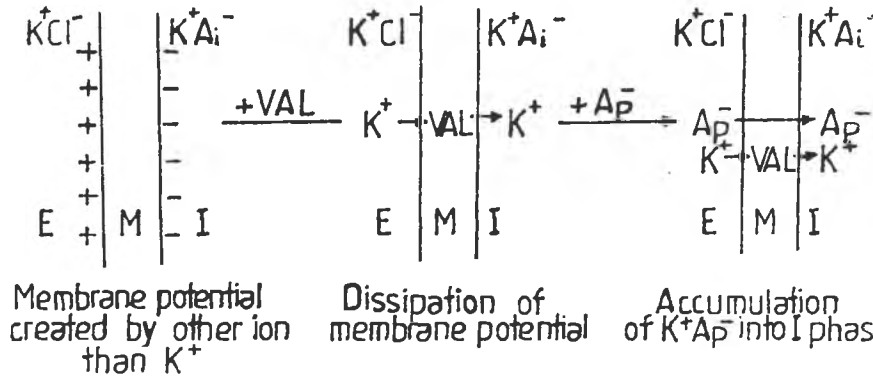


Fig. 3. *The use of valinomycin for dissipating a membrane potential and for accumulation of permeant anions.*

E — Exterior. *M* — Membrane. *I* — Interior. *VAL* — Valinomycin. A_i^- and A_p^- — Impermeant and permeant anions, respectively.

anion, followed by increased osmotic pressure, swelling and possible disruption of the system.

The best exemples for the use of valinomycin in membrane bioenergetic studies are mitochondria and mitochondrion-related systems, although chromatophores, chloroplasts and even cell suspensions have also been used in this respect. In order to understand how valinomycin and other ion translocators have been used in such systems, we shall recall the fundamental points of Mitchell's *chemiosmotic hypothesis* [17], which, despite controversy, is the major theory today explaining the mechanism of energy conversion in the process of oxidative and photosynthetic phosphorylation. According to this theory, the transfer of electrons along the components of the respiratory chain of mitochondrion is accompanied by the translocation of protons from the matrix to the exterior. In conjunction with the activity of certain antiport system, the final result is the formation of a proton electrochemical potential difference ($\Delta\mu_{H^+}$) which is expressed as

$$\Delta\mu_{H^+} = \Delta\psi - Z\Delta pH \qquad (4)$$

where $\Delta\psi$ represents the electric component (membrane potential difference), $Z = 2.3\,RT/F$ and ΔpH is the chemical component of protons (pH difference). In addition, Mitchell postulates that the ATP synthetase is a reversible proton pump, which can make ATP on the account of the energy stored in the proton gradient, in the process of back diffusion of protons into the matrix through a specific channel of the ATP synthetase.

Mitchell and Moyle [18] were the first to use valinomycin for the estimation of the membrane potential difference in rat liver mitochondria. If K^+-depleted mitochondria are put to respire in the presence of valinomycin, the equilibrium distribution of the K^+ ions across the

inner mitochondrial membrane can indeed be used to estimate the magnitude of $\Delta\Psi$ as explained for Eq. (1). Together with an estimate of ΔpH ,it was possible to find approximate value of the total electrochemical potential difference. Although this estimate was a little higher as compared to more recent measurements (by similar or different methods), the work of Mitchell and Moyle was initiating a series of experimental tests that imposed the chemiosmotic hypothesis as a valid theory. Some of these experiments, involving the use of ionophores and channel formers, will be described or, at least, mentioned throughout the rest of this paper.

Theoretically, valinomycin and other neutral ionophores should not uncouple mitochondria. This is indeed so at low concentrations of ionophore and in the absence of permeant anions. At high concentrations (higher than 100 μg/g protein) valinomycin has a slight uncoupling effect which was attributed to a possible movement of protons [18]. Moreover, in the presence of a proper cation (in particular, K^+) and a permeant anion, valinomycin induces ion influx, followed by extensive swelling and uncoupling of mitochondria, by a mechanism that was presented in Fig. 3. By contrast, under similar conditions, in submitochondrial particles (wich are structurally and functionally inverted with respect to mitochondria), there is only a slight uncoupling effect of valinomycin (see [6]). All these results are expected in view of the proposed mechanism of action of neutral ionophores and are consistent with the chemiosmotic theory of energy coupling.

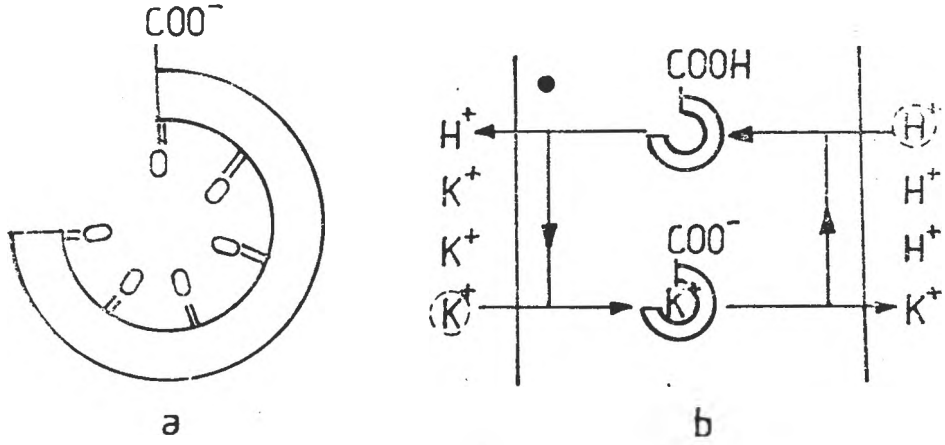
There are at least two more lines of research in connection with the use of ionophores which also sustain the chemiosmotic hypothesis. It can be seen that according to Eq. (4) it would be possible to induce ATP synthesis by an artificially imposed proton gradient. The energy can be provided by the increase of $\Delta\psi$, ΔpH or both. The first experiment of this kind was performed in 1966 by Jagendorf and Uribe [10]. They incubated chloroplasts in the absence of light in an acidic medium and observed ATP synthesis upon a fast increase of the external pH. Moreover, ATP synthesis is importantly enhanced if, in addition, a $\Delta\psi$ is generated by a proper gradient of Na^+ plus nonactin or K^+ plus valinomycin. Experiments of this kind have also been performed by Thayer and Hinkle [33] in submitochondrial particles, demonstrating that an artificially imposed electrochemical gradient can drive ATP synthesis at a rate comparable to that obtained by NADH oxidation.

The other line of investigation in support of the chemiosmotic hypothesis is represented by the so-called reconstituted membrane systems. In the modern acception, by reconstitution we understand the incorporation of membrane enzymes into artificial lipid membranes (such as liposomes) in order to reproduce their original function (or, at least, part of it). Due to the limited space, only a few, classical, examples will be presented. One of the first systems that was reconstituted was cytochrome c oxidase, the terminal enzyme of the mitocondrial respiratory chain, which was incorporated into liposomes, resulting in the so-called

cytochrome oxidase vesicles [7]. Other functional complexes of the respiratory chain have also been reconstituted by H i n k l e and collaborators [13, 25]. All of these are characterized by respiration-dependent proton translocation and uptake of K^+ in the presence of valinomycin, with a stoichiometry of K^+/O similar to that of H^+/O and close to 2. As expected, low concentrations of valinomycin do not stimulate the respiration, although higher concentrations of this ionophore or low concentrations of uncouplers stimulate the respiration several times. An illustration showing how different ionophores can be used for characterizing the membrane potential in cytochrome oxidase vesicles will be presented later, after discussing some of the other ionophores.

Before proceeding to another type of ionophore, it is worth recalling that valinomycin is a very useful tool for creating potential differences across model membranes, such as liposomes. This simple system affords an interesting study of the behaviour of various types of membranes in the presence of a potential difference. Our studies [29, 31, 32] have shown that the composition of the membrane and of the suspending medium can have important effects on surface properties of the membrane, which may affect the real or apparent magnitude of the membrane potential difference. These observations were performed in connection with the study of the mechanism of response of an optical probe to membrane potentials and they turned out to be of utmost importance in the correct utilization of that probe for measurements of magnitude and rate of membrane potential in reconstituted systems and mitochondria [29, 30, 32].

Nigericin, as discussed before, is a carboxilic acid which dissociates a proton at pH around 7. Under these conditions the resulting anion can not usually cross the membrane. Therefore, the action of nigericin is different from that of the neutral ionophores. A schematic representation of nigericin and its mechanism of action is depicted in Fig. 4 a and



F i g. 4. *A schematic representation of nigericin molecule* (a) *and its mechanism of action as an exchanger* (b).

b, respectively. It can be seen that nigericin is in fact an ion exchanger, *i.e.* it mediates an electroneutral exchange of protons for potassium ions or between other monovalent cations. The result of this process is the dissipation of the chemical gradients, in particular of the pH differences, with the preservation of the membrane potential component.

Nigericin can be used with mitochondria suspended in a medium of low K^+ concentration. When these organelles respire they create an electrochemical potential of protons (high concentration on the outside), as described by Eq. (4). Under these conditions, nigericin will preferentially promote an exchange between external protons and internal K^+ ions, the total electrochemical potential remaining unchanged. This means that the pH difference will be converted to a membrane potential difference, the entire energy of the electrochemical potential being stored into the $\Delta\Psi$ component. As we have seen before, mitochondria can very well operate (synthesize ATP) under these conditions (nigericin does not uncouple). Moreover, this ionophore can be used to estimate the electrochemical potential difference without the necessity of measuring the pH difference. Such an example will be illustrated in a later figure, along with the use of valinomycin and an uncoupling agent.

The use of nigericin and valinomycin (in separate, but corroborating experiments) has proved important also in confirming the transport mechanism of certain ions in mitochondria. For several anions, such as phosphate, various intermediates of the Krebs cycle etc., a mechanism of proton symport or hydroxyl antiport has been postulated. In the case of phosphate and a few other metabolites (pyruvate and glutamate, for example) this has been proven to be so by the use of the two ionophores, since they have different effects on the pH difference, the primary driving force of these anions. In the case of other anions (malate, citrate, aspartate, etc.), more complicated mechanisms have evolved in which protons are indirectly implicated [24, 28].

The concomitant use of nigericin and valinomycin has identical effects with those of an uncoupler. Indeed, this combination is an uncoupler, since nigericin collapses the pH gradient, while valinomycin, in the presence of a movable counterion (offered by the very action of nigericin), collapses the membrane potential. It must be mentioned that at high concentrations (especially when K^+ concentration is also high) nigericin itself may act as an uncoupler. Probably, under these conditions it can cross the membrane as anion dimers [6].

Uncouplers of the classical type act by directly dissipating the proton gradient. The mechanism of action of DNP, whose uncoupling action has been long recognized, is presented in Fig. 5. Actually, DNP and other protonophores dissipate the energy of the electrochemical potential by a futile cycle of the protons, which are returned into the matrix as soon as they are translocated by the respiratory chain activity.

Titrations with uncouplers or with uncouplers and inhibitors or other ionophores have also been important in assessing the validity of different ideas in the field of membranes and bioenergetics. For example, the

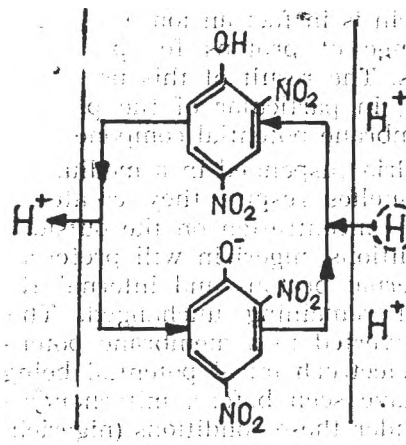


Fig. 5. *The mechanism of action of the uncoupler 2,4-dinitrophenol.*

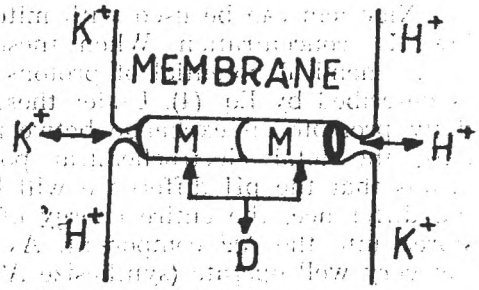


Fig. 6. *The gramicidin pore and its mode of operation.*
M — Monomer, *D* — Dimer.

observation that small quantities of the uncoupler FCCP (which only slightly decrease the magnitude of the membrane potential in rat liver mitochondria) stimulate calcium influx, whereas higher concentrations (which drastically reduce the membrane potential) promote a massive efflux of calcium, was used by Bernardi and Azzone [3] to elaborate a rather sophisticated but logical explanation for apparent contradictory results regarding the calcium fluxes in mitochondria. Complex uncoupler-inhibitor titrations have been used in discriminating between "delocalized" and "localized" chemiosmotic mechanisms (see [22]). Valinomycin, nigericin and CCCP have been used in bacterial membrane vesicles to draw important conclusions regarding the transport mechanism of organic anions in bacteria [26].

Gramicidin, in membranes, probably forms a head-to-head π (LD) helical dimer, resulting from the H-bonding of the formyl groups of the two contributing gramicidin molecules [34]. Its length is close to 30 Å (Hladky and Haydon [8]), which is enough to penetrate the hydrophobic core of the membrane. Bamberg and Läuger [1] presented evidence that the transmembrane channel is an ion-conducting dimer in equilibrium with the nonconducting monomer and that the transfer of K^+ is 3 orders of magnitude higher than the turnover of a valinomycin molecule. Its specificity is rather low, but limited to monovalent cations. In general, the larger the cation the easier it is transported, except the proton, which is the most easily translocated. Therefore, it is no wonder that gramicidin can serve as a potent uncoupler. Its general mechanism of action is presented in Fig. 6.

The complex use of several ionophores (valinomycin, nigericin and the protonophore 1799) is illustrated in Fig. 7, which can be perfectly

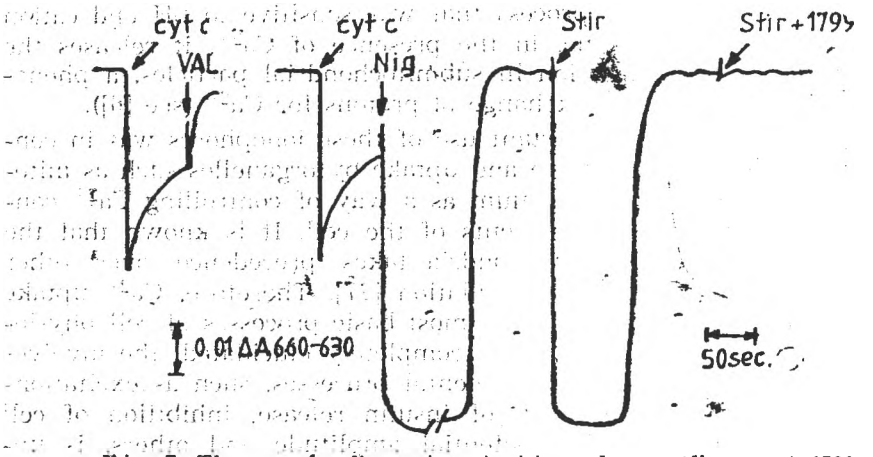


Fig. 7. *The use of valinomycin, nigericin and uncoupling agent 1799 for studying electrochemical proton potentials in cytochrome oxidase vesicles* [29, 30].

used as a summary of the most important characteristics of the membrane potential generated by respiration in cytochrome oxidase vesicles [29, 30].

3. Utilization of Ionophores for Divalent Cations

These ionophores are not so generally used as those for monovalent cations. The reason is a relative lack of specificity and a less clearly understood mechanism of action. Thus, from the two best known ionophores of this type, X-537A and A-23187, the first can transport both divalent and monovalent cations (including protons). However, in the absence of monovalent cations, it can be used quite specifically for Ca^{2+}, because it has a lower specificity for Mg^{2+}. On the other hand, A-23187, which has a greater specificity for divalent cations, is not so specific for Ca^{2+} as it is for Mg^{2+}. Such inconveniences can be easily overcome in liposomes, reconstituted systems and even vesicles obtained from natural membranes (for example, sarcoplasmic reticulum vesicles [2], synaptosomes [16], etc.), where the composition of the suspending medium and, to some extent, even of the internal medium can be controlled.

Despite complications with natural systems, X-537A and A-23187 have been used in several such systems, in the first place in the mitochondrion. A rapid decrease of the mitochondrial Ca^{2+} and Mg^{2+} concentrations, induced by A-23187, and an uncoupling of the oxidative phosphorylation (reversed by the chelator EGTA and potentiated by Ca^{2+}) were among the first relevant observations (see [9] and references therein). This uncoupling action is most probably due to an ionophore-induced cyclic Ca^{2+} movement, since it is stopped by chelators and by inhibitors of Ca movement, such as ruthenium red and La^{3+} [19]. X-537A was shown to inhibit the oxidation of glutamate, pyruvate, citrate and

other substrates through a process that was sensitive to pH and cation variations. At the same time, in the presence of Ca^{2+}, it releases the oligomycin-inhibited respiration in submitochondrial particles, a phenomenon probably due to the exchange of protons for Ca^{2+} (see [6]).

In general, the most important use of these ionophores was in connection with the calcium release and uptake by organelles such as mitochondria and sarcoplasmic reticulum as a way of controlling Ca^{2+} concentration in different compartments of the cell. It is known that the accumulation of Ca^{2+} by mitochondria takes precedence over other functions, including ADP phosphorylation [27]. Therefore, Ca^{2+} uptake by mitochondria must be one of the most basic processes of cell physiology. Although in some cases still incompletely elucidated, the involvement of Ca^{2+} in a series of fundamental processes, such as excitation-contraction coupling, stimulation of insulin release, inhibition of cell division, reduction of resting potential amplitude and others, is undoubtful [4]. One or both of the two ionophores for divalent cations have been used in gaining information about the involvement of calcium in all these processes (see [6]).

Based upon complex studies, in which A-23187 has also been used, it appears that a certain concentration of the free intramitochondrial calcium is critical even for the regulation of the activity of some important enzymes [14]. An accurate and elegant determination of the free Ca^{2+} content is in fact based exactly on the use of A-23187 or nigericin+A-23187 [5]. Several other examples and ampler discussions on the use of ionophores, in some cases, can be found in the review of Gómez—Puyou and Gómez—Lojero [6] and in more recent publications from the field of bioenergetics and cell physiology, some of them having already been mentioned in this work.

4. Conclusions

Ionophores and channel formers are powerful tools in the study of membrane bioenergetic phenomena. The manipulation of ion gradients and membrane potential differences by the use of specific ionophores (electrogenic, electroneutral etc.) and channel formers has proven especially useful in assessing the validity of the chemiosmotic theory of energy coupling in mitochondria, chloroplasts and bacteria. In many instances, the mechanism of transport of ions and metabolites across biological membranes, as well as the implication of certain ions, such as Ca^{2+}, in fundamental physiological processes have also been clarified. Recent developments in the use of ion translocators confirm the hope that they are still far from being completely exploited for the benefit of scientific investigation and discovery.

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STUDIA UNIV. BABEŞ—BOLYAI, BIOLOGIA, XXXII, 1, 1987

SEVEN *MALLOMONAS* SPECIES INVESTIGATED IN THE TRANSMISSION ELECTRON MICROSCOPE

LAURA MOMEU* and LEONTIN ŞTEFAN PÉTERFI*

SUMMARY. — Scales and bristles of seven *Mallomonas* species *(M. allorgei, M. clavus, M. annulata, M. doignonii, M. parvula, M. papillosa* and *M. pumilio* var. *munda)* have been studied in the electron microscope, two of which *(M. doignonii* and *M. parvula)* have not yet been recorded for the Romanian flora. New findings have also added to the knowledge of their ultrastructural variability and geographical distribution.

Working on the Romanian *Synuraceae,* the authors realized that some of the older yet unpublished findings are of general interest. This paper intends to complete the check-list of the Romanian *Mallomonas* species studied in the electron microscope and to contribute to the knowledge of their scale structure and distribution. Two of the species, namely *Mallomonas parvula* Dürrschmidt and *M. doignonii* Bourrelly, have not yet been recorded in Romania; the others exhibit a somewhat different fine structural scale pattern.

It should be mentioned that Asmund and Kristiansen indicate, in a recent monographic treatment [1], the occurrence of the recently described *Mallomonas retifera* Dürrschmidt [3] in samples collected in Romania [5].

Mallomonas papillosa Harris et Bradley var. **papillosa,** recorded in this paper (Fig. 1), has a somewhat different fine structure as compared with earlier Romanian findings. The scales shown in our micrograph are collar scales, provided with raised keel-like extensions on both sides of the dome. The lateral wings are less striated, smooth or lacking. The type variety of *Mallomonas papillosa* has a world-wide distribution, almost cosmopolitan; it has been recorded in the whole Europe (including the British Isles), Iceland, Greenland, North and South America (from Alaska to Chile), Malaysia, Australia, Japan etc. The first Romanian record was published by Péterfi and Momeu [7]. This population has been found in the „Tău cu Mesteceni" peat bog (pH 5.5, in April 1975).

Mallomonas annulata (Bradley) Harris (Fig. 2) is one of the most variable species as concerns the fine structure of the secondary layer of scales. The epithet given by Bradley [2] suggested the presence of ring-like decoration on the shield, forming in many cases a well-developed reticulation. The Romanian population lacks both ring-like structures and meshwork, the basal plate being covered with large, isolated papillae only.

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This species is widely distributed in the whole Europe, including Romania [6], Greenland, North and South America, Japan, Australia and Tasmania. The present population was collected in the strongly eutrophic „Tău cu Arini" bog, Sălicea, Cluj-Napoca (under ice, pH 6.0, in January 1975).

Mallomonas parvula Dürrschmidt (Fig. 3). Isolated scales and bristles of this very peculiar species were recorded for the first time in Japan [8], but since whole cells were not available, no description could be given for the alga. The species has subsequently been recorded in various parts of the world, but, unfortunately, as an isolated and unlabelled member of *Mallomonas* or *Mallomonopsis*. The first description of the species was given by Dürrschmidt in 1982 [3] based on samples collected in Chile. In Romania, scales and bristles of *Mallomonas parvula* were found in 1975, but have not yet been published.

The cells are very small, almost spherical, completely covered with bristles [1]. The scales and bristles are not very variable as concerns the fine structural pattern. The basal plate is a thin, unperforated silica membrane, bearing a very characteristic hooded V-rib; no dome has been seen in *Mallomonas parvula*. The shield is uniformly covered by regularly spaced bluntly conical papillae. Flange is smooth but provided with an upturned rim and a single but large depression. Bristles are short, slightly bent, with two teeth toward their tips.

This species is largely distributed, but often overseen or neglected. It has been found in Scotland, Holland, Denmark, Greenland, Canada, USA, Japan, Chile and Romania. The Romanian record (this paper) is based on samples collected in April 1975, from the „Tău cu Mesteceni" transitory peat bog at Sălicea, Cluj-Napoca (pH 5.5).

The scale of this species somewhat resembles those of *Mallomonas guttata* Wujek and *M. rasilis* Dürrschmidt, but readily differs from both in the lack of dome and the ornamentation of shield.

Mallomonas clavus Bradley (Figs. 4, 6—8) is a very elegant species, often overlooked for its small size, but easily recognized when available for electron microscopy. The rearmost scales, with long spikes may be absent, such cells closely resembling *Mallomonas pumilio*. The body scales have regularly arranged meshes like some varieties of *M. pumilio*, but their anterior flange is provided with a regular row of holes, instead of ribs in *M. pumilio*.

It has been found in England, Denmark, Iceland and Romania [4]. The present micrographs are based on a population collected in April 1982, from a eutrophic bog (pH 7.5) situated in the „Mestecănișul de la Reci".

Mallomonas allorgei (Deflandre) Conrad (Fig. 5). Two *Mallomonas* species possess very thick scales which are similar in structure even under the electron microscope. One of them, *Mallomonas lychenensis* Conrad, has long anterior bristles, while the other *(M. allorgei)* lacks

such bristles. In both cases, the body scales are rhomboidal, having two rows of pits; apical scales asymmetrical, rounded, provided with a variable number of pits.

Knowing that the *Mallomonas* cells may lose their bristles, the species involved can hardly be separated. It was stated [1] that isolated body scales of *Mallomonas allorgei* and *M. lychenensis* „are not always easy to distinguish from each other". The differences between them concern the fine structural features, namely the number and distribution pattern of papillae on the surface of the outer layer of the scale, the number and location of pits etc. According to the characteristics mentioned above, the isolated body scales occasionally seen in our samples (one of them being illustrated in Fig. 5) belong to *Mallomonas allorgei.*

Mallomonas allorgei is widely distributed; it has been recorded by electron microscopy in England, Federal Republic of Germany, Romania, the Soviet Union, Denmark, Canada and USA. This is our second Romanian record; the species was found for the first time in a small eutrophic bog near Sălicea, Cluj-Napoca [5]. The new record is based on samples collected in the „Mestecănișul de la Reci" (pH 7.5, in April 1982).

Mallomonas pumilio Harris et Bradley var. **munda** Asmund, Cronberg et Dürrschmidt (Figs. 9, 10). It differs from the nominate variety by its very regular and beautiful decoration pattern: penta- or hexagonal meshes, each with a group of 5 or 6 pores; anterior marginal struts clear-cut. The variety has already been recorded in Romania as *M. pumilio* var. *pumilio* [7]. Our present micrographs are based on isolated body scales found together with those of *Mallomonas annulata* („Tău cu Arini", Sălicea, Cluj-Napoca, in January 1975). *Mallomonas pumilio* var. *munda* is probably common but often overlooked; it has already been recorded in Holland, the Soviet Union, Denmark, Sweden and Chile.

Mallomonas doignonii Bourrelly emend. Asmund et Cronberg (Fig. 11). This species has been identified on the evidence of a single isolated body scale. The shield of scale is provided with 15 transversal ribs, more or less parallel, connected with few struts. Posterior flange with upturned rim; anterior flange with a regular row of perforations.

The species is known in England, Greenland and USA.

Mallomonas doignonii has been found in a sample collected from a eutrophic bog near the „Mestecănișul de la Reci" (pH 7.0—7.5, in April 1977).

Conclusions. The scales of *Mallomonas papillosa* and *M. annulata* exhibit a somewhat simplified fine structure of the secondary layer when compared to earlier findings. Two of the *Mallomonas* species namely *M. parvula* and *M. doignonii,* are new records for the Romanian flora; both of them have not yet been recorded in the southeastern part of Europe. *Mallomonas clavus* seems to be a widely-distributed, well-defined, but often overlooked species.

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FIGURE CAPTIONS

Fig. 1. *Scales and bristles of* Mallomonas papillosa *var.* papillosa. × 16,000.
Fig. 2. *Group of dome-less scales in* Mallomonas annulata. × 20,600.
Fig. 3. *Two body scales and bristle of* Mallomonas parvula. × 18,500.
Fig. 4. *Body scales of* Mallomonas clavus. × 10,300.
Fig. 5. *Isolated body scale of* Mallomonas allorgei. × 19,800.
Fig. 6. *Almost whole cell armour of* Mallomonas clavus. × 6,500.
Fig. 7. *Body scale of* Mallomonas clavus. × 23,400.
Fig. 8. *Group of rear scales in* Mallomonas clavus. × 11,300.
Fig. 9. *Body scale of* Mallomonas pumilio *var.* munda. × 33,200.
Fig. 10. *Body scale of* Mallomonas pumilio *var.* munda. × 40,000.
Fig. 11. *Isolated body scale of* Mallomonas doignonii. × 50,000.

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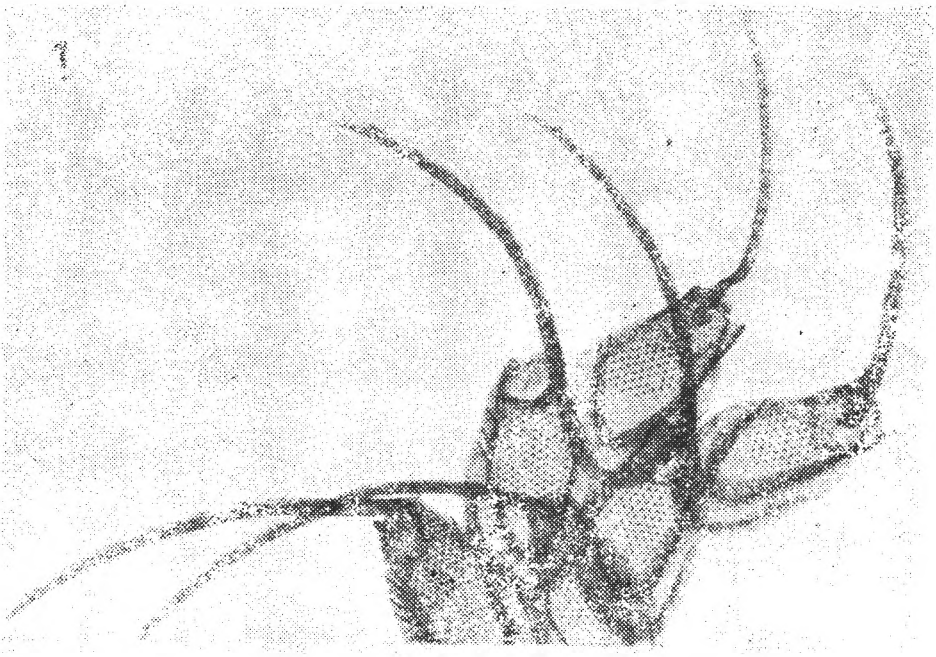

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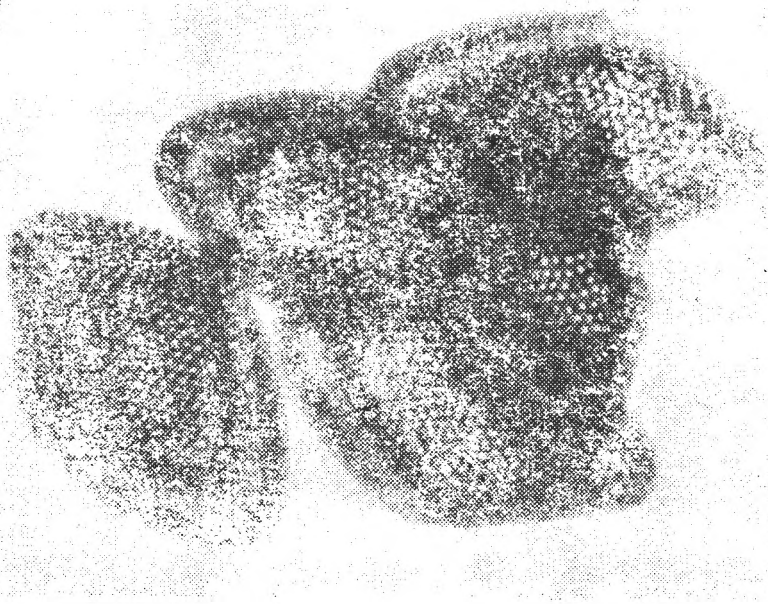


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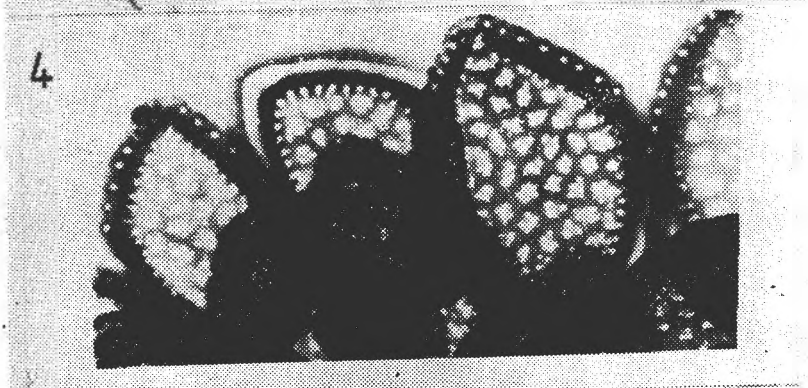
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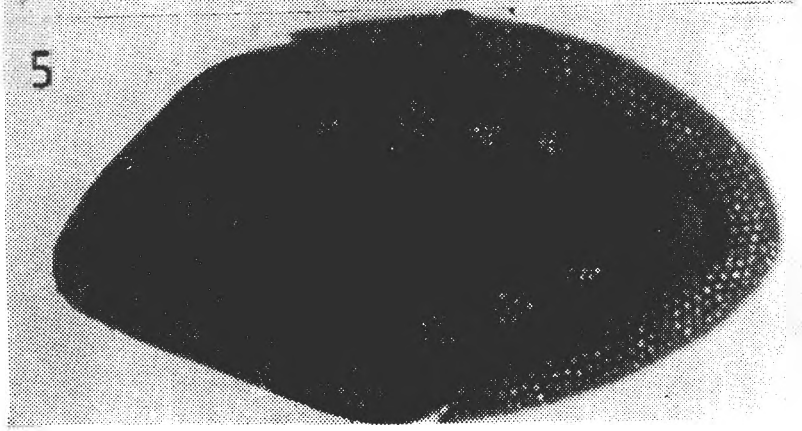
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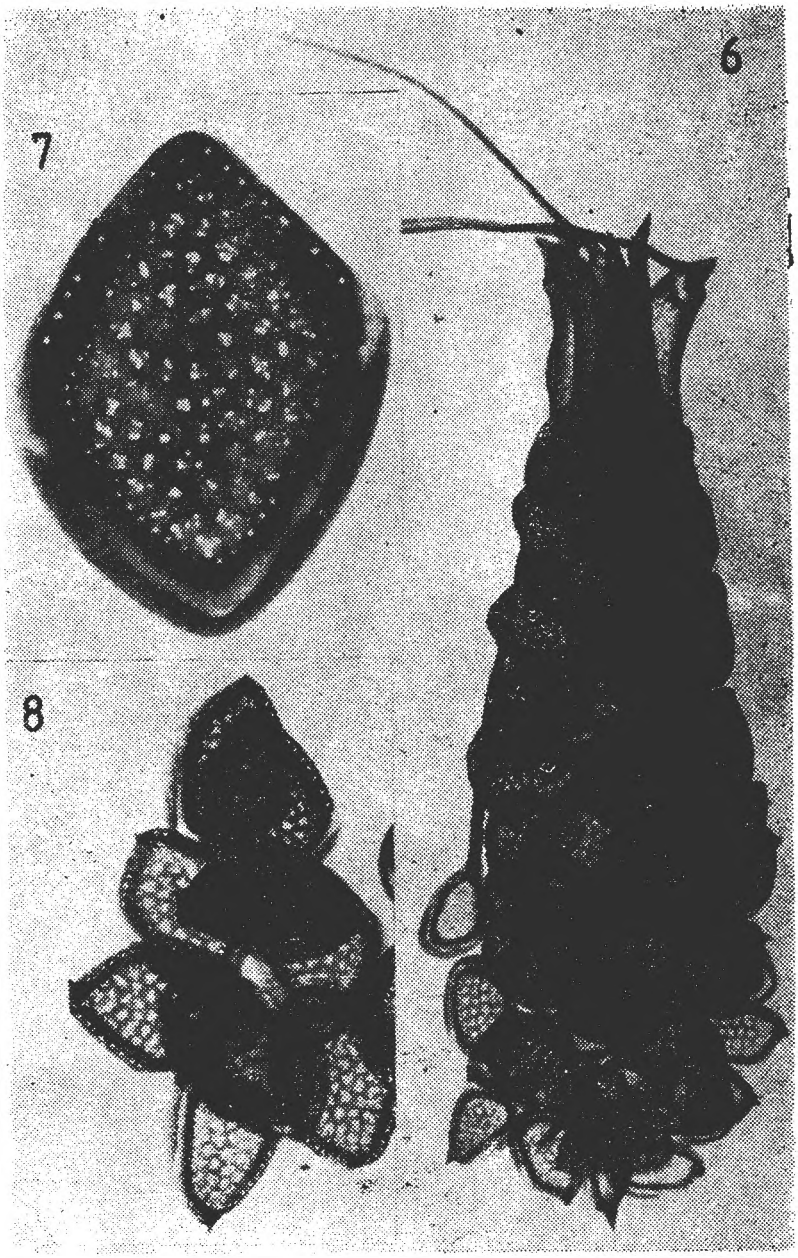
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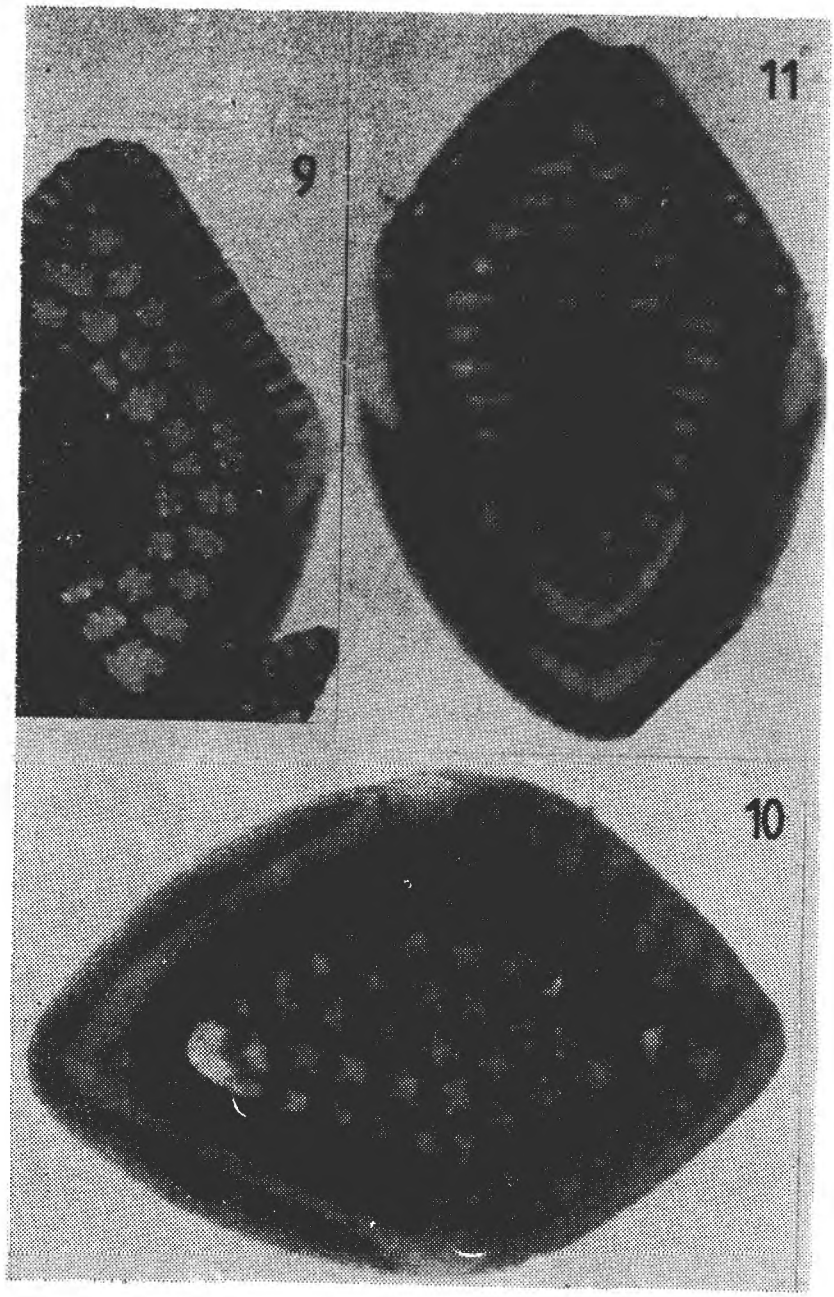
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ULTRASTRUCTURAL CHANGES IN ADIPO-GRANULAR CELLS OF THE MANTLE OF *MYTILUS GALLOPROVINCIALIS* L. AS INDUCED EXPERIMENTALLY BY HYPO- AND HYPERSALINITY*

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SUMMARY. — Mussels collected from the sublittoral zone of the Black Sea were kept under conditions of experimental hyposalinity (6‰) or hypersalinity (26‰) for 72 hours.

It has been established that both hypo- and hypersalinities act by modifying the regulatory capacity of membrane permeability systems, indicating that, during a period of 72 hours, the cellular osmoregulatory capacity is strongly affected.

Thus, hyposalinity (6‰) induces ultrastructural modifications, essentially due to an increase in membrane permeability and hence to the alterations of the exchange relations between the cells and the environment. The penetration of water, electrolytes, ions, salts, etc. is insufficiently regulated. The ultrastructural modifications indicate a decrease in the cellular metabolism and an alteration of the synthesis capacity of cells.

Hypersalinity (26‰) produces certain ultrastructural modifications due to a decrease in membrane permeability and a consecutive decrease in the cell-environment exchange. The result is an insufficient uptake of necessary nutrients by the cell and an accumulation of catabolites. Autophagolysosomal alterations, which decrease or even block the synthesis capacity, are very frequent in the cells.

Mytilus galloprovincialis belongs to the group of osmoconformers [5, 14, 15]. It is ubiquitous in the Romanian part of the Black Sea littoral [18].

Older investigations regarding the influence of salinity variations on the physiology and ethology of this species in the Black Sea were performed by P o r a *et al.* [21]. Recently, P o r a et *al.* [20], M a h a s n e h [14], M a h a s n e h and P o r a [15] and C r ă c i u n *et al.* [4] studied the osmoregulatory capacity of this bivalve under conditions of experimental hypo- and hypersalinity. So far yet, there are very few studies concerning the influence of salinity variations on its morphological structures [14]. It is worth mentioning that, in general, bibliographical data with regard to the salinity effects on histological structures are very rare and almost inexistent at ultrastructural level.

In the present paper we report our studies on the ultrastructure of adipo-granular cells in the mantle of *Mytilus galloprovincialis* from

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the Black Sea under experimentally induced hypo- (6‰) and hypersalinities (26‰). Having known that the adipo-granular cells have an intense metabolism during summer [2, 7—9], we supposed that a change in salinity should induce structural modifications observable in the electron microscope.

Materials and methods. The experiments were carried out between July 15—20, on individuals of *Mytilus galloprovincialis* L. with a mean length of 4.5—5 cm, corresponding to an age of 2—3 years [10, 11]. The mussels were collected from the intertidal zone of the Black Sea near Agigea. The average water temperature was 21°C and salinity 17‰.

The collected mussels were kept in laboratory aquaria, containing aerated sea water at 21°C and salinity 17‰, for a 72-hour period of accomodation. Then, they were distributed in three groups of 10 individuals each, in separate laboratory aquaria, for an experimental period of 72 hours, as follows: 1. control group (16‰ S); 2. group maintained in hyposalinity (6‰ S); 3. group maintained in hypersalinity (26‰ S).

Mantle pieces for the electron microscope study of adipo-granular cells were excised and pre-fixed in 2.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) at 4°C for 90 minutes. The pieces were washed in the same buffer and post-fixed in 1% osmium tetroxyde with 0.1 M phosphate buffer. The fixed tissue was dehydrated in acetone and embedded in vestopal W. The ultrathin sections were obtained with a LKB III Ultratome and contrasted with uranyl acetate and lead citrate. The examinations were performed in a Tesla BS-613 electron microscope.

Results. In all Pelecypods, the storage tissue is constituted by vesicular (Leydig) cells capable of synthesizing and storing glycogen, which in some families *(Arcidae* and *Mytilidae)* are accompanied by adipo-granular cells containing protein granules and lipid droplets [9].

It is known that there is an alternation between the development of the gonads and the storing tissue during the sexual cycle of mussels *(Mytilus edulis* and *Mytilus galloprovincialis), i.e.* the vesicular and the adipo-granular cells grow very much in the period of sexual rest (July—September) [7—9]. During this time the cells accumulate specific products that are gradually liberated by complex mechanisms (exocytosis, autophagy, partial or total lysis), at the onset of gametogenesis and during sexual maturation (October—January). In the course of the evolution of the storing tissue (July—September), the adipo-granular cells pass through several stages which constitute their functional developing cycle [2, 14]. We consider that at the half of July, when our experiments were performed, most of the adipo-granular cells reached the maturation stage, in which the processes of elaboration and deposition of the synthesized material are very intense. This fact is confirmed by the electron micrographs obtained from the control group (16‰ S), where most of these cells are found in this stage (Figs. 1 and 2).

1. Control group (16‰ S). The adipo-granular cells of this group are characterized by the multitude of secretory granules which fill almost the entire volume of the cell (Fig. 1). These granules are proteic in nature (P), with the exception of a few larger droplets of lipidic nature (L). The proteic granules have a remarkable uniformity in shape and electrondensity. They are approximately spherical, with dimensions be-

tween 300—500 nm. The cells have an oval nucleus (N) sometimes presenting infoldings. The nuclei are 3—4 μm in length and 1—1,5 μm in width and are found at the basal pole of every cell. At a larger magnification we observe that the cell is in an intense metabolic activity (Fig. 2). The endoplasmic reticulum is of granular type (RER) having numerous ribosomes attached to its surface. It is composed of several narrow profiles, with a parallel and concentric disposition around the nucleus, as well as of moderately electrondense rough vesicles spread in the cytoplasm. The Golgi complex (G) is well represented, 4—5 golgian bodies being observed in many cells. The mitochondria (M) are relatively numerous, with evident cristae and a moderately electrondense matrix. No lysosomal or vacuolar formations were observed in the cytoplasm.

2. Mussels exposed to hyposalinity (6‰ S). The adipo-granular cells in this group increased as volume and their organelles suffered a swelling process (Figs. 3 and 4). The nucleus practically doubled its volume (5×3 μm). RER lost its structural-spatial ergastoplasmic configuration, being present as short profiles (Fig. 3) and nonelectrondense vesicles (Fig. 4), spread all over the cytoplasm. Some of these vesicles are swollen and lacking the ribosomes. The Golgi complex is poorly represented, with swollen constitutive elements (Fig. 4). The result is a low synthesis activity of the proteic granules, which are present in a smaller number than in the control group (Fig. 3). The lipid droplets are somewhat more numerous. No lysosomal formations were observed. The volume density of cellular elements is reduced and the cytoplasm has a low electrondensity. Sometimes, due to the modifications induced by hyposalinity, we observe cells that lose their intercellular connections, or even cell membrane disruptions followed by dissipation of the cell content (Fig. 5).

3. Mussels exposed to hypersalinity (26‰ S). The electron micrographs obtained from this group look totally different as compared to the one observed in the previous group. The cytoplasm volume density of its elements increased considerably (Figs. 6—9). It is significant that little lysosomal formations (Lz) appear first in the cells (Fig. 6) which latter evolve into autophagolysosomes (ALz, Fig. 7). The proteic granules are relatively numerous but they suffer a process of elongation (Fig. 6) and fusion (Figs. 7 and 8). The mitochondria are relatively numerous (Fig. 6), much more electrondense and almost twice smaller than in the hyposaline group. The Golgi complex is well-developed, with many microvesicles around it, suggesting its intense activity in elaboration of primary lysosomes. RER is poorly developed, being reduced to a few narrow profiles, disposed near nucleus. The lipid droplets are relatively numerous and larger (Figs. 6—8). The nuclei have a lobular shape (Fig. 7) or become pycnotic (Figs. 8 and 9). In an advanced stage of the actions of autolysosomal formations, the cell content and, in the first place, the content of the proteic granules is lyzed and gradually changed into a relatively uniform electrondense mass. The lipid droplets persist longer in this process of self-destruction of the cells (Figs. 8 and 9). The intercellular cohesion is maintained, cells found in different degrees of affection being observed one next to another (Fig. 8). Nevertheless, we also

observed cells or fragments of cells which separated and fell in the tube lumen (Fig. 10).

Discussion. P o r a [16] and P o r a and C ă r ă u ș u [19] reported that the littoral zone of the Black Sea near Agigea is exposed to frequent variations of salinity due to water currents and dominant winds. They established that the annual mean salinity near Agigea is 16.6‰, with maximal variations between 9.3 and 26‰. These extremes always last for very short periods of time.

It is known that, generally, a variation up or down 50% of the magnitude of an environmental factor leads to the death of organisms [1, 6, 16, 17]. Experiments regarding the response of *Mytilus galloprovincialis* from the Black Sea to different salinities have demonstrated that the maximal survival takes place between 10 and 15‰ S [21]. Also, M a h a s n e h [14] has reported that mussels maintained in sea water of 6‰ and 26‰ S, respectively, do not survive more than 90—100 hrs. The cause of death is the deregulation of metabolic mechanisms essential for life. For example, recent investigations on *Mytilus galloprovincialis* from the Black Sea [12, 24] have shown that the carbohydrate metabolism is strongly affected by environmental variations. It has also been established that this mechanism is regulated in a manner similar to that in the vertebrates. Thus, the nervous influxes received from peripheral osmo- and ionoreceptors reach the central ganglia determining the secretion of insulin or glucagon for maintaining the glycaemic level within normal limits [3, 13].

The maintenance of mussels under hyposalinity (6‰) induced ultrastructural modifications in the adipo-granular cells, as illustrated by the swelling of cell organelles. The result is a decrease in the metabolic activity, manifested especially through a reduction in the synthesis capacity of its principal product, the proteic granules. There is also present a swelling process of the cells, a fact that explains the loss of the intercellular cohesion, followed, in few cases, by destruction of the cell membrane and dissipation of the cell content.

Based on these results, we consider that hyposalinity (6‰) caused the deregulation of the control systems of the membrane permeability. The membrane permeability for water increased, favouring the loss of hydric equilibrium between the cells and the environment. At the same time and in agreement with data reported by several authors [4, 14, 20, 22, 23], we consider that hyposalinity (6‰) induced a reduction in membrane permeability for ions, especially for Ca^{2+}. Thus, P r o s s e r [22] has reported that calcium ions play a very important role in stabilization of cell membrane proteins, being involved directly in the osmoregulation process. S h u m w a y [23], working on *Mytilus edulis* exposed to hyposalinity, has reported that calcium ions from the internal medium combine with the molecules of the cell membrane and decrease their permeability. The results of P o r a *et al.* [20], M a h a s n e h [14] and M a h a s n e h and P o r a [15] also confirm the results of S h u m w a y [23]. These authors, studying the concentration of Mg^{2+}, Ca^{2+}, K^{+} and Na^{+} from the hemolymph of *Mytilus galloprovincialis* kept for 72 hrs under

hypo- (6‰) and hypersalinity (26‰), respectively, observed a certain parallelism between the concentration of these ions in the hemolymph and the external medium. We suppose that the calcium ions in hyposalinity were bound, to a larger extent, to the membranes (28 mg% Ca^{2+} in hemolymph), decreasing their permeability for ions. On the contrary, the calcium ions were less bound to the membrane under conditions of hypersalinity (26‰) and, therefore, they are found in larger amounts in hemolymph (48 mg%). These results suggest implicitely an increase in membrane permeability to ions and a decrease for water. On the other hand, Crăciun *et al.* [4] have concluded that under hyposaline conditions „calcium sequestration in intracellular organelles is to be mentioned as an adaptative mechanism".

The maintenance of mussels under hypersalinity (26‰) caused ultrastructural modifications in the adipo-granular cells observable by an increase of cytoplasm electrondensity, a volume decrease of RER and mitochondria and by the presence of lysosomal formations which could finally lead to the destruction of the cells. Based on these results and on the considerations presented above, we can say that hypersalinity (26‰) alters the membrane permeability and the exchange capacity between cells and environment. In this case it appears that there is a decrease in membrane permeability for water and an increase for ions and salts. The result is an insufficient water uptake and an uncontrolled, nonselective permeation of ions and salts. The cell metabolism decreases gradually, and an accumulation of catabolites is taking place, which facilitates the increase in autophagolysosomal activity and finally the blocking of the metabolism and the death of the cells.

Conclusions. We can state that in the case of mussels, cellular osmoregulation under conditions of variable salinity takes place through complex active processes. The osmoregulatory mechanism in hyposalinity differs from that in hypersalinity. There are, thus, two different mechanisms.

Taking into account the data presented above, we consider that the electron micrographs presented by us for the mussels kept for 72 hours under hyposalinity (6‰) or hypersalinity (26‰) came from animals found at the survival limit, under and above which their metabolism can not function and the animals die. *Mytilus galloprovincialis,* as a poikilosmotic animal, is an osmoconformer with isosmotic regulation mechanisms of the intracellular fluid, mechanisms which are no longer functional under our experimental conditions.

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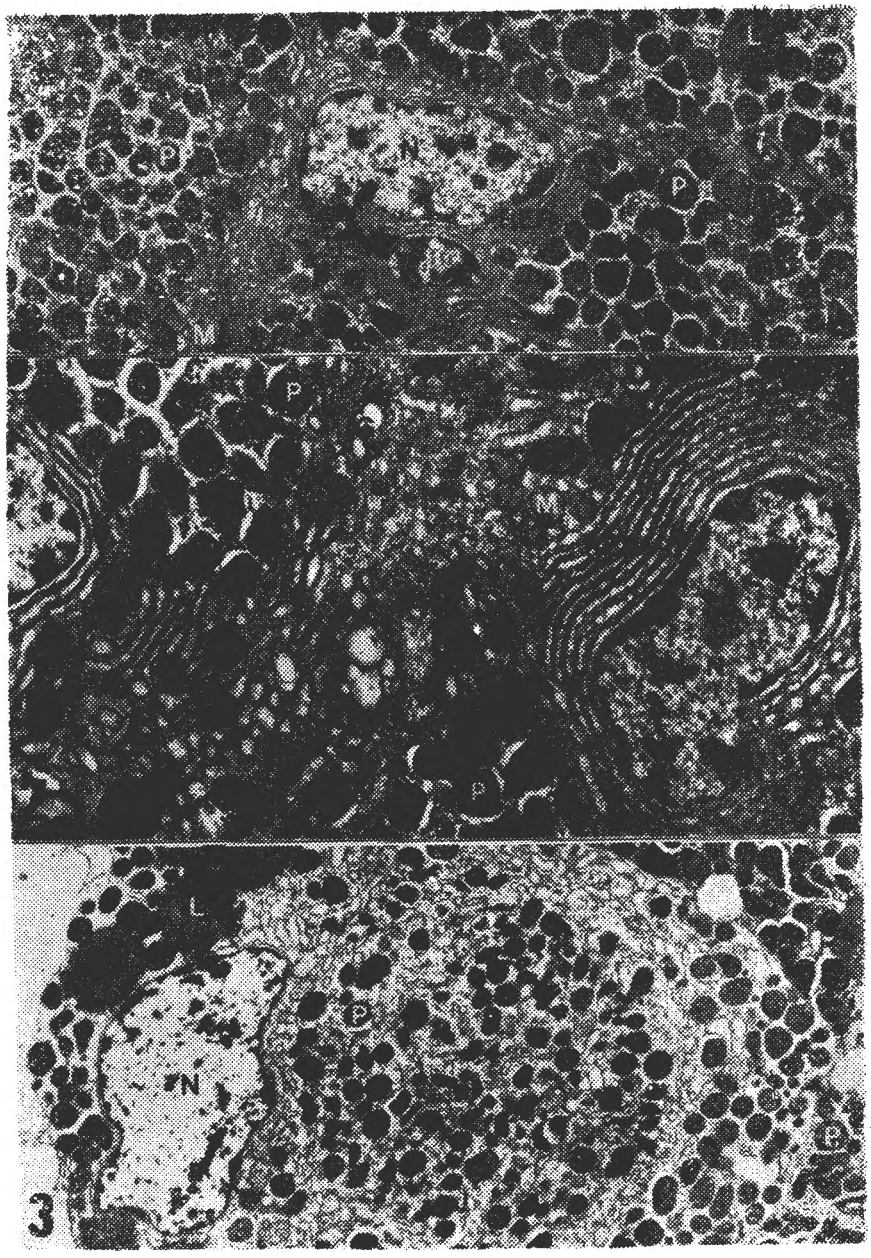
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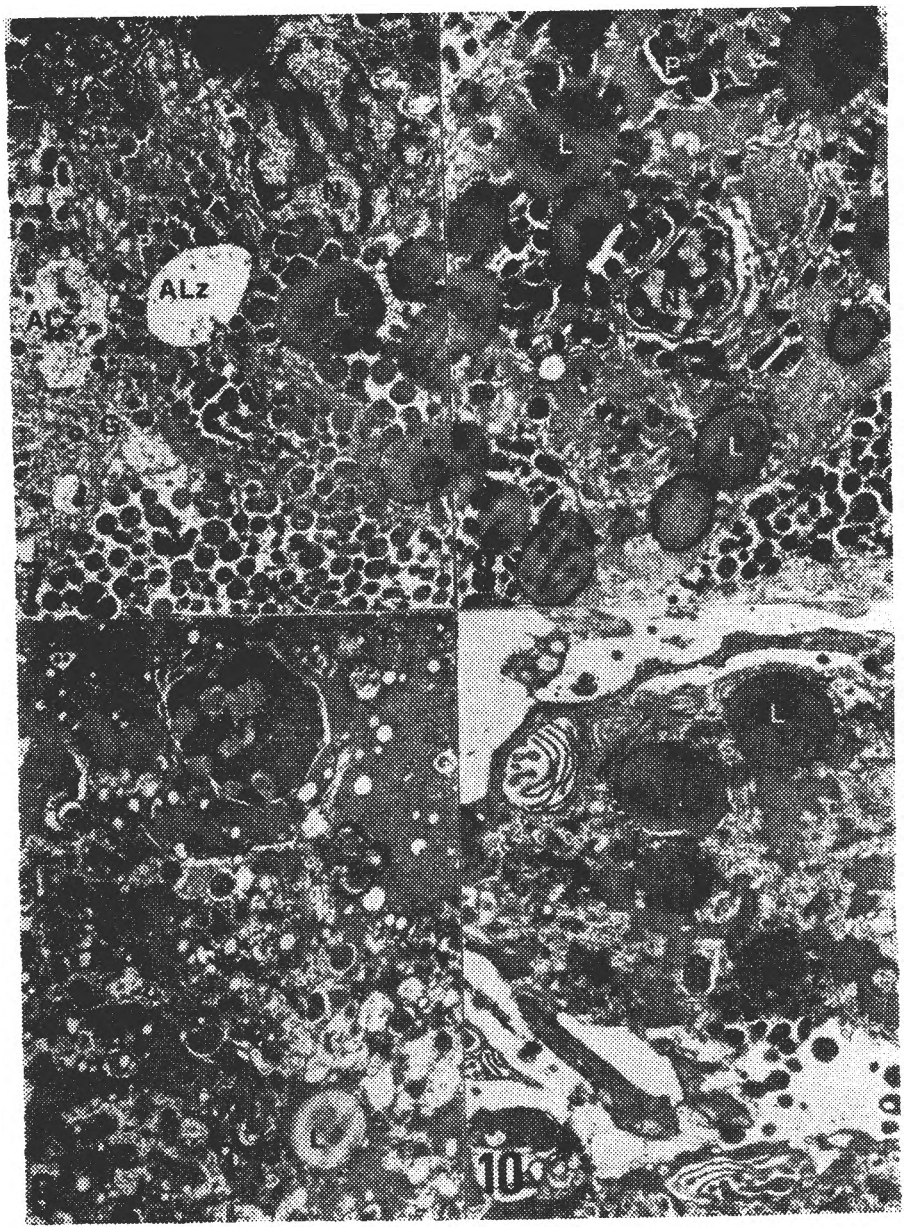
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FIGURE CAPTIONS

Fig. 1—2. *Control group (16‰ S).*
Fig. 1. × 9,450. Fig. 2. × 17,100.
Figs. 3—5. *Mussels exposed to hyposalinity (6‰ S).*
Fig. 3. × 8,550. Fig. 4. × 18,450. Fig. 5. × 9,180.
Figs. 6—10. *Mussels exposed to hypersalinity (26‰ S).*
Fig. 6. 17,100. Fig. 7. × 7,150. Fig. 8. × 8,100. Fig. 9. × 8,100. Fig. 10. ×6,930. *Alz* — Autophagolysosome. *G* — Golgi complex. *L* — Lipid droplet. *Lz* — Lysosomal formation. *M* — Mitochondrion. *N* — Nucleus. *P* — Proteic granule. *RER* — Rough endoplasmic reticulum.

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A COMPARATIVE ELECTRON MICROSCOPE STUDY OF THE REPETITIVE BANDS IN SPERMATOZOON TAILS OF TERRESTRIAL ISOPODS

CONSTANTIN CRĂCIUN*

SUMMARY. — The cross striations of the spermatozoon tails were studied in two isopod species belonging to the same family *(Armadillidae)* and the same genus *(Armadillidium)*, in two species of the family *Porcellionidae* belonging to the *Porcellio* and *Trachelipus* genera and in a species belonging to a third family *(Cylisticiidae)*, genus *Cylisticus*.

The measurements, performed on electron micrographs obtained at high magnifications (250,000—500,000 times), show that the caudal filaments have a transversal periodicity consisting of bands and major, minor and elementary subbands. They are based on constitutive units of 6 and 8 Å in width. The band width, the number, the width and succession of the elementary subbands are characteristic for each species.

Isopods represent the only group of *Crustacea* which has representatives in both aquatic and terrestrial environments. It is a group with high ecological plasticity, a feature which favoured the spreading of the species even into the most arid zones of the Globe. The terrestrial species, although relatively few in number, have very abundant populations [19]. Through their detritivorous feeding they represent an extremely important link in the economy of nature, contributing to the decomposition of the organic matter and thus to the natural fertility of the soil [17].

Due to the less distinct specific morphological characters and to the peculiarities of their postembryonal ontogenetic development, the recognition of the species is, sometimes, rather difficult [18, 23—27]. An important role in the recognition of the species is played by the knowledge of the changes of characters during ontogeny. It is our contention that the structure of the spermatozoon offers important clues in the taxonomy and phylogeny of the isopod species, genera and families.

Investigations regarding the spermiogenesis in isopods as well as the spermatozoon structure are rather scanty and almost always giving contradictory data [10, 11, 15, 28, 29]. As shown by R a d u [16], the mature spermatozoon of *Armadillidium vulgare* (Fig. 1) is composed of a long caudal filament of plasmatic origin (approximately 850 μm, variable with the species) and of a chromatic or nuclear filament, which represents the head (approximately 180 μm in length, also variable with the species). These two filaments are linked together so as to form a whip. In the extension of the two pieces there is a formation of plasmatic origin which constitutes the perforator.

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The ultrastructure of the isopod spermatozoon made the object of relatively few investigations. Blanchard *et al.* [2] studied the ultrastructure of the spermatozoon tail in *Idothea baltica*, *Cyathura* sp. and *Oniscus asellus*. Reger [20, 21] studied the spermatozoon ultrastructure in *Asellus militaris* and the comparative development of the spermatozoa in *Oniscus asellus* and in the amphipod *Orchestioidea* sp. Reger and Fain-Maurel [22] investigated the origin and distribution of the tubules of the isopod spermatozoa and spermatophores. Hollande and Fain-Maurel [12, 13] studied the spermatozoon ultrastructure in *Nerocilla bivittata* and *Anilocra sp.* and the evolution of the spermatic vesicle in *Armadillidium vulgare*. A study of the spermatozoa in two primitive crustaceans was performed by Brown and Metz [3] who also tackled certain phylogenetic implications due to similarities and differences in the spermatozoon ultrastructure of different groups of *Crustacea*. An ampler work, the doctoral thesis of Fain-Maurel [8], treats at length the spermiogenesis and the spermatozoon infrastructure in *Cymothoidae*, and from the group of terrestrial isopods the spermiogenesis in *Armadillidium vulgare*. Crăciun, in 1974 [5], studied the spermiogenesis in *Porcellio scaber* and *Trachelipus balticus*, in 1975 [6] the ultrastructure of the gamets in the terrestrial isopods *Armadillidium vulgare* and *Armadillidium versicolor* and in 1986 [7] the spermatozoon ultrastructure in *Porcellio scaber* and *Cylisticus convexus*. Cotelli *et al.* [4] also studied the spermatozoon ultrastructure in the terrestrial isopods *Armadillidium vulgare*, *Porcellio laevis* and *Oniscus asellus*.

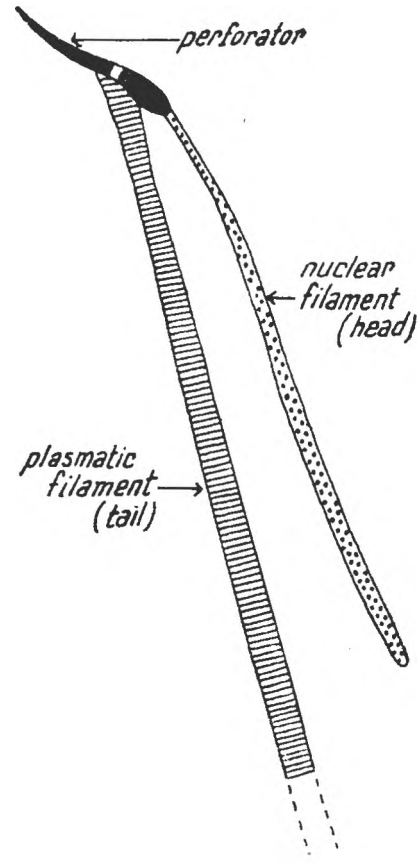


Fig. 1. *Schematic representation of the isopod spermatozoon.*

All these investigations have characterized the isopod spermatozoon as nonflagellated and atypical, since it is devoid of mobility *in vitro*, it lacks a specific linking piece between the two components and also the "2+9 filaments" structure so characteristic to the spermatozoa of the majority of living beings.

In the ultrastructural study described in the present paper we shall not insist on the overall fine structure of the spermatozoa of the 5 isopod species, because as it resulted from the works mentioned before, their spermatozoa belong to a common structural type of all isopods under discussion. However, special emphasis will be placed on the caudal filament, which is especially characteristic for the isopod group and which differs in its intimate structure from species to species.

Material and methods. The present study was performed on 5 species of terrestrial isopods from the group of higher isopods, well adapted to the terrestrial environment. These are: two species of the same family *(Armadillidiidae)* and the same genus *(Armadillidium vulgare* and *Armadillidium versicolor);* two species of the same family *(Porcellioniidae),* but different genera *(Porcellio scaber* and *Trachelipus balticus);* one species *(Cylisticus convexus)* belonging to a third family *(Cylisticiidae).* The biological material was collected in several estival periods from the protected ecological zone of the Faculty of Biology, Geography and Geology in Cluj-Napoca.

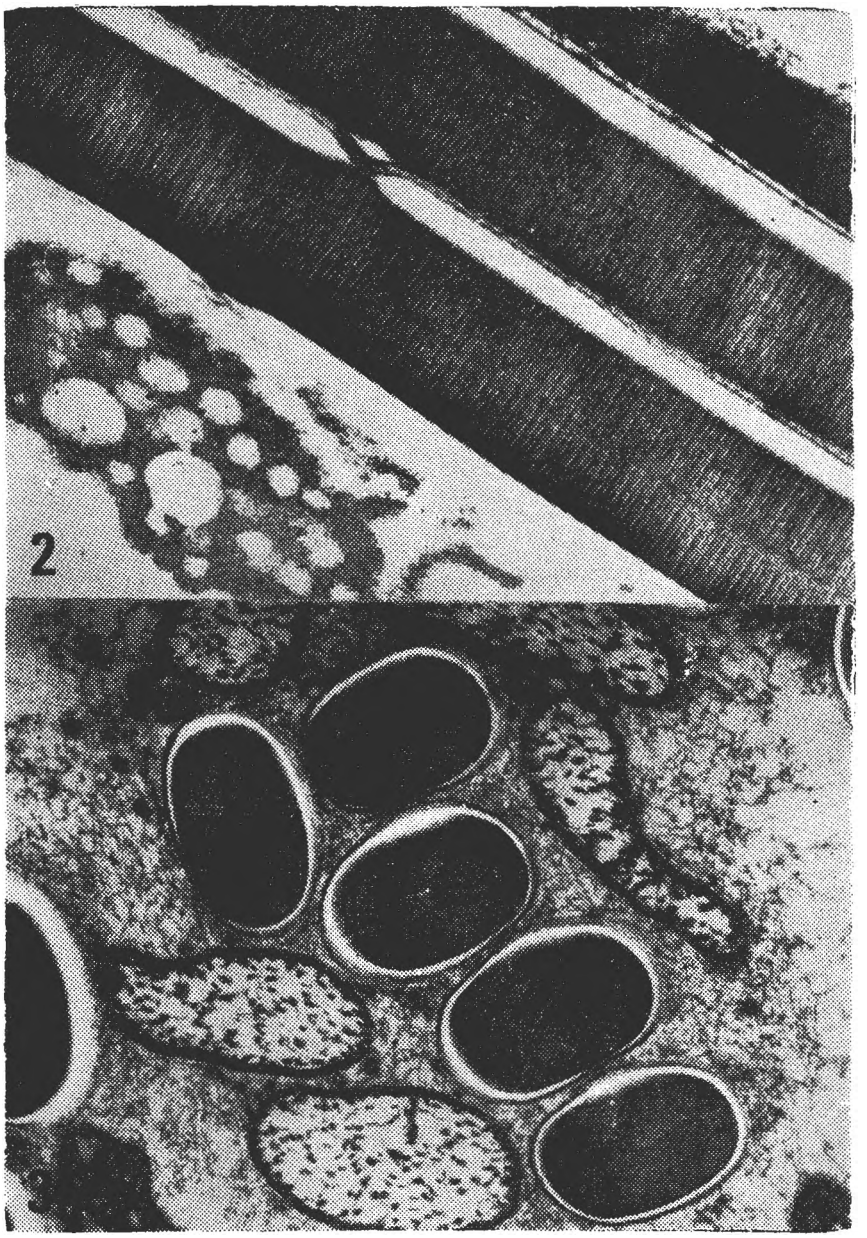
For the study of the spermatozoa, only the mature individuals were selected, from which vas deferens was obtained by pulling out the last abdominal segments. Fixation was done in a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2, and then in a 1% osmic acid solution of the same buffer. After the biological pieces were dehydrated in acetone, they were infiltrated and included in vestopal W and then cut with a LKB III ultramicrotome. The ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a TESLA BS—613 electron microscope with a 5 Å power of resolution. The positive images obtained at magnifications between 250,000 and 500,000 times were further enlarged and then studied with the aid of a stereomicroscope, equipped with an oculary micrometer. In view of establishing the thickness of the repetitive bands and subbands, tens of caudal filaments were thus analyzed for each species.

Knowing the fact that following polymerization the synthetic resin used for embedding, vestopal W, has a volume contracting coefficient of 7%, which corresponds to a linear contracting coefficient of 2.3% [14], we have applied this correction to all our measurements.

Results and discussions. The fine structure of the caudal filaments of the isopod spermatozoa reveals a transversal striation all along beginning even from the zone of connection with the nuclear filament (Fig. 2).

Previous studies performed by Radu [16], Fain-Maurel [8], Crăciun [6, 7] and Cotelli *et al.* [4] have emphasized the fact that the caudal filaments of the isopods of the genera *Armadillidium* and *Porcellio* have the shape of a slightly elastical rod of 800 μm in length. They are elliptical in cross section (Fig. 3). Their diameter is constant all along their length with the exception of a little portion from the zone of connection with the corresponding nuclear filaments, where they are thinner. Therefore, we have always avoided the anterior zones of these filaments for measurements.

The precise establishment of the tail diameters of the spermatozoa is difficult because the shape of their section is ellipsoidal and it must be measured on perfectly transverse caudal sections. The incidence of this type of sections is rather rare and one should always allow a certain degree of approximation in the dimensions obtained. In this way, we have found 390 nm for the diameter of the caudal filament in *Arma-*



Figs. 2—3. *Caudal and nuclear filaments of the* Porcellio scaber *spermatozoa.*
Fig. 2. *Longitudinal section through two caudal filaments (tails).* × 61,000.
Fig. 3. *Transversal section through caudal and nuclear filaments of the spermatozoa from a spermatophore.* × 61,000.

dillidium vulgare, 420 nm in *A. versicolor,* 378 nm in *Porcellio scaber,* 204 nm in *Trachelipus balticus* and 315 nm in *Cylisticus convexus* (see Table 2).

The cross striation of the spermatozoon tails appears similar at the first glance for all 5 isopod species. However, after rigorous measurements on our electron micrographs, we established that it differs from one species to another both in magnitude and the succession of its constitutive elements.

It results, from our studies, that this cross striation appears under the form of certain repetitive bands, which, in turn, are composed of an alternating successions of slightly electrondense (white) and intensely electrondense (black) subbands (Fig. 2). Within each band, one can always distinguish two major subbands, unequal in width. In their turn, the major subbands are each composed of 1—5 minor subbands, also unequal in width. Further, each minor subband is composed of a white-black succession of several (2—9) elementary subbands, equal or unequal, but always having at the basis constitutive elements of 6 and 8 Å. In this way, the elementary subbands can be composed of a constitutive unit, a multiple of this unit or a combination of the two constitutive units (see Table 1 and the schematic representations in Figs. 4—9).

The analysis of the repetitive bands in the *Armadillidium vulgare* (Fig. 4) and *A. versicolor* (Fig. 5) spermatozoa (family *Armadillidiidae)* reveals significant differences only with regard to the order of succession and magnitude of the elementary subbands. The number of the elementary subbands is very close (26 and 24, respectively), suggesting the genus phyliation of the two species.

From the family *Porcellionidae,* the two species analyzed belong to two distinct genera: *Porcellio scaber* (Fig. 6) and *Trachelipus balticus* (Fig. 7). The comparative ultrastructure of bands reveals much more differences than in the previous case. In the first place, there is a difference of 88 Å between the width of the two bands (see Table 1). In the second, the minor subbands are missing in *Trachelipus balticus.* In the third place, there is a difference of 10 elementary subbands between the bands of the two species discussed. As in the previous case, there are also significant differences regarding the order of succession and magnitude of the elementary subbands, although they are organized from the same constitutive units of 6 and 8 Å.

To unravel the band evolution during the development of spermatozoa, we also studied the tails of some immature spermatozoa from the testicular folicles of *Trachelipus balticus* (Fig. 8 and Table 1). The analysis of the repetitive bands of these immature spermatozoa indicates a much simplified structure as compared to the mature spermatozoon (only 10 elementary subbands versus 24). Also, the bands are 121 Å shorter and without differentiations toward the constitutive units of 6 and 8 Å.

It results from this data, that during all the period of growing and development, the structure of the repetitive bands of the spermatozoon becomes gradually more complicated reaching the stability at the ma-

Table 1

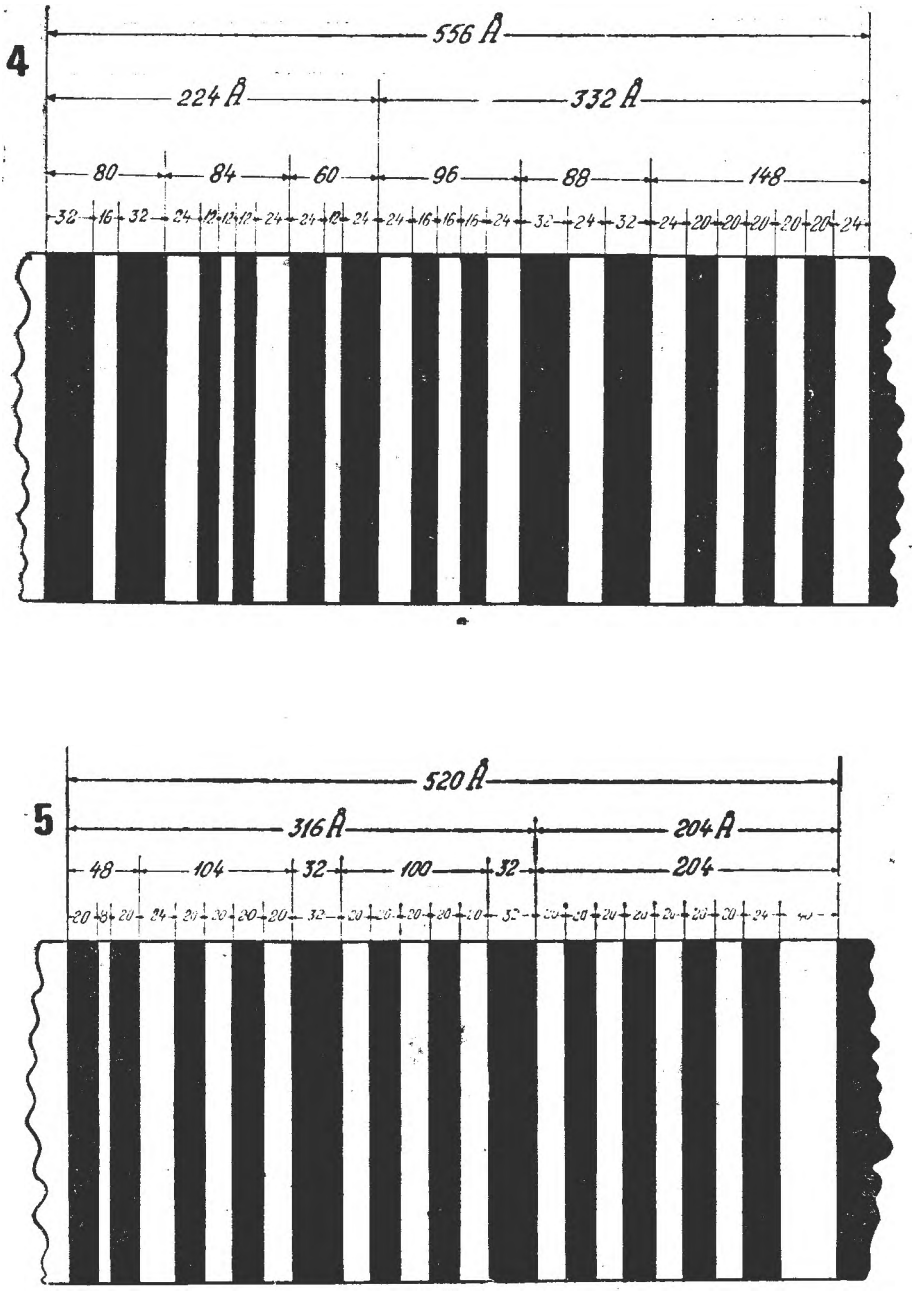
Comparative data on the ultrastructure of the studied spermatozoon tails

Family	Genus and species	Band width (Å)	Major subband width (Å)	Minor subband width (Å)	Elementary subband width (Å)	Number of elementary subbands	Constitutive units
ARMADILLIDIIDAE	Armadillidium vulgare	556	332/224	148, 88, 96/80, 84, 60	12, 16, 20, 24, 32	26	6 and 8 Å
	Armadillidium versicolor	520	316/204	48, 104, 32, 100, 32/204	8, 20, 24, 32, 40	24	6 and 8 Å
PORCELLIONIDAE	Porcellio scaber	598	330/268	84, 64, 84, 98/104, 164	6, 10, 12, 16, 18, 20, 24, 36	34	6 and 8 Å
	Trachelipus balticus (mature)	510	286/224	—	12, 18, 20, 24, 36	24	6 and 8 Å
	Trachelipus balticus (immature)	389	261/128	—	31, 32, 36, 42, 43, 50	10	—
CYLISTICIIDAE	Cylisticus convexus	502	256/246	76, 80/174, 72	8, 10, 16, 18, 20, 24, 36	26	6 and 8 Å

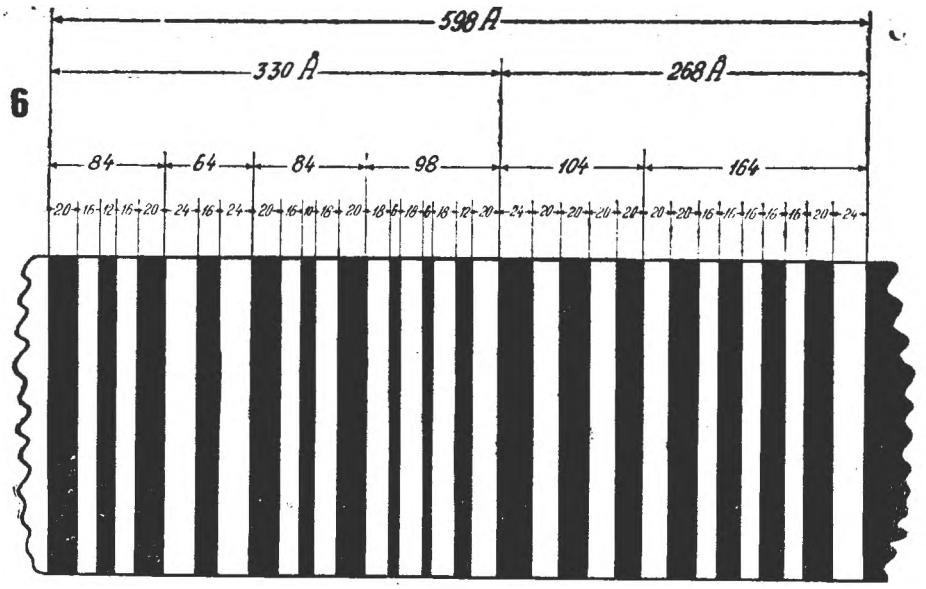
turity. We stress the fact that in order to find the real structure of the repetitive bands of the isopod spermatozoon tails, the studies must be performed only on mature spermatozoa and on perfectly longitudinal sections. This requirement explains the results of certain previous studies [8, 12] which were probably performed on immature spermatozoa, without the mentioning of this detail (see Table 2).

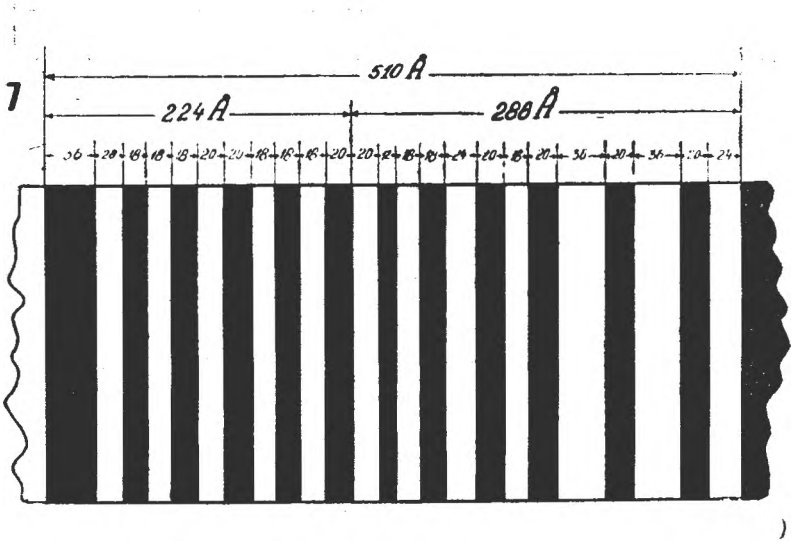
The analysis of the repetitive bands in a species coming from a family different from the two already presented (*Cylisticus convexus*, family *Cylisticiidae*, Fig. 9) would suggest certain connective associations with the two species of the genus *Armadillidium* (see Table 1). We observe that the number of the elementary subbands is identical to that of the *Armadillidium vulgare* (26) and has a minimal difference (2) as compared to *Armadillidium versicolor*. The difference between the bands is only 18 Å in width.

It is still hazardous to claim that, on the basis of all the above data, we could establish more correct phyliations between different isopod groups. However, we consider that the order of succession and width of the elementary subbands are species-specific characters, having probably a value of ultrastructural taxa. Ampler ultrastructural studies of the spermatozoon tails, comprising species of all genera and families of the order *Isopoda*, could very well contribute to the specification of the existent phyliations within this order.

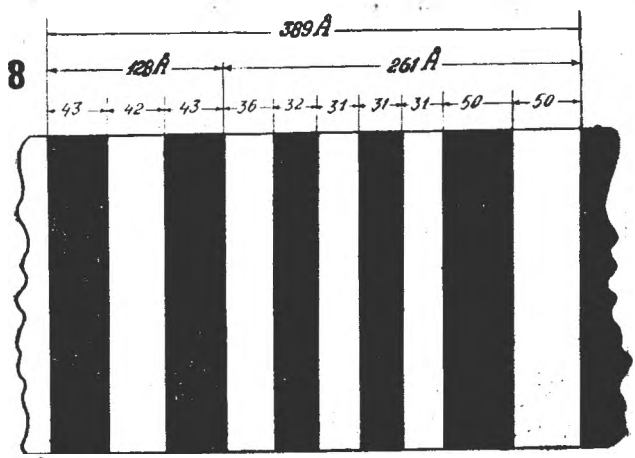


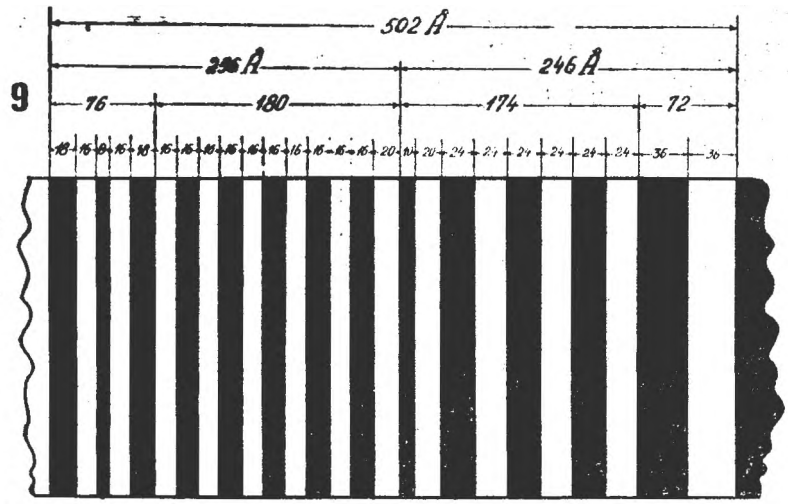
Figs. 4—5. *Schematic representation of transversal striations of a band from the tail of mature spermatozoon of* Armadillidium vulgare (Fig. 4) *and* Armadillidium versicolor (Fig. 5).





Figs. 6—7. *Schematic representation of transversal striations of a band from the tail of mature spermatozoon of* **Porcellio scaber** (**Fig. 6**) *and* **Trachelipus balticus** (**Fig. 7**).





Figs. 8—9. *Schematic representation of transversal striations of a band from the tail of immature spermatozoon of* Trachelipus balticus (Fig. 8) *and of mature spermatozoon of* Cylisticus convexus (Fig. 9).

Table 2

Comparative bibliographical data on the ultrastructure of the spermatozoon tails of different isopods and on other fibrillar structures with cross striations

Bibliographical source	Studied isopods (in phylogenetic order)			Band width (Å)	Subband width (Å)			Tail diameter (nm)
	Family	Genus and species	Way of life		major	minor	elementary	
Hollande, A. and Fain M., 1964 [12]	Cymothoidae	Nerocila bivittata	Parasite on marine fish	384	192/192	24/48	—	—
Fain, M. A., 1966 [8]	,,	,,	,,	630—690	265/365 295/395			—
Blanchard, R. E. *et al.*, 1961 [2]	Idoteidae	Idotea baltica	Marine	570	160/80/ 200/80/70	—	—	400
Reger, J. F., 1964 [20]	Asellidae	Asellus militaris	Fresh water	750—800	6 striations of 125/150	—	—	500
Blanchard, R. E. *et al.*, 1961 [2]	Anthuridae	Cyathura sp.	Fresh water	630	180/165/ 120/165	—	—	300
,, ,,	Oniscidae	Oniscus asellus	Terrestrial	620	App. 7 bands of 80 Å	—	—	240
Reger, J. F., 1966 [21]	,,	,,	,,	750—800	6 striations of 125/150	—	—	—
Cotelli, E. *et al.*, 1976 [4]	,,	,,	,,	700	—	—	—	—
,, ,,	Porcellionidae	Porcellio laevis	,,	700	—	—	—	—
Crăciun, C., 1974 [5]	,,	Porcellio scaber	,,	598	330/268	84/64 84/98 104/164	6, 10, 12, 16, 18, 20, 24	378
,, ,,	,,	Trachelipus balticus	,,	510	286/224	—	12, 18, 20, 24, 36	204
Fain, M. A., 1966 [8]	Armadillidiidae	Armadillidium vulgare	,,	400—440	270/130 290/150	—	—	—
Cotelli, F. *et al.*, 1976 [4]	,,	,,	,,	750	—	—	—	—
Crăciun, C., 1975 [6]	,,	,,	,,	556	332/224	148/88/96/ 84/80	12, 16, 20, 24, 32	390
,, ,,	,,	Armadillidium versicolor	,,	520	316/204	48/104/32/ 100/32/204	8, 20, 24, 32, 40	420
Crăciun, C., 1986 [7]	Cylisticiidae	Cylisticus convexus	,,	502	256/246	76/180/174/ 72	8, 10, 16, 20, 24, 36	315
After Blanchard, R. E. *et al.*, 1961 [2]	Paramyosin fiber			720	—	—	—	—
Giesekíng, R., 1962 [9]; Björn, R. O., 1963 [1]	Collagen fiber			700 with 5—8 subperiods	—	—	—	—

We have made the effort of correlating our data with those existing in the literature, but, unfortunately, these are still few. Moreover, the authors of these studies have established, for the spermatozoa studied by them, only the thickness of the repetitive bands and, in a few cases, that of the major subbands, without any concern for the minor and elementary subbands (see Table 2).

We have also tried to make certain analogies between the values of the repetitive band periodicities of the isopods and other structures that possess transversal striations. Among these, the collagen fiber is the best known. It has a periodicity of 700 Å, with an alternation of 5—8 white-and-black successive subbands [1, 9]. Also, paramyosin (a fibrillar protein) has a periodicity of 720 Å [2]. We consider that both these structures are succeptible of being composed of constitutive units of 6 and 8 Å, like the isopod spermatozoon tails.

These last data suggest the fact that all these structures with transversal periodicity could have in common the same basic constitutive units, which can show up in the structural organization of certain repetitive bands of approximately equal widths.

Conclusions. The isopod spermatozoon is atypical, being nonflagellated. Its caudal filament is a semi-rigid rod which possesses a cross striation composed of repetitive bands. Every band is made up of major, minor and elementary subbands. Band and subband widths and the succession of the elementary subbands are species-specific. These elements could be considered as ultrastructural taxa and used as an index of interspecific phyliations within the group of isopods.

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VARIATIONS À LONGUE ÉCHÉANCE DANS LA DYNAMIQUE D'UNE POPULATION TROGLOBIE

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SUMMARY. — Long-Term Variations in the Dynamics of a Troglobitic Population. Research on the assembly of individuals of the bathysciin beetle *Pholeuon proserpinae glaciale* which populates the warm meroclimate in the cave *Gheţarul de la Scărişoara*, undertaken in two different phases separated by a 20-year interval, allowed surveying long-term changes that might occur in this subspecies' dynamics. These changes are entirely quantitative and are probably due to influences of the outside climate oscillations on the life conditions in the colluvial biotope (superficial hypogean compartment), influences that go down to the assembly of individuals interrelated with the cave's space. It is concluded that such influences, associated to the instability of meteorological factors outside the cave, may lead to a distorted image of cave population's dynamics, when this image is built up on data ranging on a too long extent of time.

A côté de nombreux sujets d'étude que la grotte glacière nommée *Gheţarul de la Scărişoara* (Monts du *Bihor*, Roumanie) offre au point de vue glaciologique et climatologique, on doit également compter les problèmes d'écologie souterraine liés aux conditions particulières dans lesquelles vit dans cette cavité le coléoptère bathysciinae *Pholeuon* (s. str.) *proserpinae glaciale*. La dynamique de la poulation cavernicole de cette sous-espèce troglobie a été suivie pour la première fois dans le cadre d'un programme complexe de recherches, échelonnées entre 1963 et 1968 [7]. Une vingtaine d'années plus tard, les études imposées par le projet d'un aménagement touristique de la grotte et réalisées grâce à l'appui accordé par le Conseil populaire du département d'*Alba* nous ont permis de reprendre les problèmes d'écologie et une nouvelle série d'observations a pu être faite entre 1982 et 1986. Nous avons donc aujourd'hui la possibilité de comparer deux séquences distinctes de la dynamique de cette population et d'en déduire les modifications qui peuvent se manifester au but d'un intervalle de 20 ans. C'est ce que nous nous proposons de réaliser dans le présent travail.

La méthode employée pour la mise en évidence des variations de densité qui se produisent au niveau de la population cavernicole a été celle des estimations mensuelles d'effectif, à la suite d'une concentration de la faune par stimulation trophique à l'aide des appâts [1]. Plusieurs stations ont été mises en place dans la cavité, mais la comparaison dont nous venons de parler ne concerne que celle installée dans la Galerie Coman, c'est-à-dire dans la partie profonde de la grotte, au sein d'un méroclimat relativement chaud et dépourvu de toute périodicité saison-

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nière dans la variation des facteurs thermohygrométriques [9]. Les valeurs moyennes sont de 3,5°C pour la température et de 98% pour l'humidité relative et les amplitudes annuelles ne dépassent pas 0,6°C et, respectivement, 5%.

Ce trait dominant des conditions physiques du milieu représente en même temps un élément décisif pour la dynamique de *Ph. p. glaciale*, la grandeur de la population dans les limites du méroclimat chaud étant déterminée par des facteurs dépendants de la densité (dans le sens de D a j o z [2]). Come l'influence que la structure méroclimatique de la cavité a sur la dynamique de la population cavernicole sera analysée d'une manière plus détaillée dans un autre travail, nous n'insistons plus maintenant sur cette question. En échange, on doit présenter, même brièvement, les principaux aspects qui caractérisent les fluctuations d'effectif durant la première série d'observations (1963—1968), afin de pouvoir réaliser ensuite la comparaison avec les données de la seconde série (1982—1986) et d'en détacher les conclusions concernant les changements qui peuvent intervenir selon un plus grand pas de temps.

Considéré au niveau de toute la période de 5 années, l'ensemble d'individus (dans le sens de D e l a y [3]) qui peuple la zone à méroclimat chaud a présenté de fortes variations d'effectif (Tableau 1, Fig. 1), en évident contraste avec la stabilité thermohygrométrique du milieu. Ces variations se caractérisent pour la plupart par des augmentations et des diminutions successives, ainsi que par une sévère réduction de la densité durant les mois d'automne. On doit noter également le fait que la grandeur de l'ensemble d'individus a manifesté une tendance progressive

Tableau 1

Effectifs mensuels de Pholeuon proserpinae glaciale **en 1963—1968 et 1982—1986 dans le méroclimat chaud de la Grotte de Scărișoara**

Mois	1963	1964	1965	1966	1967	1968	1982	1983	1984	1985	1986	Médiane
Jan.		11	126	187	433	221		44	25	185	327	185
Fév.		270	84	325	320	250		102	186	78	75	186
Mar.		90	74	87	145	154		76	43	115		89
Avr.		51	184	290	334	(164)		77	139	142		153
Mai		161	132	302	236	175		150	264	9		168
Jun.		80	274	362	242	244		219	235	44		239
Jul.		130	63	372	164	94		218	228	224		191
Aug.		(131)	(56)	(218)	(113)		67	29	(20)	153		90
Sep.	25	92	49	64	(62)		14	(20)	29	8		29
Oct.	38	19	6	(232)	12		27	37	(35)	45		35
Nov.	6	26	3	401	(22)		52	76	114	104		52
Déc.	(8)	(76)	(95)	(417)	32		(35)	22	92	95		76
Σ	77	1137	1146	3257	2115	1302	195	1070	1410	1202	402	
N		95	96	271	176			89	118	100		

Observation : Les valeurs entre parenthèses sont interpolées.

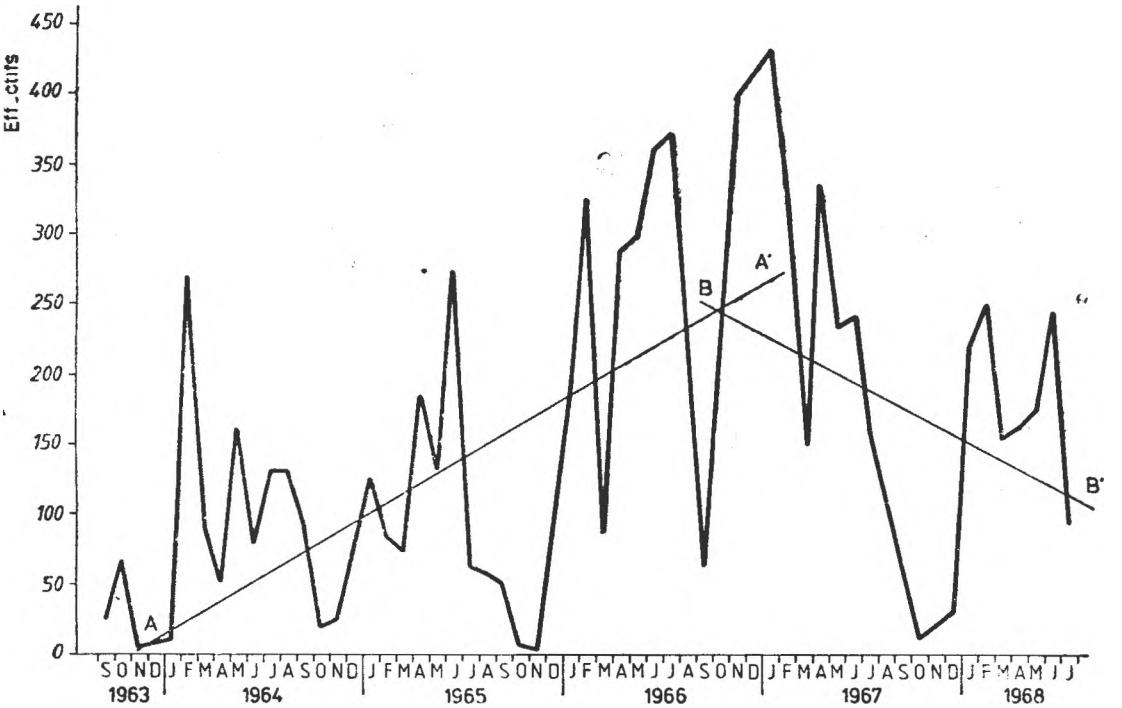

Fig. 1. *Effectifs mensuels de* Pholeuon proserpinae glaciale *durant l'intervalle 1963—1968. A—A'* et *B—B'* — Tendances de longue durée.

d'augmentation (Fig. 1, A—A'), déterminée sans doute par la présence permanente des appâts dans la grotte, mais que cette grandeur a diminué au cours des deux dernières années (Fig. 1, B—B'), comme suite du prélèvement systématique de 50% des échantillons mensuels.

Certains éléments spécifiques pour la dynamique de l'ensemble d'individus sont mieux mis en évidence par le diagramme de la variation saisonnière (Fig. 2), qui a été établi par la méthode des séries de rapports [13]. Il s'agit — nous le rappelons très sommairement — de rapporter chacun des effectifs mensuels à une grandeur de référence, qui est l'effectif du mois de janvier, et d'exprimer finalement les résultats comme pourcents d'une base commune, représentée par la moyenne arithmétique de la série de ces rapoprts. On arrive ainsi à éliminer justement les effets de la tendance de longue durée sur les éléments qui concernent les variations de densité durant un cycle annuel.

Le diagramme comporte deux aspects particuliers. Il montre, en premier lieu, la façon bien nette dont se manifeste la réduction automnale des effectifs. Étant donnée que cette réduction est accompagnée d'une augmentation statistiquement significative du taux des jeunes imagos, encore immatures au point de vue sexuel, elle peut constituer

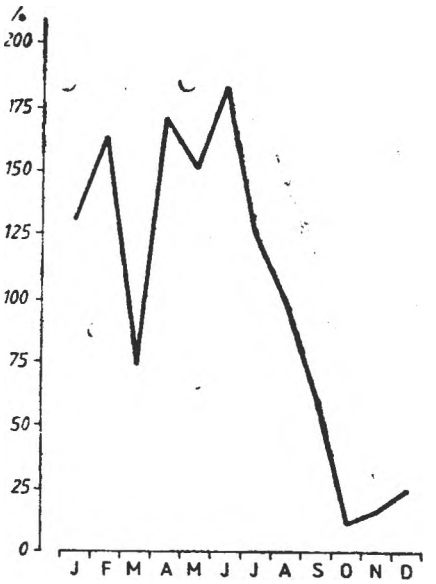

Fig. 2. *Variation saisonnière pour l'intervalle 1963—1968, établie par la méthode des séries de rapports.*

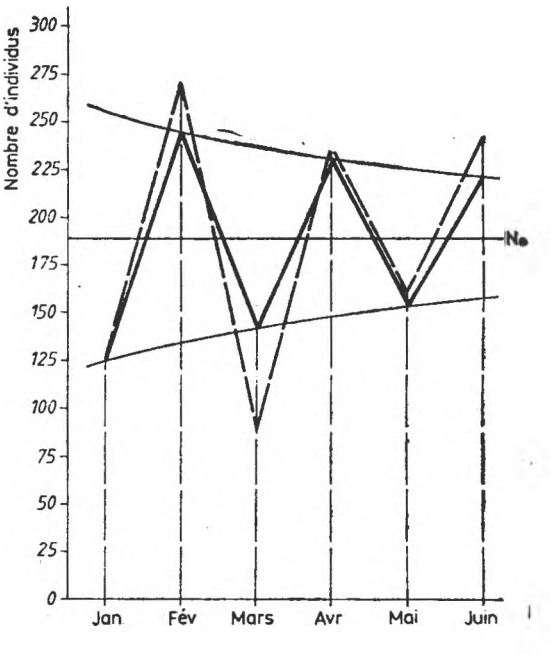

Fig. 3. *Modèle théorique de l'autorégulation des effectifs pour l'intervalle 1963—1968 (trait continu), superposé aux oscillations réelles (trait interrompu).* *No* — Valeur d'équilibre.

l'indice d'un maximum reproductif, ayant comme cadre non pas l'espace de la grotte, mais le réseau de fissures du massif calcaire. La situation paraît bine correspondre au scénario que Jeannel [4] a proposé pour la reproduction des coléoptères troglobies spécialisés. En second lieu, ce diagramme reproduit avec fidélité au cours des premiers 6 mois de l'année la succession d'augmentations et de diminutions de la densité. Comme les données se raportant à cette période confirment l'existence d'une corrélation significative entre les effectifs absolus et ceux relatifs, il apparaît que la grandeur de l'ensemble d'individus est contrôlée par un processus d'autorégulation, conformément au modèle établi par MacArthur et Connel [6]. Ce processus se réalise par des déplacements alternatifs des insectes entre la grotte et le réseau de fissures et a comme élément de référence la capacité biotique des deux habitats souterrains. Une telle interprétation est confirmée d'une manière très satisfaisante par le modèle théorique de l'autorégulation (Fig. 3), qu'on peut construire à partir de la corrélation mentionnée ci-desus [8].

Notons, enfin, que la densité la plus forte correspond dans la variation saisonnière au mois de juin, mais sans qu'elle puisse être considérée comme l'équivalent d'un maximum estival proprement dit, car elle n'est que très peu différente par rapport aux valeurs précédentes.

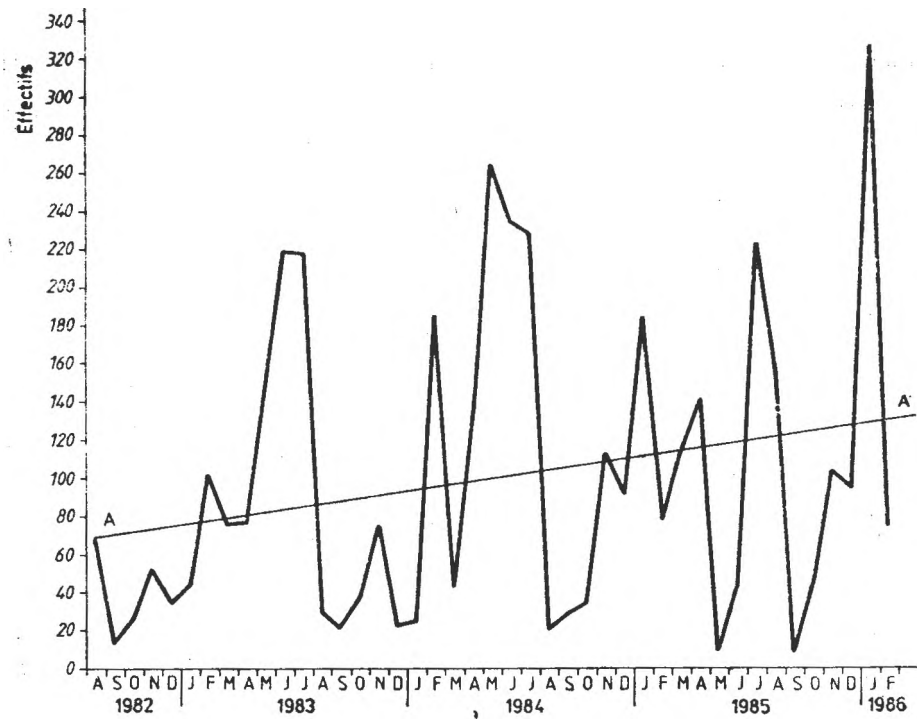

Fig. 4. *Effectifs mensuels de* Ph. p. glaciale *durant l'intervalle 1982—1986.*

Après ce rapide exposé des paramètres qui ont pu être détachés en tant qu'éléments caractéristiques des fluctuations d'effectif enregistrées durant l'intervalle 1963—1968, il convient d'analyser plus en détail les données furnies par la seconde série de recherches, entreprises entre 1982 et 1986.

La succession des effectifs mensuels le long de cette seconde série (Tableau 1, Fig. 4) ne présente guère de différences fondamentales vis-à-vis de celle de la première série de données (Fig. 1). On remarque, en effet, la même alternance entre de faibles et de fortes valeurs de la densité et la même réduction automnale du nombre d'individus se trouvant dans l'espace de la grotte, ainsi que la tendance progressive d'augmentation de la grandeur de la population. Cette tendance est pourtant de moindre anvergure, la pente de la droite de régression par laquelle elle est illustrée étant plus faible (Fig. 4, A—A'), comme suite du fait que le calcul de la fonction linéaire qui correspond à cette droite mène à l'équation numérique (Tableau 2):

$$N = 1{,}436\ n + 67{,}90, \qquad (1)$$

Tableau 2

Calcul de la tendance de longue durée de l'ensemble d'individus durant l'intervalle 1982—1986

N°. d'ordre des mois	Effectifs (y_i)	u_i	$u_i y_i$	Valeurs régul.	Écarts	Écarts %
1	67	−21	−1407	69,34	−2,34	−3,4
2	14	−20	−280	70,77	−56,77	−80,2
3	27	−19	−513	72,21	−45,21	−62,6
4	52	−18	−936	73,65	−21,65	−29,4
5	35	−17	−595	75,08	−40,08	−53,4
6	44	−16	−704	76,52	−32,52	−42,5
7	102	−15	−1530	77,95	24,05	30,9
8	76	−14	−1064	79,39	−3,39	−4,3
9	77	−13	−1001	80,83	−3,83	−4,7
10	150	−12	−1800	82,26	67,74	82,3
11	219	−11	−2409	83,70	135,30	161,6
12	218	−10	−2180	85,14	132,86	156,0
13	29	−9	−261	86,57	−57,57	−66,5
14	20	−8	−160	88,01	−68,01	−77,3
15	37	−7	−259	89,45	−52,45	−58,6
16	76	−6	−456	90,88	−14,88	−16,4
17	22	−5	−110	92,32	−70,32	−76,2
18	25	−4	−100	93,76	−68,76	−73,3
19	186	−3	−558	95,19	90,81	95,4
20	43	−2	−86	96,63	−53,63	−55,5
21	139	−1	−139	98,06	40,94	41,7
22	264	0	0	99,50	164,50	165,3
23	235	1	235	100,94	134,06	132,8
24	228	2	456	102,37	125,63	122,7
25	20	3	60	103,81	−83,81	−80,7
26	29	4	116	105,25	−76,25	−72,4
27	35	5	175	106,68	−71,68	−67,2
28	114	6	684	108,12	5,88	5,4
29	92	7	644	109,56	−17,56	−16,0
30	185	8	1480	110,99	74,01	66,7
31	78	9	702	112,43	−34,43	−30,6
32	115	10	1150	113,87	1,13	1,0
33	142	11	1562	115,30	26,70	23,2
34	9	12	108	116,74	−107,74	−92,3
35	44	13	572	118,17	−74,17	−62,8
36	224	14	3136	119,61	104,39	87,3
37	153	15	2295	121,05	31,95	26,4
38	8	16	128	122,48	−114,48	−93,5
39	45	17	765	123,92	−78,92	−63,7
40	104	18	1872	125,36	−21,36	−17,0
41	95	19	1805	126,79	−31,79	−25,1
42	327	20	6540	128,23	198,77	155,0
43	75	21	1575	129,67	−54,67	−42,2
	4279		9512			

$\bar{x} = 22 \quad \bar{y} = 99{,}5 \quad \Sigma u_i^2 = 6622$

$$a = \frac{\Sigma u_i y_i}{\Sigma u_i^2} = \frac{9512}{6622} = 1{,}436$$

$$b = -ax + y = -1{,}436 \cdot 22 + 99{,}5 = 67{,}90$$

$$N = 1{,}436\, n + 67{,}90$$

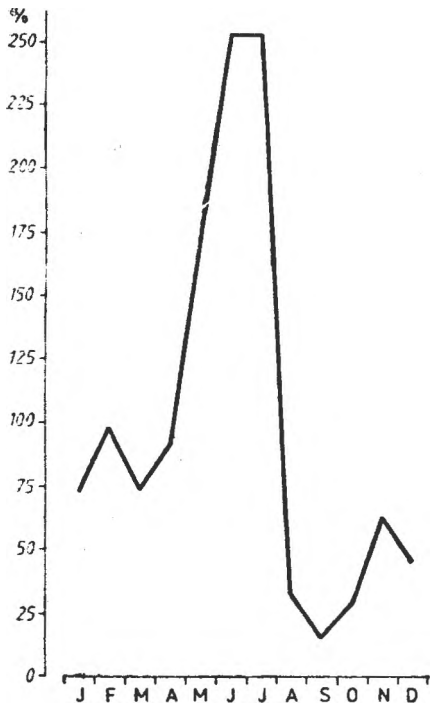

Fig. 5. *Variation saisonnière pour l'intervalle 1982—1986 (données finales du Tableau 3).*

dans laquelle le coefficient a=1,436 est nettemnt plus petit que celui établi pour la première série de données (a=7,02) [9]. Le taux de croissance de l'ensemble d'individus, qui résulte du rapport entre l'effectif théoretique final et l'augmentation annuelle, n'est dans ces conditions que de 13,0%, c'est-à-dire environ 2,5 fois plus réduit que celui (de 31,4%) déterminé pour la première série de données.

En étroite liaison avec cette constatation on doit noter le fait que la grandeur générale de l'ensemble d'individus est à son tour plus faible durant le second intervalle, l'effectif moyen annuel n'étant que de 102 exemplaires, par rapport à 160 exemplaires en 1963—1968, et l'effectif maximum n'atteignant maintenant que 327 individus (en janvier 1986), contre 433 individus dénombrés en janvier 1967 (Tableau 1). Il paraît que la manière pas tout à fait satisfaisante dont on a respecté jusqu'à présent les normes de protection de la réserve scientifique établie dans la Grotte de *Săcrișoara* (et qui renferme la zone occupée par *Pholeuon)* n'a pas resté sans conséquences à l'égard des conditions de vie de la population de coléoptères troglobies.

Des différences bien plus marquées entre les deux séries de données apparaissent lorsqu'on fait la comparaison entre les variations saisonnières des effectifs. Le diagramme correspondant à la seconde série (Fig. 5) — obtenu à la suite de l'élimination de la tendance de longue durée par la méthode des séries de rapports (Tableau 3) — se caractérise en premier lieu par la même réduction très accentuée de la densité au cours de l'automne, le minimum étant enregistré cette fois-ci un mois plus tôt, c'est-à-dire en septembre. Mais à l'encontre de la configuration que cette variation saisonnière a pris durant les années 1963—1968 (Fig. 2), le maximum estival, qui couvre les mois de juin et juillet, devient maintenant beaucoup plus prononcé. Cet aspect distinctif peut se trouver en relation avec certaines particularités concernant la dynamique globale de la faune souterraine terrestre.

Le sous-genre *Pholeuon* (s. str.) a été considéré jusqu'à présent comme étant strictement troglobie. Des recherches entreprises dans le bitope colluvial (milieu souterrain superficiel) de la Vallée d'*Ordîncușa*

Tableau 3

Calcul de la variation saisonnière durant l'intervalle 1982–1986

Ibm — Indices à base mobile (valeurs médianes, exprimées en pour-cents, des séries de rapports entre deux mois consécutifs).

Ibf — Indices à base fixe (valeurs rapportées au mois de janvier, conformément aux égalités : $\frac{F}{J} \cdot \frac{M}{F} = \frac{M}{J} \ldots \frac{D}{N} \cdot \frac{N}{J} = \frac{D}{J}$.

Ic — Indices corrigés par le facteur K, déduit de la valeur du rapport $\frac{J}{J}$ et représentant la grandeur du taux mensuel de la tendance de longue durée.

Ic% — Indices corrigés exprimés en pour-cents par rapport à leur moyenne arithmétique.

Mois	Rapport numérique					Ibm	Ibf	Ic	Ic%
	1982	1983	1984	1985	1986				
J/D		1,26	1,14	2,01	3,44	164		100	73,0
F/J		2,32	7,44	0,42	0,23	137	137	135	98,5
M/F		0,75	0,23	1,47		75	103	101	73,7
A/M		1,01	3,23	1,23		123	127	125	91,2
M/A		1,95	1,90	0,06		190	241	237	173,0
J/M		1,46	0,89	4,89		146	352	346	252,6
J/J		1,00	0,97	5,09		100	352	346	252,6
A/J		0,13	0,09	0,68		13	46	45	32,8
S/A	0,21	0,69	1,45	0,05		45	21	21	15,3
O/S	1,93	1,85	1,21	5,63		189	40	39	28,5
N/O	1,93	2,05	3,26	2,31		218	87	86	62,8
D/N	0,67	0,29	0,81	0,91		74	64	63	46,0
								1644	

$$J/J = \frac{74 \cdot 164}{100} = 121$$

$$\log K = \frac{\log 100 - \log 121}{12} = -0{,}0068991\,;\ K = 0{,}98424$$

$$\overline{Ic} = \frac{1644}{12} = 137$$

[11], c'est-à-dire aux environs de la Grotte de *Scărișoara*, ont démontré pourtant que ces coléoptères peuvent accéder à ce secteur du domaine hypogé mis récemment en évidence au voisinage de la surface des terrains karstiques et également non karstiques [5]. Par conséquent, on peut admettre que le modèle conçu à partir des données obtenues sur la faune souterraine du bassin de *Valea Iadului* [10, 12] est applicable dans le cas de *Pholeuon* aussi. Nous rappelons que ce modèle illustre les migrations que les animaux effectuent entre les divers niveaux du domaine souterrain (principalement le biotope colluvial, le réseau de fissures et les grottes), selon le changement saisonnier de la position de l'optimum écologique. De telles migrations mènent à une concentration de la faune dans les cavités karstiques au cours de l'été, quand l'insola-

tion et la sécheresse rendent les conditions physiques moins favorables dans le biotope colluvial. Et il va sans dire que l'ampleur de cette concentration dépend des particularités de la météorologie externe et qu'elle peut varier au cours du temps entre des limites assez larges.

Il est donc possible que le maximum estival plus accentué qui se manifeste au cours de la seconde période soit dû à un contexte climatique moins propice pour la présence des *Pholeuon* dans les secteurs superficiels du massif karstique, de sorte que ceux-ci ont migré en plus grand nombre dans les secteurs profonds, y compris l'espace de la grotte où le méroclimat particulièrement stable est mis hors les oscillations météorologiques.

Le poids bien plus grand que le maximum estival a dans la variation saisonnière de l'ensemble d'individus durant la seconde période détermine un autre trait distinctif de cette variation. Il s'agit du fait que la succession d'augmentations et de diminutions de l'effectif qui sortait si nettement en évidence dans le diagramme correspondant à la première série de données (Fig. 2) devient maintenant tout à fait mineure. On peut néanmoins la déceler dans l'intervalle octobre-avril (Fig. 5), c'est-à-dire décalée de deux mois par rapport à la série précédente, et le calcul montre que la corrélation entre les effectifs absolus et les effectifs relatifs est statistiquement significative à un seuil de 1% (Tableau 4);

Tableau 4

Calcul de la corrélation entre les effectifs absolus (N) et les effectifs relatifs (ΔN) durant l'intervalle 1982—1986
(écarts numériques par rapport à la droite de régression correspondant à la tendance de longue durée)

N (x_i)	ΔN (y_i)	$x_i - \bar{x}$	$y_i - \bar{y}$	$(x_i - \bar{x}) \cdot (y_i - \bar{y})$	$(x_i - \bar{x})^2$	$(y_i - \bar{y})^2$
52	−17	−46,4	−21,8	1011,52	2152,96	475,24
35	+9	−63,4	4,2	−266,28	4019,56	17,64
44	+58	−54,4	53,2	−2894,08	2959,36	2830,24
102	−26	3,6	−30,8	−110,88	12,96	948,64
76	+1	−22,4	−3,8	85,12	501,76	14,44
76	−54	−22,4	−58,8	1317,12	501,76	3457,44
22	+3	−76,4	−1,8	137,52	5836,96	3,24
25	+161	−73,4	156,2	−11465,08	5387,56	24398,44
186	−143	87,6	−147,8	−12947,28	7673,76	21844,84
43	+96	−55,4	91,2	−5052,48	3069,16	8317,44
114	−22	15,6	−26,8	−418,08	243,36	718,24
92	+93	−6,4	88,2	−564,48	40,96	7779,24
185	−107	86,6	−111,8	−9681,88	7499,56	12499,24
78	+37	−20,4	32,2	−656,88	416,16	1036,84
115	+27	16,6	22,2	368,52	275,56	492,84
104	−9	5,6	−13,8	−77,28	31,36	190,44
95	+232	−3,4	227,2	−772,48	11,56	51619,84
327	−252	228,6	−256,8	−58704,48	52257,96	65946,24
1771	87			−100691,84	92892,28	202590,52

$\bar{x} = 98{,}4$; $\bar{y} = 4{,}8$;

$$r = \frac{-100691{,}84}{\sqrt{92892{,}28 \cdot 202590{,}52}} = -0{,}73$$

$N = -1{,}084\,N + 111{,}462$

$m = n - 2 = 16$ D.L.

$p < 1$

la première condition requise par une autorégulation de la densité est ainsi accomplie. On doit pourtant préciser dès le début que le résultat le plus satisfaisant est obtenu à partir non pas des valeurs numériques brutes des effectifs mensuels, mais des valeurs régularisées par élimination de la tendance de longue durée (écarts des ordonnées de la série des effectifs mensuels par rapport à la droite de régression) (Tableau 2).

La fonction linéaire de cette corrélation

$$\Delta N = -1{,}36\,N + 2{,}17 \qquad (2)$$

a un coefficient a=—1,36 plus faible en valeur absolue que celui obtenu pour la première série de données (a=—1,86) [9], mais toujours supérieur à 1. En conséquence, le modèle théorique de l'autorégulation des effectifs déduit de l'équation (2) (Tableau 5) reste du même type que celui établi pour la série précédente, c'est-à-dire comprenant des oscillations dont l'amplitude décroît dans le temps, et ce modèle correspond d'une manière acceptable aux variations réelles pour l'intervalle octobre -mars (Fig. 6). La différence qui existe par rapport à la première série n'est donc que d'ordre quantitatif et concerne le fait que la tendance d'amortissement des ces oscillations est maintenant bien plus marquée.

On peut par conséquent en conclure que, durant l'intervalle 1982—1986, la manifestation d'un plus fort maximum saisonnier a eu comme résultat un effacement de l'action des facteurs cénotiques dans le contrôle de la densité, ce qui est tout à fait normal sin on a en vue qu'un

Tableau 5

Calcul des valeurs théoriques (Nt) du modèle de l'autorégulation des effectifs pour l'intervalle 1982—1986 à partir de la fonction linéaire de la corrélation N/ΔN

Mois	No	N	Nt	N réel
Oct.	—62	+86	24	—62
Nov.	24	—30	—14	—18
Déc.	—14	+21	7	—16
Jan.	7	—7	0	21
Fév.	0	+2	2	—5
Mar.	2	—1	1	—3
Avr.	1	0	1	27

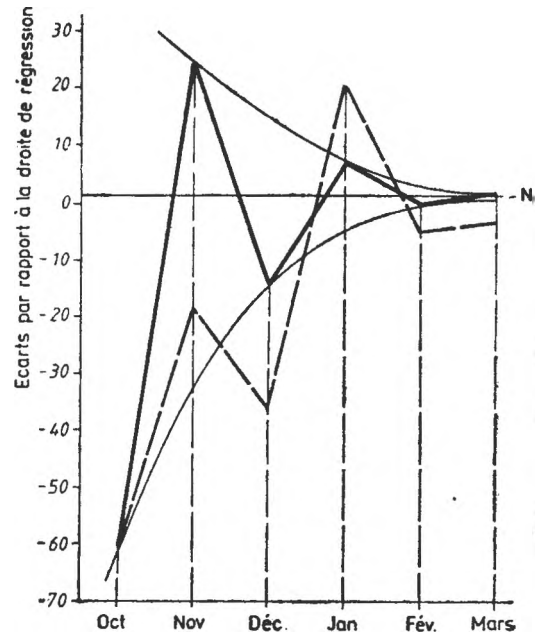

Fig. 6. *Modèle théorique pour l'autorégulation des effectifs dans l'intervalle 1982—1986.*
Mêmes détails que dans la Fig. 3.

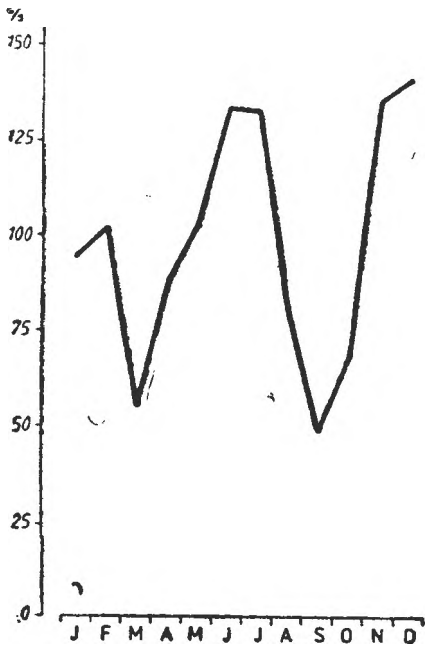

Fig. 7. *Variation saisonnière globale pour l'intervalle 1963—1986.*

tel maximum, déterminé par des paramètres physiques, agit toujours comme un élément de perturbation dans le mécanisme d'autorégulation.

La principale constatation qui se détache de cette mise en parallèle des deux séries de données concernant la dynamique de l'ensemble d'individus qui peuplent la zone à méroclimat stable de la Grotte de *Scărișoara* est que les modifications qui apparaissent à longue échéance dans les aspects caractéristiques pour les fluctuations d'effectif ne sont que purement *quantitatives*. Outre le fait qu'un tel résultat est parfaitement logique et qu'il démontre un degré très satisfaisant de fidélité pour la méthode d'étude employée, la similarité que nous venons de constater constituie un motif suffisant pour justifier l'essai d'obtenir un tableau global sur l'ensemble de données dont nous disposons.

Un tel essai abouti malheureusement à des images déformées sur la dynamique de la population cavernicole. En effet, le diagramme de la variation saisonnière (Fig. 7) ne conserve pratiquement aucun des traits caractéristiques pour cette dynamique. L'abaissement automnale de la densité, qui se manifeste toujours au mois de septembre, est doublé d'une réduction du même ordre de grandeur au mois de mars et perd donc presque entièrement sa signification. Une situation analogue apparaît à l'égard du maximum estival, qui est suivi à son tours par une nouvelle augmentation de la densité en novembre-décembre. Enfin, les oscillations qui suggèrent le contrôle par autorégulation des effectifs cavernicoles et qui peuvent être reconnues durant l'intervalle novembre-avril n'ont qu'une amplitude si faible, que la corrélation entre les effectifs absolus et ceux relatifs reste non significative. La valeur la plus grande du coefficient de corrélation, avec une signification statistique à un seuil de 1%, est tout de même obtenue en partant des écarts par rapport aux droites de régression calculées pour chacune des deux series de données (c'est-à-dire après l'élimination de la tendance de longue durée), mais l'équation numérique de cette corrélation linéaire

$$\Delta N = -0{,}91\ N + 16{,}53, \qquad (3)$$

avec un coefficient a=—0,91 inférieur à 1 en valeur absolue, conduit à un modèle théorique tout à fait différent (Fig. 8), représenté par une courbe croissante qui atteint très vite la valeur d'équilibre; ce modèle

n'est évidemment pas acceptable par rapport aux oscillations réelles.

L'explication de cet échec est bien simple. Elle réside dans les décalages que nous avons mentionnés à plusieurs reprises et qui se manifestent entre les deux séries de données justement à l'égard des principaux éléments caractéristiques pour la dynamique de l'ensemble d'individus. Lorsqu'on superpose ces deux séries, de tels décalages ont comme effet l'effacement des points les plus importants de la variation saisonnière et l'image qu'on obtient finalement devient de toute façon dépourvue de signification.

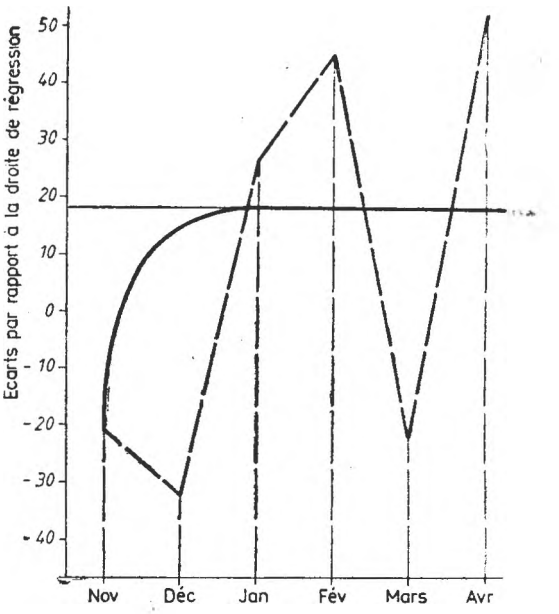

Fig. 8. *Modèle théorique de l'autorégulation des effectifs pour l'intervalle 1963—1986.*
Mêmes détails que dans la Fig. 3.

Conclusions. La dynamique de l'ensemble d'individus qui occupe le méroclimat stable de la grotte *Gheţarul de la Scărişoara* garde à longue échéance ses principaux traits caractéristiques. Ces traits consistent en: un maximum estival qui dérive des migrations saisonnières déroulées entre les divers secteurs du massif karstique, y compris la biotope colluvial; un minimum automnal qui répond le plus probablement à une périodicité reproductive de même saisonnière; une phase d'autorégulation des effectifs cavernicoles, comprise entre les deux moments précédents. Il est donc démontré que les particularités écologiques qui caractérisent les populations cavernicoles dérivent sans aucune doute non pas de certaines circonstances occasionnelles, mais bien des conditions spécifiques dans lesquelles se trouve chacune de ces populations.

Au but d'un intervalle de l'ordre de 20 ans, les modifications qui peuvent être décelées dans la dynamique de cet ensemble sont de facture seulement quantitative. Elles concernent principalement le maximum estival et sont probablement dues aux variations qui se produisent dans le contexte météorologique de l'éxtérieur, en reflétant ainsi des changements dans le conditions de vie du biotope colluvial.

Étant donné que la population cavernicole, y compris l'ensemble d'individus se trouvant en relation avec la zone la plus profonde de la grotte, n'arrive pas à être mis hors les influences de la météorologie externe, le caractère aléatoire de ces influences rend riscant l'éssai de dresser le tableau de la dynamique de la population à partir de données échelonnées sur une trop longue période de temps.

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ÜBER AMPHIBIEN UND REPTILIEN AN DER OBEREN WALDGRENZE IM RETEZAT-GEBIRGE

BOGDAN STUGREN* und IOAN GHIRA**

SUMMARY. — **On Amphibians and Reptiles at the Timberline in the Retezat Mountains.** There are only two species of amphibians and three species of reptiles above the timberline in the Retezat Mountains. Individual densities of populations are different on limestones and non-calcareous (metamorphic rocks of volcanic origin) habitats in the subalpine zone. So, *Rana temporaria* is more abundant on volcanic soils than on limestones. On the contrary, *Lacerta vivipara* shows higher densities on limestones. *Vipera berus* has so far been found in the subalpine zone of the Retezat only on non-calcareous soils. *Triturus alpestris* and *Bombina variegata* occur above the timberline only on limestone soils.

Die Amphibien und Reptilien des Hochgebirges im Retezat-Massiv wurden bisher kaum untersucht. Alpenmolch *(Triturus alpestris),* Grasfrosch *(Rana temporaria)* und Bergeidechse *(Lacerta vivipara)* werden aus dem Retezat ohne genauere Fundortangabe erwähnt [1, 4, 5]. Für die Kreuzotter *(Vipera berus),* welche in den Transsilvanischen Alpen zu Hause ist [8], sind nur zwei genaue Fundorte angegeben [5]. Bestandsaufnahmen und Gesamtbiomasse (GB)-Schätzungen fehlen jedoch.

Hier wird versucht die kleinräumige Verbreitung von Amphibien und Reptilien im oberen Ökoton des Nadelwaldes, im Krummholz und im Felsengelände darzustellen. Das Material wurde in den Jahren 1984—1986 von dem zweiten Autor gesammelt. Es ist im Kreismuseum Deva aufbewahrt. Alle Schätzungen der Belastung der Standorts durch die Population wurden auf eine Fläche von 250 m^2 bezogen, welche die Aufnahmefläche darstellt.

Triturus alpestris (Laur.). Laut unserem Material, steigt die Häufigkeit des Alphenmolchs mit der Höhenlage an. In der Valea Soarbele, innerhalb der Nadelwaldstufe bei 1 250 m ü. M. wurden 3 Exemplare gefangen. Am Grenzfichtenwald (Ökoton-Wald [2]) bei 1 550 m ü. M.,. oberhalb der Stîna Soarbele wurden 11 Exemplare gesammelt (GB= 32,5 g), Viel höher, im Gletschersee Täul Soarbele bei 1 700 m ü. M., wurden 22 Stücke gesammelt (GB=93,5 g). Alle Fundorte liegen im Bereich der Kalkschiefer.

Rana temporaria L. An und oberhalb der Waldgrenze ist der Grasfrosch keine häufige Art. Seine Bestände sind gering. Auf der Strecke von Valea Soarbele bis Stîna Scorota wurden nur 2 Exemplare erbeutet (GB=52,5 g). Die Standortbelastung (SB) ist ebenfalls gering: 0,21 $g.m^{-2}$. Im Kalkschiefergebiet konnten wir keine weiteren Grasfrösche finden.

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Dagegen ist der Grasfrosch auf metamorphen Graniten viel häufiger. Im Krummholz von Legföhren *(Pinus montana)* in der Valea Judele bei 1 900 m ü. M. wurden 3 Stücke erbeutet (GB=186 g, SB=0,74 g.m^{-2}). Im Glazialkar Căldarea Bucura (2 041 m ü. M.) wurden weitere 6 Stücke gesammelt, darunter 3 Erwachsene und 3 Jungfrösche mit einer GB=183,4 g und SB=0,73 g.m^{-2}.

Bombina variegata (L.). Die Gelbbauchunke wurde bis jetzt im Hochgebirge des Retezat-Massivs und auch anderer Gebirgsmassiven von Siebenbürgen nicht vorgefunden. Der höchstgelegene, bisher festgestellte Fundort in Siebenbürgen ist „Băile Puciosu" (Büdös Fürdö) in den Ostkarpaten bei 1 300 m ü. M. [11]. In der Hohen Tatra (ČSSR) erreicht die obere Grenze der Gelbbauchunke 1 450 m ü. M. [7]. Der hier gemeldete Fundort liegt zwischen 1 650—1 700 m ü. M. in der Nähe der Stîna Soarbele im Kalkschiefergebiet, wo insgesamt, am 26.6.1986, 34 Stücke gesammelt wurden (GB=198 g; SB=0,79 g.m^{-2}).

Lacerta vivipara Jacquin. Im Kalkschieferbereich des Hochgebirges wurden im April 1986 insgesamt 9 Stücke (GB=42,5 g; SB=0,17 g.m^{-2}) in der Valea Iarului im Ökoton Fichtenwald/subalpiner Rasen bei etwa 1 500—1 600 m erbeutet. Im Bereich des Nationalparks Retezat, auf metamorphem Granit-Substrat scheint die Bergeidechse viel spärlicher zu sein. In der Valea Rovine, bei 1 950—2 000 m ü. M. im Krummholz von Legföhren wurden nur 4 Jungtiere erbeutet (GB=12,5 g; SB=0,05 g.m^{-2}). Auf ähnlichem lithologischen Substrat, in der Valea Judele bei etwa 1 900 m ü. M., an der oberen Grenze von Legföhren wurden 12 Jungtiere gesammelt (GB=14,9 g; SB=0,05 g.m^{-2}).

Vipera berus (L.). Im Hochgebirge des Retezats konnten wir die Kreuzotter nicht auffinden. Die Art kommt aber im Nationalpark Retezat auf Böden, welche auf metamorphen Graniten liegen, vor, wo sie häufig zu sein scheint und bedeutende produktionsbiologische Leistungen aufweist (Tabelle 1).

Tabelle 1

Produktionsbiologische Charakteristika der Kreuzotter (Vipera berus) **aus der subalpinen Zone im Nationalpark Retezat**

No.	Standort	m ü. M.	Gewicht (g)	Gewicht des Fettkörpers (g)	Bemerkungen
1 ♀	Ökoton Krummholz/nackter Felsen; Aug. 1984	1 950	127	9,5	in Flüssigkeit konserviertes Material
2♂ Juv.	ibidem	1 500	15,5	—	idem
3 ♂	Schutzhütte Pietrele, am Fichtenwaldrand; Sept. 1985	1 450	42,5	—	idem
4 ♀	Schutzhütte Gemene, am Fichtenwaldrand; Aug. 1985	1 770	61,5	—	idem
5 ♀	Wissenschaftliches Reservat, am Fichtenwaldrand; Aug. 1986	etwa 1 700	34		lebendiges Material
6 ♀	idibem	etwa 1 700	62,5		idem; melanotische Form

Diskussion. Im Hochgebirge des Retezats bilden Amphibien und Reptilien keine grossen Bestände. Diese Tatsache scheint nicht von dem rauhen Hochgebirgsklima, sondern von den orographischen Verhältnissen bedingt zu sein. Die Vegetationsbedeckung ist weithin von Schutthalden und Blockmeeren im Bereich der oberen Waldgrenze und in der subalpinen Zone verhindert [9]. Daraus folgt, dass auch das Vorhandensein geeigneter Standorte für Amphibien und Reptilien spärlich wird. In dieser Kampfzone der Bäume und des Waldes gegen rauhe Klima-Bedingungen [12] finden Amphibien suboptimale bzw. relativ Pessimum-Bedingungen der Standortsgestaltung, obwohl das rauhe Klima vom subarktischen Gepräge keine unüberwindbare Schranke für diese poikilothermen Wirbeltiere darstellt.

Im Gebirgszug des Urals erreicht dagegen *R. temporaria* an der oberen Waldgrenze hohe Individuenzahlen [10]. Im Sarek-Hochplateau (Schwedisch-Lappland) ist *R. temporaria* im Birkengürtel häufig [3]. Der Reichtum an stehenden Gewässern begünstigt dort das Populationswachstum des Grasfrosches, eine Bedingung, die im Hochgebirge des Retezats fehlt.

Vergleichende Angaben über Individuendichten von *L. vivipara* und *V. berus* aus dem Hohen Norden sind uns nicht bekannt.

Die Bindung von Amphibien und Reptilien an gewisse Substrate ist im Hochgebirge des Retezats stark ausgeprägt. Grasfrosch und Bergeidechse sind demgemäss als gesteinsgebundene Tiere im Sinne von Holdhaus [6] zu bezeichnen.

Schlussfolgerungen. Die Artenliste der Hochgebirgsformen von Amphibien und Reptilien ist für das Retezat und die Südkarpaten im Allgemeinen mit der Gelbbauchunke *(B. variegata)* zu bereichern. Kleinräumige Verbreitung ist im Hochgebirge des Retezats durch die Standortsbeschaffenheit bzw. das lithologische Substrat (Klakschiefer und metamorphe Granite) und nicht als Folge von klimatischen Bedingungen zu erklären. Die geringen Bestände von Amphibien und Reptilien sind auf lokale orographische Verhältnisse (Schutthalden, Blockmeere) zurückzuführen.

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VARIATION OF THE CONTENT OF FREE AMINO ACIDS IN ERI SILKWORM *(PHILOSAMIA RICINI)* PUPAE DURING DIAPAUSE AS INFLUENCED BY THE TREATMENT OF LARVAE WITH KERATROF

MANUELA DORDEA*, LIVIU FLOCA* and TIBERIU PERSECĂ*

SUMMARY. — Eri silkworm *(Philosamia ricini)* pupae, derived from keratrof-treated larvae, were kept at 4±1°C and sacrificed at the beginning, middle and last part of the diapause. Haemolymph, fat body, tegument and ovary were analyzed to determine their free amino acid content.

In haemolymph, the content of free amino acids was low initially, rose slightly at the middle of the diapause and decreased evidently at its end and during emergency. This reflects the succession of metabolic processes connected with histolysis and, afterwards, with the genesis of adult tissues (ovary, allar buds). The great amounts of the fibroin-forming amino acids serine, glutamic acid, phenylalanine-leucine and glycine in haemolymph suggest that the metabolic chains of larvae are maintained during tne pupal diapause, too.

The great amounts of arginine, histidine and phenylalanine-leucine in tegument during the entire pupal diapause make it possible to assume that the tegument may represent a storage organ for amino acids.

The treatment with keratrof, which induces, during larval development, an increase in the biosynthesis of silk proteins, does not influence significantly the protein metabolism in pupae and the genesis of adult tissues.

Owing to the great economic interest for sericulture, promoted nowadays by the introduction of some new species of silk-producing *Saturniidae, Philosamia ricini* became, in our country, the object of various scientific investigations with both theoretical and practical importance. Some investigations were focussed on the stimulation of silk synthesis by treating larvae with different biostimulators [2, 3]. The treatment of larvae with keratrof (hydrolyzate of a scleroprotein analogous to silk fibroin) resulted in larger amounts of fibre materials. One of the factors determining the effect of keratrof is the evident increase in the content of free amino acids (FAA), especially of the fibroin-forming ones, in the silkgland of Eri silkworm, this increase being accompanied by a decrease in the FAA content of the haemolymph.

The present study aims at establishing the influence of the treatment of larvae with keratrof on the variation of FAA during pupal development and diapause.

Material and methods. Eri silkworm *(Philosamia ricini)* pupae, derived from keratrof-treated larvae, were used. Unlike *Bombyx mori* that undergoes an egg diapause, *Philosamia ricini* undergoes a pupal diapause. Egg hatching and larval development proceeded in laboratory under special standard conditions [1]. The

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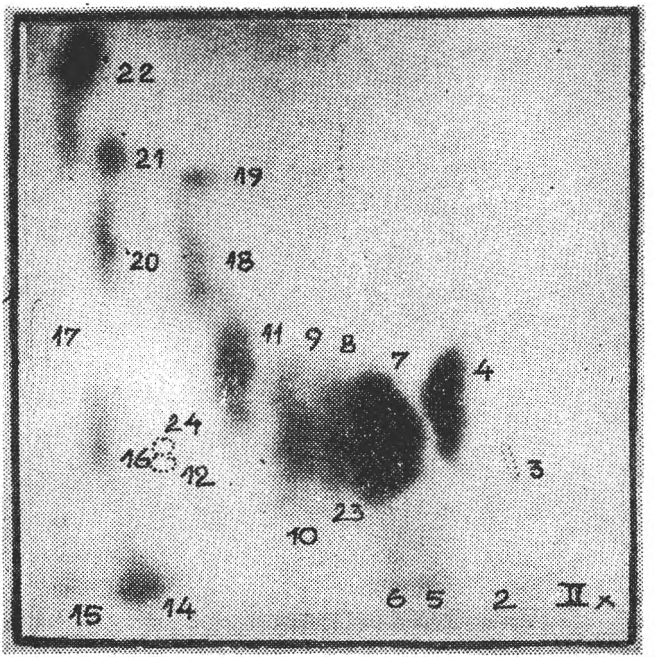

Fig. 1. *FAA from haemolymph in control pupae at the beginning of diapause.*

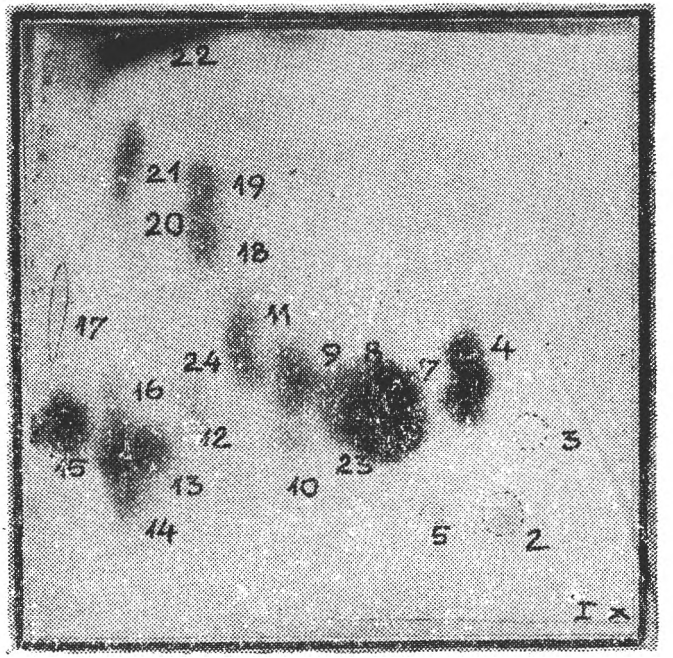

Fig. 2. *FAA from* **haemolymph** *in pupae after treatment* **with** *keratrof, at the beginning of diapause.*

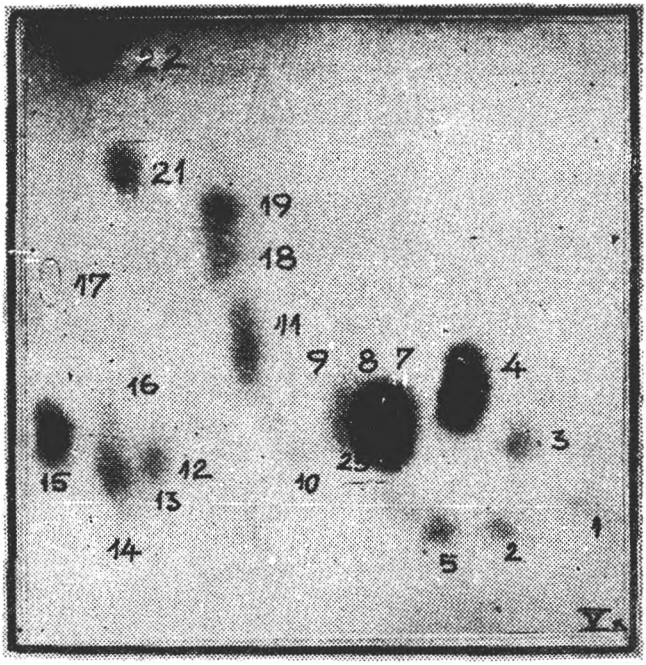

Fig. 3. *FAA from fat body in control pupae at the beginning of diapause.*

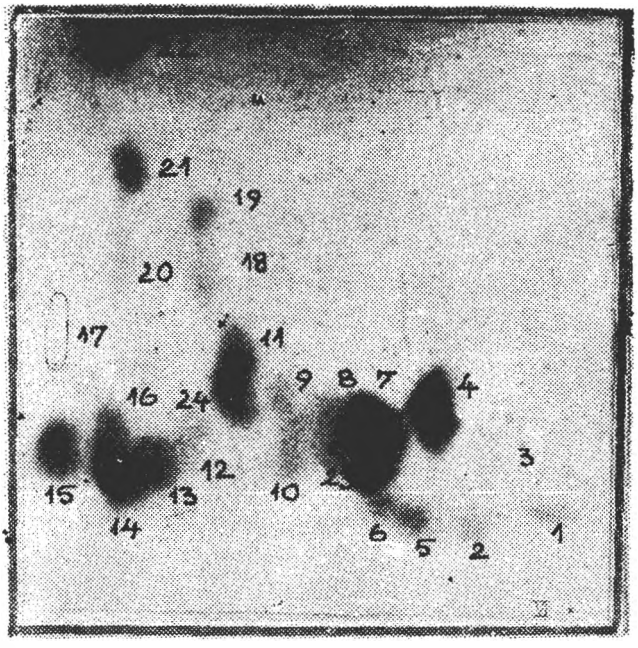

Fig. 4. *FAA from fat body in pupae after treatment with* **kera-***trof. at the beginning of diapause.*

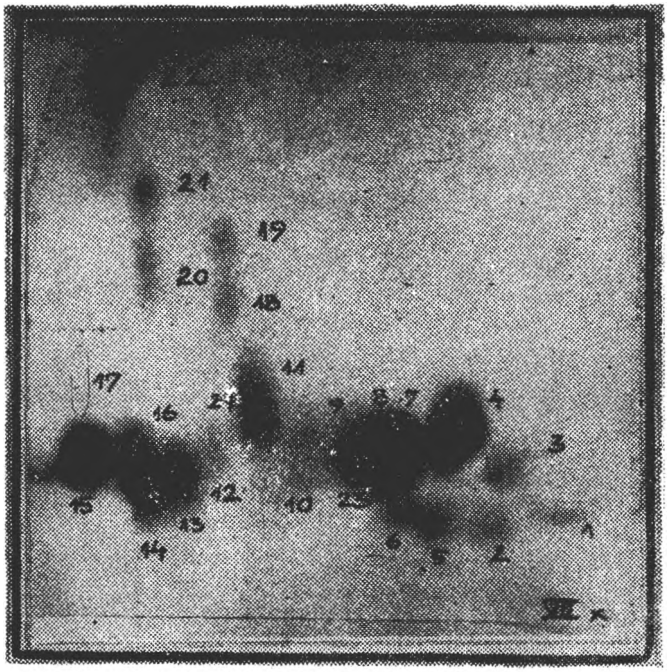

Fig. 5. *FAA from tegument in control pupae at the beginning of diapause.*

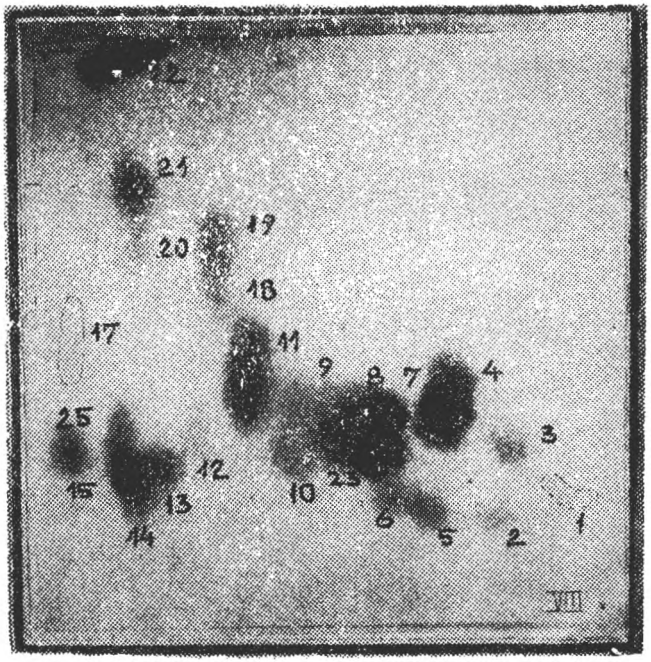

Fig. 6. *FAA from tegument in pupae after treatment with keratrof, at the beginning of diapause.*

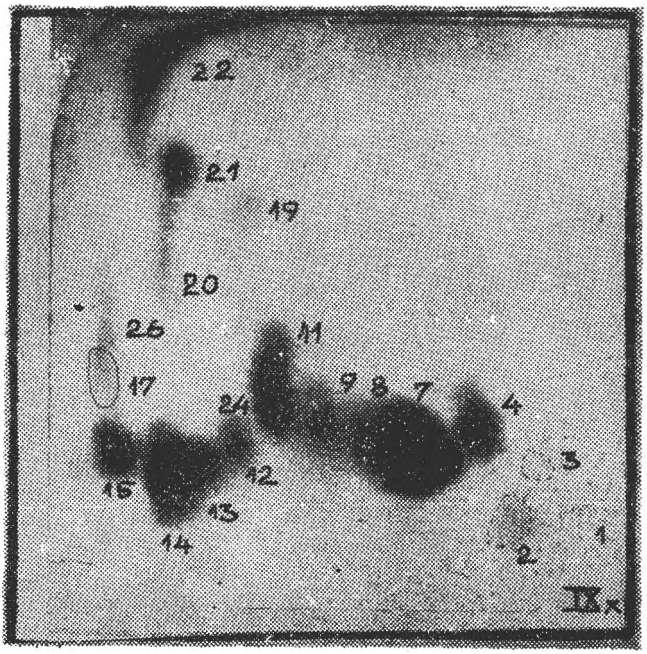

Fig. 7. *FAA from haemolymph in control pupae at the middle of diapause.*

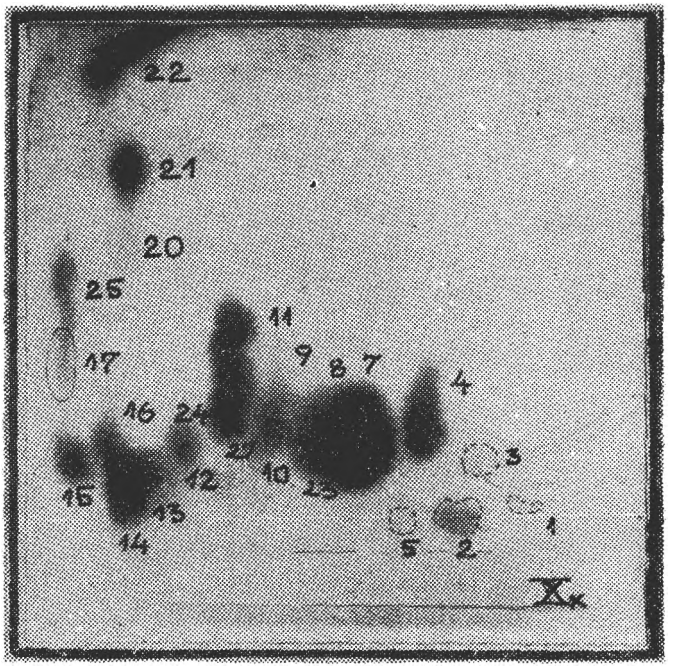

Fig. 8. *FAA from haemolymph in pupae after treatment with kevatrof, at the middle of diapause.*

keratrof solution was sprayed on larvae as well as on leaves, the tegument adsorption being the most important way of entering the body. Pupae were kept at 4±1°C and sacrificed at the beginning, middle and last part of the diapause. Imagoes, after emergency, were also tested.

The FAA patterns in haemolymph, fat body, tegument and ovary were determined by applying the paper chromatographic methods described elsewhere [7]. Qualitative and quantitative analyses were performed by using standard chromatograms for comparison.

Results and discussion. At the beginning of pupal diapause, a variable pattern of FAA was recorded in all tissues. Serine, glutamic acid, phenylalanine-leucine and alanine had high levels in haemolymph (Fig. 1). The treatment with keratrof did not alter qualitatively the pattern of FAA, but decreased their quantity, excepting that of arginine, ornithine and lysine (Fig. 2).

The fat body was richer in FAA after keratrof treatment (Fig. 4) as compared with the control (Fig. 3). Large amounts of ornithine, lysine, arginine and phenylalanine-leucine were recorded. γ-Aminobutyric acid and threonine, present in small amounts in treated pupae, were absent or appeared as traces in the control ones (Fig. 3).

No appreciable changes occurred in the FAA pattern of the tegument. Nevertheless, the amounts of ornithine, lysine, arginine and histidine were larger in the control (Fig. 5) than in the treated pupae (Fig. 6).

Biochemical analyses on ovaries at the beginning of pupal development could not be performed because at this stage they are not differentiated.

During the middle of pupal diapause, the FAA content slightly increased in all studied tissues. The haemolymph from both control (Fig. 7) and treated pupae (Fig. 8) was rich in most FAA, especially in serine, glutamic acid, alanine and phenylalanine-leucine. But smaller amounts of ornithine, lysine, arginine and tyrosine were noticed after keratrof treatment.

In the fat body (Fig. 9), the majority of FAA were found in greater quantities during the middle of pupal diapause than at its beginning (Fig. 3). The same great quantities of FAA, especially of phenylalanine-leucine, methionine-valine and serine were recorded also in pupae after keratrof treatment (Fig. 10). The absence of γ-aminobutyric acid was noticed in both control and treated pupae. Amounts of ornithine, arginine und lysine were smaller in the treated pupae (Fig. 10) than in the control ones (Fig. 9).

In the control pupae (Fig. 11), in comparison with the treated ones (Fig. 12), the tegument contained larger amounts of most amino acids, mainly of serine, lysine, ornithine, glutamic acid and phenylalanine-leucine.

As diapause proceeds, histogenesis takes place in ovaries Their FAA content was high (Fig. 13). The keratrof treatment diminished it significantly (Fig. 14).

During the last stage of diapause, the FAA content decreased evidently in haemolymph, but rose in tegument, fat body and ovary

(Fig. 15). At the level of the sample size applied on chromatographic papers we could not detect methionine, valine, tyrosine, γ-aminobutyric acid and proline in the haemolymph of newly emerged flies (Fig. 16).

Our results are in agreement with those of Pant and Agrawal [4, 5] and Pant and Lacy [6], who also found great amounts of serine, glutamic acid, alanine, phenylalanine-leucine and histidine in haemolymph during pupal development. But, unlike them, we could not detect asparagine, hydroxyproline and citruline. The FAA pattern of tissues was comparable with that of haemolymph, although, in contrast with the observations by Pant and Lacy [6], we could not record taurine and great quantities of proline.

It is evident from our findings that in haemolymph the FAA content is low initially, rises slightly at the middle of the diapause and decreases evidently at its end and during emergency. This reflects the succession of metabolic processes connected with histolysis and, afterwards, with the genesis of adult tissues (ovary, allar buds). Histolysis leads to an increase in the FAA content of haemolymph. The FAA are then utilized for the synthesis of tissue proteins of the imago. The great amounts of the fibroin-forming amino acids serine, glutamic acid, phenylalanine-leucine and glycine in haemolymph suggest that the metabolic chains of larvae are maintained during the pupal diapause, too.

The great amounts of arginine, histidine, lysine and phenylalanine-leucine in tegument during the entire pupal diapause make it possible to assume that the tegument may represent a storage organ for amino acids.

The treatment with keratrof, which induces, during larval development, an increase in the biosynthesis of silk proteins, does not influence significantly the protein metabolism in pupae and the genesis of adult tissues. However, the viability and the degree of hatching of the eggs layed by treated flies remain to be studied.

Conclusions. 1. Variation of FAA during pupal diapause reflects the succession of metabolic processes connected with histolysis and, afterwards, with the genesis of adult tissues.

2. Treatment of larvae with keratrof does not influence significantly the protein metabolism in pupae and the genesis of adult tissues.

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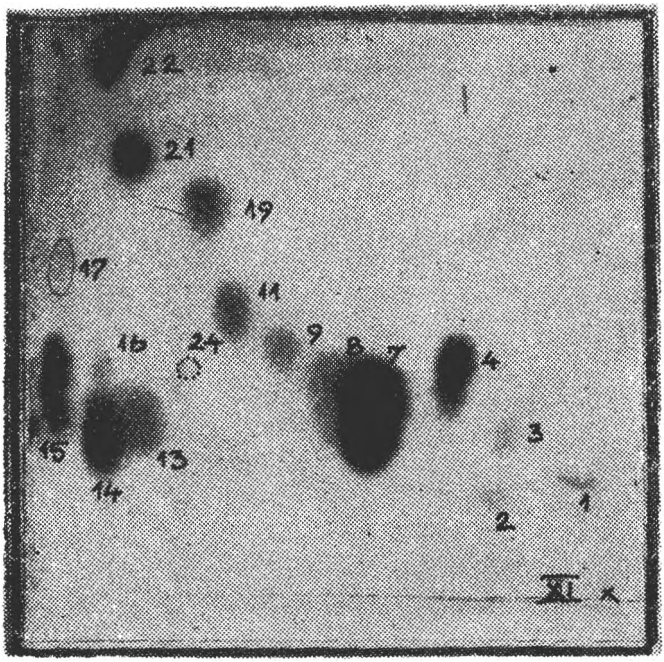

Fig. 9. *FAA from fat body in control pupae at the middle of diapause.*

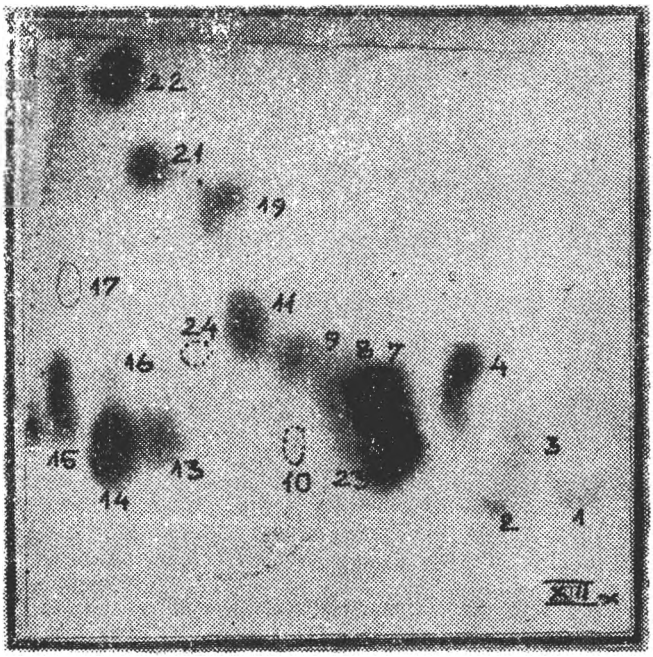

Fig. 10. *FAA from fat body in pupae after treatment with keratrof, at the middle of diapause.*

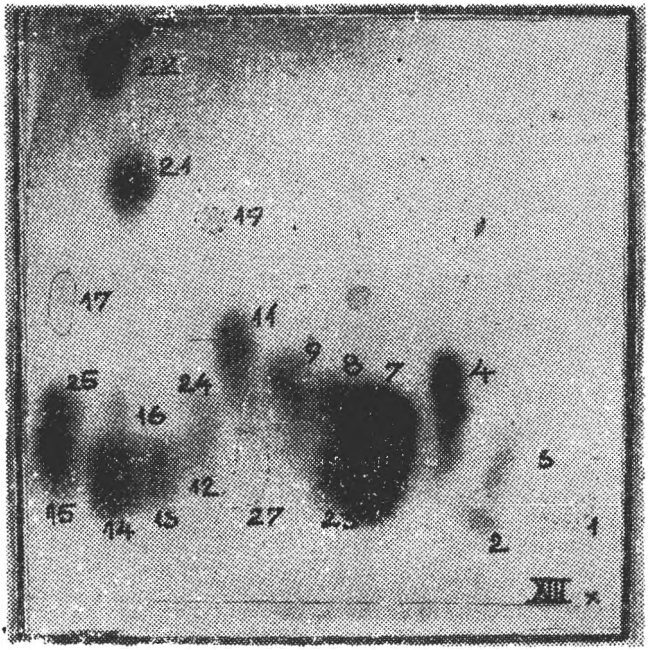

Fig. 11. *FAA from tegument in control pupae at the middle of diapause.*

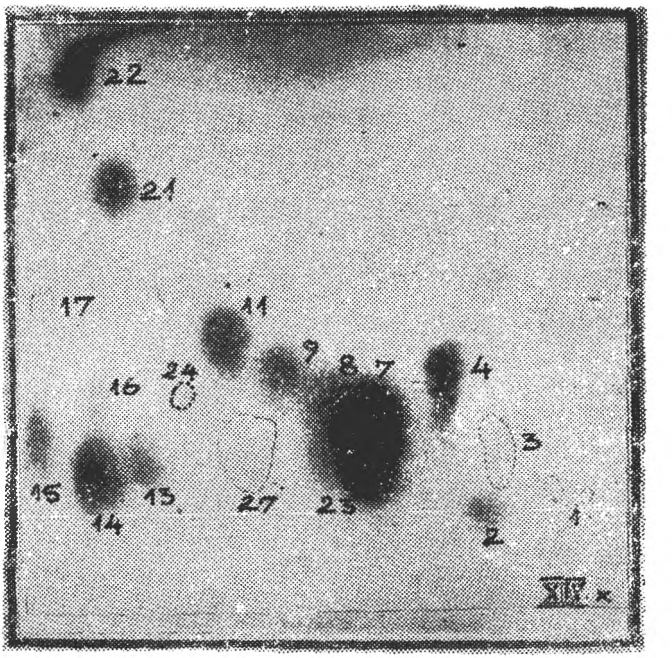

Fig. 12. *FAA from tegument in pupae after treatment with keratrof, at the middle of diapause.*

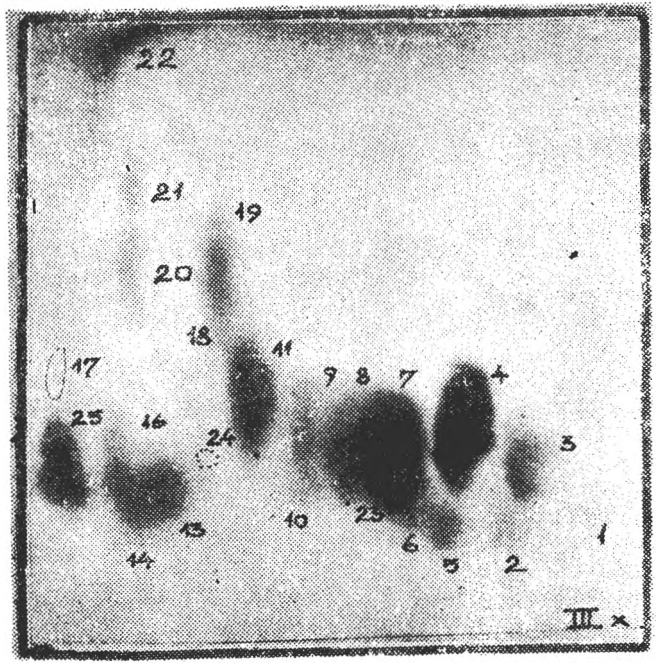

Fig. 13. *FAA from ovary in control pupae at the middle of diapause.*

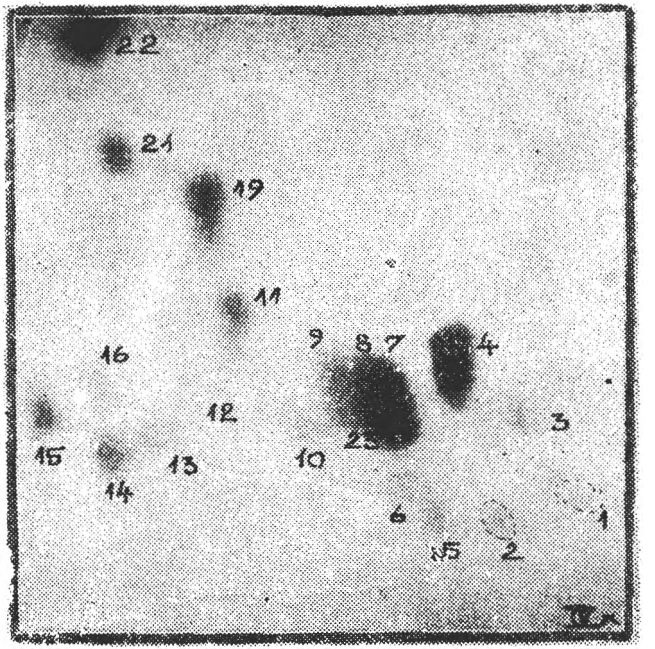

Fig. 14. *FAA from ovary in pupae after treatment with keratrof, at the middle of diapause.*
1 — Cysteic acid. 2 — Cystathionine. 3 — Aspartic acid. 4 — Glutamic acid. 5 — Cystine 6 — ? 7 — Serine. 8 — Glycine. 9 — Threonine. 10 — ? 11 — Alanine. 12 — Glucosamine. 13 — Ornithine. 14 - Lysine. 15 — Arginine. 16 — Histidine. 17 — Proline 18 - ? 19 — Tyrosine. 20 — γ-Aminobutyric acid. 21 — Methionine + valine. 22 — Phenylalanine + leucine. 23 — Taurine. 24—27 — ?

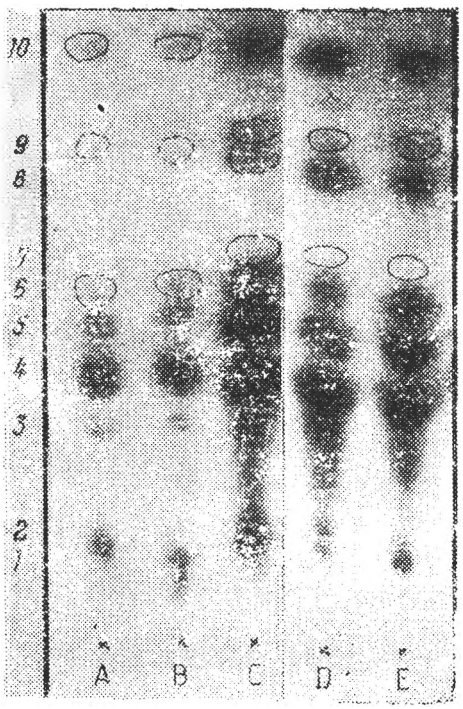

Fig. 15. *FAA in pupae at the last stage of diapause.*
A, B — Haemolyph. C — Tegument. D — Fat body. E — Ovary.

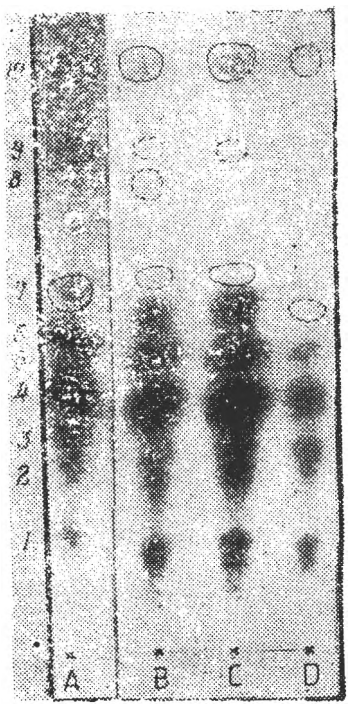

Fig. 16. *FAA in pupae after emergency.*
A — Ovary. B — Fat body. C — Tegument. D — Haemolymph. 1 — Cysteic acid. 2 — Lysine + ornithine. 3 — Histidine + arginine. 4 — Glutamic acid + serine. 5 — Aspartic acid + glycine + threonine. 6 — Alanine. 7 — Proline. 8 — Tyrosine + γ-aminobutyric acid. 9 — Methionine + valine. 10 — Phenylalanine + leucine.

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DYNAMICS OF TOTAL PROTEIN, TOTAL LIPID AND GLYCOGEN CONTENTS DURING DEVELOPMENTAL CYCLE IN BEE WORKERS (*APIS MELLIFICA* L.)

PANTE GHERGHEL*

SUMMARY. — Total protein, total lipid and glycogen contents from the whole body during developmental cycle in bee workers increase from the egg stage until the 6th day of the larval stage, after which they decrease continuously until the workers are ready to perform tasks outside the beehive. From the three categories of substances, proteins are predominant, followed by lipids and glycogen, with the exception of the 5th and 6th day of the larval stage, when the lipid content is a little higher than that of the proteins. The glycogen appears as a storage substance in the second half of the larval stage and is exhausted during the pupal stage. Adult bee workers do not store glycogen, although their food is predominantly glucose and fructose.

After investigating, in two previous works, the dynamics of certain biochemical parameters from the whole body, during the ontogeny of the Colorado beetle (*Leptinotarsa decemlineata* Say) [2] and of the oak hairy caterpillar (*Lymantria dispar* L.) [3], we have selected in the present work the bee workers, as representatives of the *Hymenoptera*, because, although belonging to one of the best known and economically most important group of insects, they have not been characterized in this respect.

Material and method. The biological material was obtained from a strong bee family, containing approximately 50,000 individuals. In order to have the exact age of the individuals taken under study, the honeycombs and the date of the egg depositions were marked. The experiments took place in august 1986.

Lipid extraction was done with chloroform-methanol (2 : 1) as described by Folch *et al.* [1] and the determination was performed with the phosphovanilinic reagent, by the method of Zollner and Kirsch [7]. Proteins were extracted by precipitation with 10% trichloroacetic acid and determined from a 1 N NaOH solution by the method of Lowry *et al.* [4]. Glycogen was determined on the basis of the anthrone reaction, as described by Roe and Dailey [5].

The moments of the developmental cycle in which the biochemical analyses were performed are indicated in Table 1. Each result is an average of at least 4 parallel samples, each containing from 3 to 20 individuals, depending on the developmental stage and insect age.

Results and discussion. It results from Table 1 that over a period of only 6 days of the postembryonal development the body weight increases from 0.185 mg, characteristic for an egg or a recently hatched larva, up to 155 mg, characteristic for a fully developed larva, at the end of the feeding period. In other words, a fully grown larva is about 838 times heavier than a recently hatched one. Such an intensive growth during such a short interval is possible due to the special nutritional

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Table 1

Dynamics of the total protein, total lipid and glycogen contents during the developmental cycle of bee workers
(Apis mellifica L.)

Developmental stage	Day	Wet weight		Content								
				Proteins			Lipids			Glycogen		
		mg/individual	+%*	mg/individual	+%	%**	mg/individual	+%	%	mg/individual	+%	%
Egg	1	0.185±0.012	100	0.020±0.004	100	10.75	0.013±0.003	100	6.98	0	0	0
Larval	2	3.05±0.02	1615	0.457±0.01	2285	15.20	0.193±0.10	1484	6.42	0	0	0
	3	29.30±0.51	15752	3.356±0.10	16780	11,45	2.171±0.02	16700	7.40	0	0	0
	5	131.00±6.27	70430	15.340±1.35	76700	11.71	16.569±1.15	127453	12.64	7.173±0.55	0	5.47
	6	155.12±1.15	88397	15.512±1.15	77560	10.00	16.976±3.84	130584	10.94	14.212±0.38	0	9.16
Pupal	1	138.00±6.00	74193	15.405±0.71	77025	11.16	12.140±0.74	93384	8.79	5.213±0.39	0	3.77
Adult	1	123.20±1.12	66236	14.150±0.53	70750	11.48	6.079±0.61	46761	4.93	0	0	0
	20	80.32±1.00	43182	11.062±0.22	55310	13.77	8.409±0.20	64684	10.46	0	0	0

* — With respect to egg. ** — From the net weight.

qualities possessed by the royal jelly, on which the worker larvae are fed during the first 3 days of the larval stage, as well as to the honey and beebread used until the end of the feeding period. Such an intense growth is due also to the fact that the larvae do not have to make any effort to find the food, which is abundantly present in the honeycomb cells, where the development takes place. Although belonging to invertebrates, the bees are among those animal species which take care of their progeny in an optimal way. Because the bee larvae „float into a nutritive bath" which, among others, contains much glucose and fructose, we believe that the intimate mechanism of the penetration of these substances into their organism deserves a special study.

The body weight of a 1 day old bee worker as compared to the fully grown larvae is 21% smaller. This decrease can be explained by the loss of moulting, larval and pupal exuviae and by the energy consumption. It is somehow surprising that the 20 days old bee workers, which leave the beehive in search for nectar and pollen, have a smaller body weight than immediately after emergence. Probably, during the 20 days in which the workers perform a series of activities within the bee family, feeding on honey and beebread, a certain „restructuring" takes place in their organisms, assuring an „optimal condition" for their future activity outside the beehive. Beside the fat body, we would like to point (among such changes) to the getting into function of a new and important metabolic centre, that is represented by the wing muscles, where intense metabolic reactions occur, which liberate the energy needed for muscle contractions.

The protein content per individual, from the egg to the 6 days old larva, increases after an ascending curve from 0.020 to 15.512 mg, after which it diminishes continuously until the workers begin their activity outside the behive. Expressed as percentage, the protein content along the developmental cycle oscillates between 10 and 15.20%, the average value being 11.94%. Thus, from the three categories of substances (proteins, lipids and glycogen) the protein content varies within the narrowest limits, its level being maintained almost constant during the entire developmental cycle.

The lipid content per individual evolves after a curve similar to that of the protein content, oscillating between 0.013 and 16.976 mg/individual. In the last two days of the larval feeding the lipid content exceeds the protein content by little, although the food of the larvae is poor in lipids. Such a massive accumulation of lipids is possible as a consequence of a very intense conversion of carbohydrates (glucose and fructose), which predominate in the food. During the pupal stage the lipid content decreases from 12.140 to 6.079 mg/individual. In this stage the lipids are widely utilized as an energy source in the process of organ morphogenesis of the adult individuals. Expressed as percentage, the lipid content oscillates between 4.93 and 12.64%, which are much larger limits than in the case of the proteins.

By the methods we employed only glycogen was detected during the second part of the larval stage, when it accumulates rapidly, reaching

14.212 mg/individual on the 6th day, a value close to the protein and lipid contents. But, during the pupal stage, the glycogen is utilized intensely along with the lipids as an energy source, so that it cannot be found in recently emerged individuals or in the 20 day old bees.

As it results from the data presented above, the largest accumulations of substances, during the entire developmental cycle, occur at the end of the larval stage. This is also valid for the oak hairy caterpillar (*Lymantria dispar* L.) [3], but this does not feed as an adult whereas the bee workers take up large quantities of food. The Colorado beetle (*Leptinotarsa decemlineata* Say), which in the adult stage still uses much food, accumulates during this stage the largest quantity of proteins and lipids from the entire developmental cycle [2]. From the three species studied so far, no one stores important glycogen reserves as an adult, whereas in all three species glycogen reaches its highest content at the end of the larval stage. Probably, in the adult stage, the carbohydrate reserves are represented to a large extent by trehalose, which is very characteristic for the insect haemolymph [6].

Conclusions. 1. The total protein, total lipid and glycogen contents from the whole body of the bee workers increase from the egg stage until the 6th day of the larval stage, after which they decrease continuously until the workers are ready to perform tasks outside the beehive.

2. From the three categories of substances, proteins are predominant, followed by lipids and glycogen, with the exception of the 5th and 6th day of the larval stage, when the lipid content is a little higher than that of the proteins.

3. The glycogen appears as a storage substance in the second half of the larval stage and is exhausted during the pupal stage. Adult bee workers do not store glycogen, although their food is predominantly glucose and fructose.

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PROTEIN METABOLISM AND GROWTH RATE IN CHICKENS FED ON DIETS CONTAINING DIFFERENT AMOUNTS OF *SPIRULINA*

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SUMMARY. — Young chickens were fed on diets containing *Spirulina* in different amounts. The growth rate and metabolic effects of the diets were determined. The results indicate a more marked effect of 2 and 4% *Spirulina* diets compared to 6% *Spirulina* diet. After a first decrease in growth, the 2 and 4% *Spirulina* diets resulted in an increased protein synthesis, reduced amino acid oxidation and increased growth rate, which were not observed in the case of the 6% *Spirulina* diet. Stopping the treatment, an enhanced growth occurred in the case of the 6% *Spirulina* diet but not in the case of the 2 and 4% *Spirulina* diets.

Cyanobacteria of the genus *Spirulina* have high concentrations of protein: 50—60% of the wet weight [2]. Although they contain low concentrations of some essential amino acids (lysine and histidine [5]), an attempt to substitute some traditional protein sources with *Spirulina*, in the food of different animal species, was made. In rats the *Spirulina* diet, supplemented with essential amino acids, had similar efficiency as to the traditional one [5]. In chickens the *Spirulina* content of the food must not exceed 10%; when the content of *Spirulina* was higher, a lowering in growth was noticed [1].

In this paper we report some metabolic effects of *Spirulina*-supplemented chicken diets, and the correlation of these effects with the growth rate.

Materials and methods. The experiments started on chickens aged 14 days. The animals were divided into 4 groups: control group (C) fed on a standard diet; group 6S fed on standard diet containing 6% *Spirulina;* group 4S fed on standard diet supplemented with 4% *Spirulina;* group 2Se fed on standard diet containing 2% *Spirulina* and 0.5% Eridiarom (plant extract against diarrhoea). Beginning with the 45th day the *Spirulina* treatment was stopped, and all experimental groups returned to the standard diet. The growth of chickens was followed to the age of 64 days.

Metabolic determinations were performed on chickens aged 45 days. The following parameters were determined: the concentration of myofibrillar, sarcoplasmic and connective proteins [3], the total protein content (sum of the former three); the oxidation rate of leucine, the synthesis rate of protein from leucine and glucose, all three radiobiochemically. All determinations were performed on muscle tissue (musculus pectoralis major).

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The oxidation rate of leucine and the synthesis rate of proteins from leucine were determined by incubation of tissue slices in labelled leucine solution (2×10^5 dpm per ml, with ^{14}C-leucine) containing blood plasma. The synthesis rate of proteins from glucose was determined by incubation with labelled glucose (2×10^5 dpm per ml with (U-^{14}C) glucose) in Krebs-Henseleit phosphate buffer. The oxidation rate of leucine was evaluated based on the radioactivity of CO_2 (dpm of CO_2 per mg protein), the synthesis rate of protein based on radioactivity of proteins (dpm per mg, specific radioactivity).

The radioactivity measurements were done in a BF-5003 liquid scintillation spectrometer (Berthold, Wildbad, Federal Republic of Germany), a PPO-POPOP mixture being used as scintillator.

The statistic evaluation of differences between means was made according to the paired Student test. Aberrant individual values were previously eliminated according to Chauvenet's criterion.

Results. Changes in the growth rate of experimental groups are summarized in Table 1. We found a significantly enhanced growth rate during the first 10 days of treatment in all experimental groups as compared to group C. In the next 10 days we noticed a marked decrease in growth rate in groups 2Se and 4S, but no change occurred in group 6S. In the last 10 days of treatment a significant increase in growth rate occurred in groups 2Se and 4S without changes in group 6S. When the treatment was stopped, the modifications were as follows: group 6S had a significantly increased rate of growth, group 4S showed a slight increase in this rate ($0.1 > p > 0.05$), the modifications in group 2Se were clearly not significant ($p > 0.25$).

The weight of 45 days old chickens and the results of metabolic determinations are summarized in Table 2. The chickens of group 2Se had slightly smaller weight than the chickens of group C ($0.1 > p > 0.05$). The total protein content of the muscle was slightly decreased in group 2Se ($0.1 > p > 0.05$). The sarcoplasmic protein content was not changed in experimental groups, but myofibrillar protein content was significantly lower in group 2Se. The connective protein fraction showed significantly increased levels in groups 2Se and 4S. The radioactivity of CO_2 origi-

Table 1

Evolution of growth rate in chickens fed on Spirulina diets

Period (days)	Experimental groups			
	C	6S	4S	2Se
14–24	18.67±0.41 (19)	22.69±0.49** (20)	22.85±0.58** (20)	20.48±0.52* (20)
25–34	20.83±0.66 (19)	20.77±0.73 (20)	15.77±0.92** (20)	13.93±1.11** (20)
35–44	24.86±1.84 (14)	25.70±0.84 (15)	30.83±1.65* (15)	33.37±1.06** (15)
45–64	19.08±1.65 (14)	25.41±1.07* (15)	22.86±1.03 (15)	16.75±2.40 (15)

Values (means±SE) are given in g weight gain per day per individual.
* — Significant difference at $p < 0.5$. ** — Significant difference at $p < 0.001$.
The number of animals is given in brackets.

Table 2

Protein metabolism in chickens fed on Spirulina diets

Metabolic parameters	Experimental groups			
	C	6S	4S	2Se
Protein content	265±9.2 (8)	264.6±8.1 (7)	250±9.2 (8)	239.2±9.7 (8)
Sarcoplasmic proteins	108±7.3 (8)	105.6±5.6 (7)	107.3±3.3 (8)	100.5±4.1 (8)
Myofibrillar proteins	135.1±5.1 (8)	140.7±5.7 (7)	115.1±9.2 (8)	111.9±1.9** (8)
Connective proteins	21.5±2.4 (8)	18.3±1.4 (7)	27.8±0.7* (8)	27.72±0.6* (8)
Radioactivity of CO_2	3.92±0.13 (6)	3.43±0.01* (6)	1.39± 0.05** (6)	2.1±0.07** (6)
Radioactivity of protein in incubation with glucose	16.66±5.1 (4)	13.68±1.07 (5)	20.59±3.44 (6)	16.64±2.21 (5)
Radioactivity of protein in incubation with leucine	4.17±0.6 (6)	3.80±0.91 (6)	5.17±0.85 (5)	8.33±1.43* (5)
Weight	650±39 (5)	709±65 (5)	628±59 (5)	543±30 (5)

Values (means ± SE) are given in mg per g tissue for sarcoplasmic, myofibrillar, connective and total protein contents; in dpm per mg protein for radioactivity of CO_2, radioactivity of protein in incubation with glucose and radioactivity of protein in incubation with leucine; in g for weight.
* — Significant difference at $p < 0.5$. ** — Significant difference at $p < 0.001$.
The number of determinations is given in brackets.

nated from labelled leucine was significantly reduced in all experimental groups as compared to C.

The rate of protein synthesis from glucose showed no significant modifications. The synthesis rate of proteins from leucine was significantly higher in group 2Se; no other statistically significant modifications occurred. In order to estimate whether the oxidation of leucine or the use of leucine in protein synthesis prevails, we calculated a coeffecient according to the following formula:

$$c = \frac{R_{CO_2}}{R_P}$$

where R_{CO_2} is the radioactivity of CO_2 after incubation with labelled leucine, and R^d is the radioactivity of proteins after the same incubation. In groups C and 6S the value of c was close to 1 (0.940±0.138 for C and 0.902±0.212 for 6S), thus the participations of leucine in oxidation and protein synthesis were balanced. In groups 4S and 2Se the values of c were far below 1 (0.268±0.05 for 4S and 0.252±0.041 for 2Se), *i.e.* the participation of leucine in protein synthesis prevailed as compared to leucine oxidation. The differences between group C and groups 4S and 2Se, respectively, are statistically significant ($p < 0.01$ for both groups).

Discussion. We had 4 „static" parameters of protein metabolism in the experiment (the concentrations of protein fractions and the total protein content) and three „dynamic" parameters of the process (the oxidation rate of leucine and the synthesis rate of proteins from glucose and leucine). It is worth mentioning that the rates of leucine oxidation and the use of leucine in protein synthesis are good measures of overall

protein metabolism in muscle [4]. One can hypothesize that the static parameters reflect the result of some processes which had run before the determinations were done, while the dynamic ones show the actual tendency which could indicate a future state of the static parameters.

Taking into account this working hypothesis, the results of the metabolic determinations are correlated with the growth rate. The decreased growth rate of chickens between 25—34 days (groups 4S and 2Se) could be responsible for both the decreased myofibrillar protein content and the increased connective protein content of muscle tissue in these groups. The noticed predominance of protein synthesis in the chickens aged 45 days (groups 4S and 2Se) must be in correlation with the increased rate of the 35—45 days old chickens of the same groups.

The growth rate of chickens and the results of metabolic determinations could be correlated as seen. But why do these modifications appear?

The results indicate a more marked effect of 2 and 4% *Spirulina* diets as compared to the 6% *Spirulina* diet. The group 6S showed a behaviour very similar to that of group C, in almost all cases. We cannot give an explanation of this observation.

Another strange result: after the *Spirulina* diet was stopped and replaced by the standard one, a significant increase of growth rate appeared in group 6S, but no significant changes occurred in groups 4S and 2Se. After a first decrease in growth, the 2 and 4% *Spirulina* diets resulted in an increased protein synthesis, reduced amino acid oxidation and increased growth rate, which were not observed in group 6S. Stopping the treatment, an enhanced growth occurred in group 6S but not in the groups 4S and 2Se. The causes of these modifications could be found by the use in experiments of some *Spirulina* fractions (extracts), because, we think, there are more than one factor involved in the modifications which occurred in the growth rate and the protein metabolism.

Conclusions. A *Spirulina*-supplemented diet induces correlated modifications in the growth rate and the protein metabolism of chickens. The effects seem to be positive from the chick-breeder's point of view, but further research is needed for an explanation of the modifications.

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ENZYMATIC ACTIVITIES OF SOME GREEK FOREST SOILS

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SUMMARY. — Thirteen soil samples were collected from forests located on the Central Continental Plateau of Greece and analyzed enzymologically. Dehydrogenase, invertase, phosphatase, catalase and levanase activities and nonenzymatic H_2O_2-splitting capacity were found to be present in each sample. Levansucrase activity was demonstrated in 9 samples. Dehydrogenase, invertase, catalase, levansucrase and levanase activities were, in general, higher in the organic horizon than in the mineral one, while the reverse was true for phosphatase activity and nonenzymatic H_2O_2-splitting capacity. The nature of the geological formations from which the soils derived and that of the main forest species covering the soils had a strong influence on the level of soil dehydrogenase and invertase activities and a slight influence on the other activities.

There are no literature data available on the enzymatic activity in Greek soils. But one of the soil biological properties, nitrification, was thoroughly studied by Nakos [7—9] in many forest areas of Greece.

The present work aims at determining enzyme activities in some Greek forest soils.

Material and methods. The sampling sites are located on the Central Continental Plateau of Greece. The studied soils, derived from limestone, flysch or metamorphic rock formation, are covered by forests whose main species is *Abies cephalonica*, *Pinus halepensis*, *Fagus silvatica* or *Quercus* sp. A soil under predominantly evergreen Mediterranean vegetation was also studied. The soils were sampled from organic (A_0) and/or mineral (A_1) horizons. In all, 13 samples were taken. A general characterization of the sampling sites and some physico-chemical properties of the soil samples are presented in Table 1***. The air-dried and sieved samples were analyzed to determine their dehydrogenase, invertase, phosphatase and catalase activities and their nonenzymatic H_2O_2-splitting capacity as well as their levansucrase and levanase activities.

Dehydrogenase activity was determined in reaction mixtures without added glucose (actual dehydrogenase activity) and with added glucose (potential dehydrogenase activity) by using the colorimetric method of Casida *et al.* [1]. The activity is expressed as mg triphenylformazan/3 g soil/24 hours at 37°C. Invertase activity was assayed polarimetrically [3], and recorded as difference in optical rotation ($\Delta\alpha°$)/5 g soil/24 hours at 37°C. For the determination of phosphatase activity, the colorimetric method of Krámer and Erdei [6] was used. This activity is given in mg phenol/3 g soil/24 hours at 37°C. Catalase activity and nonenzymatic H_2O_2-splitting capacity were estimated in active and

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Table 1

General characterization of the sampling sites and some physico-chemical properties of the soil samples

Sample number	Sampling site	Geological formation	Slope	Aspect	Main forest species	Horizon	pH	Organic matter (%)
1	Aghia Triada, Attica district	Limestone	40—70	Northern	Abies cephalonica	A_0	6.6	50.23
2	Aghios Petros, Attica district	Limestone	<40	Northern	Abies cephalonica	A_0	6.6	50.23
3	„ „	„ „	„	„ „	„ „	A_1	6.5	11.59
4	Parnitha mountain	Flysch	40—70	Southern	Abies cephalonica	A	6.2	10.55
5	Parnitha mountain	Flysch	<40	Northern	Abies cephalonica	A_0	6.2	48.30
6	„ „	„	„	„ „	„ „	A_1	5.7	3.58
7	Parnitha mountain	Limestone	40—70	Northern	Pinus halepensis	A_0	6.4	55.47
8	Phillis, Attica district	Flysch	40—70	Northern	Pinus halepensis	A_0	5.9	41.41
9	Kissos Magnisias	Metamorphic	40—70	Northern	Fagus silvatica	A_0	4.3	40.71
10	„ „	„ „	„	„ „	„ „	A_1	4.3	5.10
11	Anavra Magnisias	Limestone	40—70	Northern	Quercus sp.	A_0	5.8	28.29
12	„ „	„ „	„	„ „	„ „	A_1	5.7	5.38
13	Skiathos Magnisias	Metamorphic	40—70	Northern	Mediaterranean vegetation, predominantly evergreen	A	6.3	1.87

inactivated (autoclaved) samples, by using a technique based on Kappen's [2] permanganometric method. They are registered as mg H_2O_2/1 g soil/1 hour at 20°C. Levansucrase and levanase activities were studied qualitatively by means of paper chromatography [4, 5]. The presence or absence of activities was noticed after incubating the reaction mixtures with added sucrose and levan, respectively, for 10 days at 37°C.

Results. Results of the quantitative analyses are shown in Table 2. One can deduce from this table that each of the 13 forest soil samples studied is enzymatically active.

Potential dehydrogenase activity of all samples is higher than their actual dehydrogenase activity. The organic horizon is more dehydrogenase-active than the mineral one. Both actual and potential dehydrogenase activities are highest in sample 2 (organic horizon of a limestone-derived soil under *Abies cephalonica* forest). The influence of the nature of geological formations on the dehydrogenase activity becomes evident when comparing samples 2 and 3 with samples 5 and 6: both mineral and organic horizons of the limestone-derived soil under *A. cephalonica* forest manifest higher dehydrogenase activity as compared

Table 2

Dehydrogenase, invertase, phosphatase and catalase activities and nonenzymatic H_2O_2-splitting capacity of some Greek forest soils

Sample number	Dehydrogenase		Invertase	Phosphatase	Catalase	Nonenzymatic H_2O_2-splitting
	Actual	Potential				
1	2.400	3.900	0.21	4.200	50.51	2.03
2	4.600	5.324	2.03	4.620	47.46	5.08
3	1.262	2.712	0.41	5.220	47.12	8.47
4	0.850	2.050	0.21	5.580	54.24	1.69
5	4.024	4.974	1.14	5.765	48.47	7.45
6	0.837	1.675	1.14	5.340	44.74	10.17
7	3.200	3.350	1.21	5.000	N.D.	N.D.
8	2.125	3.200	1.85	5.160	50.85	5.08
9	0.950	3.550	1.35	5.970	45.76	8.47
10	0.175	0.450	0.41	5.760	44.74	1.69
11	3.412	5.174	1.51	4.170	53.90	1.69
12	0.525	0.975	0.21	6.036	46.78	8.47
13	0.225	0.425	1.05	5.042	48.13	3.39

N. D. — Not determined.

to the same horizons of the flysch-derived soil also under *A. cephalonica* forest. Dehydrogenase activity of the organic horizon in the limestone-derived soil under *Pinus halepensis* forest exceeds the activity of the same horizon in the flysch-derived soil also covered by *P. halepensis* forest (compare sample 7 with sample 8). The influence of the main forest species on the dehydrogenase activity can be established by comparing samples 1, 7 and 11 which all are organic horizons of soils derived from limestone and having the same slope and aspect: the organic horizon under *Quercus* sp. forest is more dehydrogenase-active than that under *A. cephalonica* or *P. halepensis* forest. The soil under Mediterranean vegetation exhibits a very low dehydrogenase activity.

Invertase activity is, in general, higher in the organic horizon than in the mineral one. This activity, like dehydrogenase activity, gives the greatest value in sample 2. Thus, sample 2 is more invertase-active than sample 5, but invertase activity in sample 3 is less than that in sample 6. In contrast to dehydrogenase activity, invertase activity in the organic horizon of the limestone-derived soil under *P. halepensis* (sample 7) is lower than that registered in the organic horizon of the flysch-derived soil also under *P. halepensis* (sample 8). The result of a comparison of samples 1, 7 and 11 is that the organic horizon under *Quercus* sp. exhibits greater invertase activity than the same horizon under *A. cephalonica* or *P. halepensis*. The soil covered by Mediterranean vegetation is moderately invertase-active.

In opposition to dehydrogenase and invertase activities, phosphatase activity is generally higher in the mineral than in the organic horizon. This means that in the studied Greek forest soils the clay minerals should have a major contribution to the accumulation of phosphatase molecules. Phosphatase activity in these soils manifests only slight variations depending on geological formations and main forest species.

Catalase activity (*i.e.* enzymatic splitting of H_2O_2) is much stronger than nonenzymatic H_2O_2-splitting capacity in each analyzed sample. The organic horizon is somewhat more catalase-active than the mineral one, but, in general, the reverse is true for the nonenzymatic H_2O_2-splitting capacity. Like phosphatase activity, catalase activity in the analyzed soils varies only to a slight extent in dependence of geological formations and main forest species.

Table 3 contains the results of the qualitative analyses concerning levansucrase and levanase activities. Levansucrase activity is present in 9 samples and lacks in samples 9 and 10 (organic and mineral horizons of a soil derived from metamorphic rocks and covered by *Fagus silvatica* (forest) and in sample 13 (soil under Mediterranean vegetation). Sample 11 (organic horizon of a limestone-derived soil under *Quercus* sp.) exhibits the highest levansucrase activity. Levanase activity is demonstrated in each analyzed sample. Samples 2, 5 and 8 (collected from organic horizons) are the most levanase-active.

Table 3

Levansucrase and levanase activities in some Greek forest soils

Sample number	Levansucrase	Levanase
1	+++	++
2	++	+++
3	+++	++
4	++	++
5	++++	+++
6	+	++
7	++	++
8	+	+++
9	−	+
10	−	+
11	+++++	++
12	N.D.	N.D.
13	−	++

N. D. — Not determined.

Conclusions. 1. Each of the 18 samples collected from the organic and/or mineral horizons of some Greek forest soils exhibits dehydrogenase, invertase, phosphatase, catalase and levanase activities and nonenzymatic H_2O_2-splitting capacity. Levansucrase activity is present in 9 samples.

2. Dehydrogenase, invertase, catalase, levansucrase and levanase activities are, in general, higher in the organic horizon than in the mineral one. The reverse is true for phosphatase activity and nonenzymatic H_2O_2-splitting capacity.

3. The nature of geological formations from which the soils derived and that of the main forest species covering the soils have a strong influence on the level of soil dehydrogenase and invertase activities. The other activities in the studied soils vary only to a slight extent in dependence of geological formations and main forest species.

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RECENZII

Revoluţia biologică *(La révolution biologique)*, Coordonatorul seriei „Probleme globale ale omenirii" (Coordonnateur de la série „Problèmes globals de l'humanité"): Mihai Drăgănescu, Coordonatorul lucrării (Coordonnateur de l'ouvrage): G. Zarnea. Editura Academiei Republicii Socialiste România, Bucureşti, 1985, 175 pages avec 27 figures et 6 tableaux.

Les problèmes de la vie représentent aujourd'hui, et resteront de même pour l'avenir, l'essence des questions majeures auxquelles le genre humain se donne de la peine à répondre: la défense de la vie et la conservation des structures au niveau écosystémique, la qualité de la vie, l'évolution de la matière vivante, les caractéristiques et les limites de la vie, les perspectives de les connaître — voici seulement quelques-uns des sujets dont la recherche scientifique s'occupe et lesquels sont commentés sous le rapport scientifique et philosophique. Le tome que nous venons de présenter réunit dans un recueil 15 ouvrages d'une large palette d'idées appartenant à l'ensemble des problèmes susmentionnés, dans le but de prévoir les attitudes conceptuelles propres au progrès des sciences biologiques et les moyens de mettre en valeur ces connaissances dans l'économie globale de l'avenir.

Les auteurs des ouvrages réussissent à mobiliser les spécialistes et à préparer l'opinion publique pour la révolution biologique devenue imminente dans le dernier quart du XX-ème siècle. On fait une analyse critique du contexte historique dans lequel s'accomplit cette révolution dont l'apogée peut être atteint seulement après une étude approfondie sur la matière vivante, ce qu'il est possible en vertu des connaissances très avancées d'électronique et d'informatique (Mihai Drăgănescu). La révolution biologique la suppose et l'implique la révolution biochimique („La place et le rôle de la biochimie dans l'ensemble de la 'révolution biologique' se reflètent en plan méthodologique et en plan conceptuel" — Mihai Şerban), ce que signifie, au fond, la révolution biotechnologique ou bioindustrielle. Sur la signification de la dernière on a conclu: „L'énumération des perspectives ouvertes par la révolution biologique prouve de larges possibilités d'application effective dans différents domaines, desquels il ne manque pas les sciences médicales, la production agroalimentaire, la production de nouvelles sources d'énergie et de matières destinées à la chimie, et même dans le rétablissement écologique dans la nature par le remplacement des moyens chimiques par les produits biologiques naturels. On peut conclure que les biotechnologies modernes offrent de grandes possibilités et de grandes perspectives pour l'umanité" (G. Zarnea).

En contexte de la „révolution biologique" sont soumis à une discussion critique des concepts modernes concernant les relations existentes entre l'ordre taxonomique et l'organisation systémique de la matière vivante (Nicolae Botnariuc), ainsi que le démarche systémique dans la biologie, interprété par les mathématiques et par modelage systémique (C. Bălăceanu-Stolnici); d'autre part, dans l'anthropologie, sont présentées les principales directions dans la recherche et leurs significations pour la préparation et le développement de la révolution biologique (Olga Necrasov).

Du domaine de perspective de la révolution biologique appartiennent aussi les connaissances sur le plus grandiose processus biologique, situé au fondement même de l'existence de la vie sur la Terre — la photosynthèse, un mécanisme biologique très élaboré d'échange de substance, d'énergie et d'information entre la matière vivante et son milieu (Lucian Atanasiu); puis les phénomènes de transport à travers les biomembranes (Dan-Georg Mărgineanu), ainsi qu'un vaste champ de „l'ingénierie génétique", fort plein de promesses, duquel est présentée la technologie de l'ADN recombinant (L. M. Popa et coll.) et, plus en détail, les relations fonctionnelles de la cellule avec l'information génétique (Radu Meşter).

C'est à prévoir qu'un ouvrage sur la révolution biologique, aux fondements de laquelle vont se situées les „bio-

technologies" (dans leurs diverses acceptions), ne peut pas manquer de discussion scientifique sur l'avenir de l'agriculture et sur la science d'actualité — l'écologie agricole, considérée parmi „les modalités les plus propres à la production des denrées", ainsi que pour l'obtention du combustible et d'autres matériaux (Ioan Puia et Viorel Soran). Avec optimisme, mais pas sans réalisme en même temps, les auteurs font un commentaire très érudit sur les techniques classiques et celles nonconventionnelles, d'ordre agroécologique, qui pourrions servir l'agriculture de l'avenir en vue d'une productivité accrue dans les agroécosystèmes.

Les éléments de prévision dans l'agroécologie sont ressaisits par le même couple d'auteurs (V. Soran et I. Puia) dans un autre démarche, plus général, concernant au pronostic écologique et au modelage mathématique das l'exploitation du milieu.

Un remarcable horizon d'érudition scientifique dégagent encore des autres travaux, où sont mises en discussion, avec opinion critique ferme, fondée sur une position scientifique rigureuse, mais sans omettre des alternatives eventuelles ou possibles, les fondements théoriques de la biologie de l'avenir. La discussion sur la qualité de la vie (Petre Papacostea et coll.), ou sur la corrélation entre la révolution biologique et la conscience écologique (Nicolae Boşcaiu), ou, enfin, sur la nouvelle révolution imminente dans la conception biologique (Carol Wittenberger) est séduisante pour chaque lecteur, en lui procurant une très aggréable satisfaction intellectuelle.

Ce volume, par son contenu riche en idées généreuses et originales, il est à souhaiter se retrouver dans la bibliothèque de chaque biologiste, sinon de tout intellectuel.

ANA FABIAN

Annual Review of Microbiology, Volume 39 *(Revista Anuală de Microbiologie, Volumul 39)*, Edited by (Sub redacţia:) L. N. Ornston, A. Balows and (şi) P. Baumann, Annual Reviews Inc., Palo Alto, California, 1985, 772 pagini cu 73 figuri şi 41 tabele.

Volumul cuprinde 28 lucrări de sinteză, elaborate, în total, de 49 autori. Subiectele tratate sînt variate; ele prezintă o importanţă teoretică sau/şi practică pentru o serie de domenii şi ramuri ale microbiologiei.

Citologia, biochimia şi fiziologia microorganismelor. T. Hase se ocupă cu creşterea intracelulară şi structura rickettsiilor. Citologia acrasiomicetelor din genul *Dictyostelium* este tratată de C. L. Rutherford, R. A. Vaughan, M. J. Cloutier, V. Naranan, D. A. Brickey şi D. K. Ferris. E. R. Kashket se ocupă cu problema măsurării forţei motrice protonice la bacterii. În lucrarea lor, E. A. Delwiche, J. J. Pestka şi M. L. Tortorello acordă o atenţie deosebită fiziologiei cocilor Gram-negativi din genul *Veillonella*. Alte lucrări se referă la metabolismul carbonului în speciile de *Rhizobium* (M. Stowers), biosinteza şi compoziţia polizaharidelor extracelulare şi parietale ale bacteriilor Gram-negative (I. W. Sutherland), reducerea bacteriană a oxidului de trimetilamină (E. L. Barrett şi H. S. Kwan), secreţia proteinelor de către *Escherichia coli* (D. Oliver), mecanismul de acţiune a antibioticelor asemănătoare kirromicinei (A. Permeggiani şi G. W. M. Swart). H.-P. Meyer, O. Käppeli şi A. Fiechter trec în revistă cercetările privind controlul creşterii în culturile microbiene.

Virologie. R. E. F. Matthews se ocupă cu taxonomia virusurilor, iar R. I. B. Francki prezintă o sinteză a cercetărilor privind virusurile satelit ale plantelor. O serie de oncogene retrovirale şi oncogenele celulare analoage sînt tratate de către L. Ratner, S. F. Josephs şi F. Wong-Staal. Subiectul lucrării lui C. Bazinet şi J. King este mecanismul de asamblare a bacteriofagilor cu ADN dublucatenar.

Microbiologie şi imunologie medicală. Mecanismul virulenţei bacteriene este tratat de R. R. Brubaker. Cu problema supravieţuirii bacteriilor în infecţii se ocupă M. R. W. Brown şi P. Williams. Lucrarea lui R. C. Good este o descriere sintetică a micobacteriilor patogene oportuniste. Toxinele proteice şi glicoproteice de origine vegetală şi fungică cu acţiune inhibitoare asupra proteosintezei la eucariote sînt trecute în revistă de către A. Jiménez şi D. Vázquez. Tema lucrării lui J. C. Boothroyd este „Variaţia antigenică a tripanosomelor africane".

Microbiologia insectelor. Bacteriile nesporogene patogene pentru insecte sînt tratate de O. Lysenko.

Microbiologie ambientală. R. A. MacLeod prezintă o sinteză asupra cercetărilor sale de microbiologie marină. Lucrarea lui J. T. Staley și A. Konopka este consacrată măsurării *in situ* a activităților microorganismelor nefotosintetice din habitatele acvatice și terestre. Bacteriile sulfat-reducătoare și coroziunea anaerobă a metalelor constituie subiectul lucrării lui W. A. Hamilton.

Microbiologie industrială. C. A. Dahl Sawyer și J. J. Pestka se ocupă cu bacteriile prezente în alimente în fluxul preparării acestora. Tema lucrării lui O. Ciferri și O. Tiboni este potențialul industrial și biochimia algelor albastre (cianobacteriilor) din genul *Spirulina.* Indepărtarea sulfului din combustibilii fosili cu ajutorul bacteriilor (în primul rînd cu specii de *Thiobacillus* și cu *Sulfolobus acidocaldarius)* și aspectele tehnologice ale acestui proces sînt tratate în lucrarea lui D. J. Monticello și W. R. Finnerty.

Paleomicrobiologie. Tema lucrării lui A. H. Knoll este „Distribuția și evoluția vieții microbiene în era proterozoică superioară".

Volumul constituie o sursă comprehensivă și modernă de informații pentru specialiștii din diversele domenii ale microbiologiei.

ȘTEFAN KISS

A mezőgazdaság kemizálásának talajbiológiai kérdései *(Soil-Biological Problems of the Chemization of Agriculture),* Szerkesztette (Edited by) B. Tóth, Akad. Bizottság, Veszprém, Hungary, 1985, 282 pages with 54 figures and 67 tables.

This volume is a collection of review articles, grouped into 8 chapters.

Chapter I, written by J. Szegi and entitled „The chemization and the soil-biological problems of plant nutrition", deals with the rhizosphere and phyllosphere.

In Chapter II, F. Gulyás reviews „The soil-biological effects of phosphorus and potassium fertilization".

Chapter III, „Microbiological transformation of plant residues", consists of 4 subchapters: „Plant residues as the principal source of carbohydrates" (F. Gulyás and J. Szegi); „Decomposition of cellulose" (J. Szegi and F. Gulyás); „Microbiological transformation of lignin" (F. Gulyás), and „Decomposition of plant residues reaching the soil and turnover of organic matter in soils" (B. Tóth).

Chapter IV, entitled „Effects of nitrogen fertilization on the microbiological decomposition of corn stalk and wheat straw", comprises 3 subchapters. The first subchapter dealing with „The effect of different forms of nitrogen nutrient on the soil-microbiological processes during the decomposition of corn stalk" was written by B. Tóth. The authors of the second and third subchapters („Mineralization of wheat straw and corn stalk in soils treated with different nitrogen fertilizers"; „Effect of nitrogen fertilizers on the intensity of soil respiration in laboratory experiments") are B. Tóth, J. Szegi and F. Gulyás.

Chapter VI, dedicated to the soil-biological effects of trace elements, comprises two subchapters: „Effects of microelements on the cellulolytic activity of carbonate sandy soils" (F. Gulyás and I. Kádár) and „Effect of Kardonit (a urea-dolomite fertilizer) on the leguminous plants and rhizobia" (A. S. Kiss).

The topic of Chapter VI is „The interrelationship between higher plants and N_2-fixing microorganisms" (J. Szegi).

„The soil-biological problems of the recultivation of disturbed soil surface" are treated in Chapter VII (J. Szegi).

The last chapter, „Soil biology of the pesticides", was elaborated by M. Kecskés.

Each review is based on the personal investigations of the respective author(s) and on a comprehensive, up-to-date bibliographical information. The excellent quality of the illustrations should also be emphasized.

The volume is addressed to a broad circle of readers interested in understanding the biological bases of soil productivity in the context of modern agriculture.

ȘTEFAN KISS

Die Anwendung enzymatischer und mikrobiologischer Methoden in der Bodenanalyse *(The Application of Enzymatic and Microbiological Methods in Soil Analysis),* Herausgeber und Verleger (Editor and Publisher): Landwirtschaftlich-chemische Bundes-

anstalt Linz/Donau, Austria, 1986, 383 pages with 131 figures and 65 tables.

This volume comprises the proceedings of a soil-enzymological and microbiological seminar held in Linz on June 5—6, 1986, namely *a*) the introductory and opening speeches made by the heads of the organizing institutions, Professor W. Beck, Director of the Federal Agricultural-Chemical Experiment Station Vienna and Linz and Professor O. Nestroy, President of the Austrian Soil Science Society; *b*) 6 papers communicated in plenary sessions; *c*) 16 poster presentations; *d*) the discussions, and *e*) the list of the 140 participants.

Of the 6 papers communicated in plenary sessions, three are reviews. Associate Professor F. Schinner deals with „The importance of microorganisms and enzymes in soil" for the biological cycles of elements and for the nutrition, health and productivity of plants.

The author of the second review is Professor Gg. Hoffmann, a leading member of Professor Ed. Hofmann's school, whose major contributions to the foundation of modern soil enzymology are recognized all over the world. This review, entitled „Soil enzymes as characteristics of the biological activity and the turnover of organic materials in soils", is devoted chiefly to summarizing the main achievements of Ed. Hofmann's school, and consists of 5 parts: 1. beginnings of soil enzymology; 2. prehistory of soil enzymology; 3. Ed. Hofmann's working orientation in soil enzymology (principle of the methodology; use of soil samples as enzyme preparations; aim of the investigations); 4. mode of action and origin of soil enzymes (evidence of the enzyme nature; microorganisms as producers of soil enzymes), and 5. possibilities to characterize special soil conditions with enzyme activities (phosphatases as indicators of P availability; enzyme methods for characterizing the effects of harmful substances on the biology of a vineyard soil).

In the third review, Dr. Th. Beck emphasizes that the application of enzymatic and microbiological methods in the analysis of agricultural soils aims at evaluating the more or less strong effects of the present-day soil management techniques on the microbial ecosystem as a whole and, consequently, on the content and availability of plant nutrients in soils and on the structure and humus content of soils. Then, the classification and short description of these methods are given. Three groups of methods are delineated. The first group includes the methods for studying microbial populations. With the methods of the second group, the metabolic activity is measured. The actual activity, measured in short-term experiments, is direct *(e.g.* C-mineralization, *i.e.* respiration; N-mineralization, *i.e.* ammonification) or indirect (enzyme activities) The potential activity, determined in long-term model experiments, is related mainly to the C- and N-cycles. The methods of the third group are used for the determination of microbial biomass, by counting the microorganisms or by assaying the ATP content or the respiration of soil or by applying the fumigation procedure.

The other 3 papers communicated in plenary sessions and the 16 poster papers describe new investigations on a variety of topics.

Much attention has been paid to developing new or improved methods. The methodological aspects of soil sampling and storage of soil samples for enzyme activity determinations were dealt with by R. Öhlinger, A. Eibelhuber and J. Fischerlehner, and W. von Mersi and F. Schinner, respectively. F. Holz worked out partially automated photometric continuous flow methods for determining the activity of 6 soil enzymes (catalase, saccharase, alkaline phosphatase, β-glucosidase, amylase and proteinase). G. Bachman, A. Baumgarten and H. Kinzel are the authors of an improved method for assaying soil respiration (CO_2 evolution) and microbial biomass. K. Alef and D. Kleiner described a method for estimating arginine ammonification in soil samples. R. Margesin and F. Schinner compared different methods for determining the ATP content of soils. K. Vlassak and L. M. J. Verstraeten elaborated a simple and sensitive nitrate determination assay by using nitrate reductase. A. Baumgarten, M. Müllebner and H. Kinzel suggested the method of star diagrams for comparative presentation of soil-biological parameters.

Other topics are related to the soil-biological effects of fertilization (E. Kandeler; R. Öhlinger), liming (F. Schinner, R. Finkernagel, R. Schifferegger and

A. Xander), pesticide application (E. Schuster and D. Schröder), simulated acid rain (R. Finkernagel and F. Schinner).

The soil-biological comparison of the conventional and biological farmings was the topic of 3 papers (R. Öhlinger; P. Gehlen and D. Schröder; C. Siegenthaler). M. Müllebner and H. Kinzel compared different biological parameters of cultivated and quasi-natural (meadow) soils. The acetylene reduction (N_2 fixation) and N-mineralization in meadow, forest and arable soils was studied by S. Boltenstern and H. Kinzel. The paper of K. von der Emde deals with thermophilic actinomycetes developing during composting of municipal refuse.

The papers reflect a high level of investigations. It also results from the papers and discussions that some aspects (*e.g.* enzymological differentiation of soil types; relation between enzymatic and microbiological factors and soil erodability) need further, intensive multidisciplinary research.

We may conclude that the volume as a whole is a valuable contribution to the development of soil enzymology and microbiology.

ȘTEFAN KISS

Iustinian Petrescu, **Lumi geologice dispărute** *(Extinct Geological Worlds)*, Editura Dacia, Cluj-Napoca, 1986, 316 pages with 90 drawings, 18 figures and 32 colour photographs.

Elaborated following the investigation of an extensive bibliography and of a rich museum fossil material, the book represents an original synthesis regarding the paleogeographic and paleoclimatic evolution of Earth in close correlation with the plant and animal kingdoms, since the most ancient times until Actual.

The first part of the book (78 pages) includes two chapters. Chapter 1, "The significance of fossils" brings information regarding the most important fossil burial grounds and famous museums and paleontological collections from our country and abroad. The author briefly analyzes the importance of fossils in the establishment of the age of the geological strata, in specifying the paleoclimate, paleogeography and environmental conditions from diverse biotopes, in the deposition of the useful sedimentary rocks (coals, petroleum and gases, biogenic limestone and siliciferous rocks) as well as in the discovery of the ways followed by the vegetal and animal world in the historical process of evolution. A few pages are dedicated to debating the delicate problem of the geological time. In treating the problem of mass extinction of different groups of beings the author presents briefly the internal causes (phylogenetic aging, diminution of the evolutionary potential) and the external ones (climatic changes, tectonic phenomena, vertical oscillations of the seas, radioactivity and cosmic radiation) responsible for such effects. In Chapter 2, "From fossils to... art and literature", there are presented the most important artistic preoccupations of the fossil man. Also, there are reviewed the most representative Romanian and universal creations of science-fiction and usual literature dealing with many aspects of the tumultuous life of past geological eras. The relationship between paleontology and cinematographic art is treated in a critical way.

The second part of the book (238 pages) consists of 4 chapters.

Chapter I, "Precambrian era — the dawn of ancient worlds", deals with Precambrian paleogeography, climate and ancient organisms.

Chapter II, "Primitive life in the Paleozoic era", comprises: paleogeography and the Paleozoic climate; the first petrified forests; halting places through the jungles of the Carboniferous period; plants and animals in the Paleozoic seas and the first assault of the continental grounds.

Chapter III, "The Mesozoic — the era of dinosaurs", includes: paleogeography and climate of the Mesozoic era; new pages in the biography of stegocephals; aquatic lizards; the fabulous world of dinosaurs; flying monsters, and the first birds and mammals.

Chapter IV, "The Neozoic era — prelude of present worlds" deals with the following topics: paleogeography and climate of the Neozoic era; the great changes in plant world; from "petrified daisies" to... the seaurchins; fossil birds; the tumultuous biography of mammals, and Man — the crowning of evolution.

We should also mention that the reading of the book provides a complex

image on the phylogeny of the vegetal and animal kingdoms. If the phylogenetic trees of the animals and plants are imagined indeed as trees, then these will have a small part of their roots stuck into the Precambrian and the rest into the Paleozoic strata. In Paleozoic, too, the phylogenetic trees get their most important branches; only those corresponding to birds and mammals (in the case of the animals) and to angiosperms (in the case of the plants) await the Mesozoic era. The "fruits" of the phylogenetic trees (*i.e.*, the species) had a temporary character. Only 1% of the ever existed species are contemporary, the rest of 99% having been extinguished in the geological past. They are extinct worlds, as the title suggests, whose vestiges were preserved as high price documents in the strata of the Earth crust, as true archives, the mistery of which is unraveled by the paleontologist and geologist.

To offer an image about some of the extinct vegetal and animal groups, the author resorts to the presentation of their closest related descendants of today. Thus, we can imagine mastodonts and mamouths easier if we know elephants, while ostriches are a good comparing term for the giant fossil birds. The descriptions achieved on these occasions have gotten additional valencies, in comparison with the traditional description of zoology or botany, as a result of the interpretation of data from the paleontologist's viewpoint.

The book is attractive not only through its rich and interesting scientific content, presented with the ability of a good writer, but also through its illustrative material represented by colour photographs and drawings which complete the text of the book in a happy way. The book also contains an English "Content". An index of the main systematic categories (species, genera etc.) enhances the value of the book as a working instrument for the investigator. However, the book is addressed not only to the specialists, but, as the author himself mentions in the preface, also "to other readers, eager to find and know fragments from... the odyssey of the disappeared geological worlds".

PANTE GHERGHEL

Dieter Schmidt, **Echsen in Terrarien,** Neumann Verlag, Leipzig—Radebeul, 1981, 96 Seiten mit 17 Abbildungen und 5 Tabellen.

Der Autor beschreibt in 10 Kapiteln, wie zahlreiche Echsenarten im Terrarium gezüchtet werden, die Art ihrer Vermehrung, ihre Ernährung und Krankheiten.

Im ersten Kapitel, „Echsen im Terrarium", wird beschreiben, dass in der DDR alle Amphibien und Reptilien, mit Ausnahme von Seefrosch, Teichfrosch, Grasfrosch und Moorfrosch, unter Naturschutz stehen.

Ebenfalls im ersten Kapitel wird auch von den international geschützten Echsen gesprochen, die im „Red Data Book" (die Liste der vom Aussterben bedrohten Tiere) eingetragen sind. So gibt es Echsenarten folgender Kategorien: bedrohte, sehr bedrohte, seltene, ungefährdete und unsichere, also einige die zu den Kategorien 1, 2 oder 3 gehören.

Das 2. Kapitel, „Was sind überhaupt Echsen?", enthält eine Beschreibung des charakteristischen Echsentypus, welche von einigen erklärenden, im Kapitel enthaltenen Seiten und von Zeichnungen begleitet wird.

Das 3. Kapitel, „Terrarien für Echsen", enthält die Beschreibung der wichtigsten Terrarientypen (im Freien und im Zimmer), sowie die Art und Weise, wie sie mit Pflanzen, Boden, Wasserbecken und anderen, dem Leben der Echsen notwendigen, physikalisch-klimatischen Bedingungen, zu versehen sind.

Das 4. Kapitel, „Über den Umgang mit unseren Pfleglingen", enthält eine Tabelle mit dem von einigen Echsenarten erreichten Alter, welches zwischen 7 *(Anolis sagrei)* und 54 Jahren *(Anguis fragilis)* schwankt. Es wird desgleichen auch die Art der Gefangennahme der Echsen mit Hilfe einer, auf einem Stab befestigten, Nylon- oder Rosshaarschlinge beschrieben.

Das 5. Kapitel, „Nur Mehlwürmer?", enthält eine Liste mit der Nahrung der Echsen: Regenwürmer, Nacktschnecken, Spinnen, Heuschrecken, Blattläuse, Käfer, Fliegen, Schmetterlinge, Fische, Vögel, Säugetiere und Futterpflanzen. Es folgt eine eingehendere Tabelle über die Arten, welche den Echsen als Nahrung dienen, über das Lebensmedium dieser Tiere, ihre Nahrung, Generationsfolge und Anmerkungen.

Im 6. Kapitel, „Ist eine Winterruhe notwendig?", wird von der Kontrolle der Tiere nach der Zeit des Winterschlafes gesprochen.

Im 7. Kapitel, „Vermehrung — Krönung der Tierhaltung", meint der Autor, dass sich auch tropische Arten bei Einhaltung bester Lebensbedinglungen im Terrarium vermehren können. Am Ende des Kapitels wird eine Tabelle mit der Art, der Bruttemperatur und der Brutzeit (in Tagen) für einige Reptilien angeführt (zum Beispiel für den grünen Leguan, *Iguana iguana*, 28—34°C, Dauer 76 Tage, Zauneidechse, *Lacerta agilis*, 30°C, Dauer 35—36 Tage).

Im 8. Kapitel, „Krank — was nun?", wird behauptet, dass Vorbeugen besser als Heilen ist, da die Krankheiten, welche die Terrariumreptilien anfallen, sehr vielfältig sind. Einige, wie Amöbendysenterie, Kokzidien, Bandwürmer, Fadenwürmer, Infektionskrankheiten, Stoffwechselstörungen, Tumore, Legenot, Verletzungen, Prolaps, Anomalien der Körperform und -farbe, Unfälle bei Narkotisierung, führen oftmals zu Verlusten in der Reihe der Bewohner des Terrariums.

Das 9. Kapitel, „Echsenfamilien — nicht nur Systematik", befasst sich mit der kurzen Beschreibung der 17 Gattungen (es wird auch ein Schema angeführt).

Im 10. Kapitel, „Echsen fürs Terrarium", werden die lebenswichtigen Bedingungen, das Vorkommen und die Art und Grösse von 79 Echsen, welche möglicherweise in einem Terrarium aufgezogen werden können, in einer sehr ausgebreiteten Tabelle beschrieben.

Leider ist die Literaturliste kurz, es werden bloss die wichtigsten Arbeiten zu diesem Thema angeführt. Das Material ist Liebhabern der Vivaristik, den Herpetologieamateuren, den Systematikern und auch anderen Fachleuten auf diesem Gebiet zugänglich.

DAN FIOR SÎRBU

Insecte vectoare şi generatoare de disconfort *(Vector and Discomfort-Generating Insects).* Sub redacţia: (Edited by) Ionela Bîlbîe şi (and) Gabriela Nicolescu, Editura medicală, Bucureşti, 1986, 346 pages with 172 figures and 22 tables.

This book is a monograph elaborated by a group of specialists, whose majority are co-workers of the Medical Entomology Laboratory of the "Cantacuzino" Institute in Bucharest. The work deals with the problem of vector and discomfort-generating insects in a multidisciplinary (biological, medical, chemical, etc.) way. It is based on long investigations in the laboratory and under natural conditions, and on an extensive bibliography, selectively presented. It consists of 3 parts.

Part I (201 pages) treats in separate chapters the principal insects of medico-sanitary importance: mosquitoes (G. Nicolescu), chironomidae (N. Botnariuc), phlebotomi (G. Nicolescu), synanthrope flies (A. Enescu and C. Ceianu), cockroaches (A. Enescu), bedbugs (I. Ghiurcă), lice (A. Enescu) and fleas (M. Suciu). In each case, the authors insist on morphological characters with taxonomic importance and also on certain biological and ecological aspects, especially on those important in supervising, control and fighting, such as the duration of the developmental cycle and the number of generations per annum as a function of environmental conditions, the habitat of different species, the trophic regime and the feeding behaviour, the circadian rhythm of activity, etc. In treating of these insects, the epidemiological aspects also take an important place.

Due to the fact that mosquitoes are the most important insects regarding man's health, the largest number of pages (124) are dedicated to them. The corresponding data are grouped into 4 chapters: "Mosquitoes" (G. Nicolescu), "Vector role of anopheles in malaria epidemiology" (I. Bîlbîe), "Present situation of the anophelism in the Danube Plain and Dobruja" (I. Bîlbîe) and "Anophelism peculiarities in Moldavia during the period of interruption of malaria transmission" (C. Teodorescu). The chapter on mosquitoes also comprises a determination key of the species based on the 4th age larvae and adult individuals. In the case of synathrope flies, a key for family determination is also given. In the case of the other vector and discomfort-generating insects, the identification is based on the description of the most important species. We think that the chironomidae, that are much more useful as larvae than detrimental as adult individuals, were treated in this work in order to avoid confusion with the mosquitoes which they resemble at a superficial entomological exa-

mination as well as to avoid the useless fighting against them.

The first chapter of Part II (128 pages) deals with the "Conventional insecticides and other substances used in insect control" (S. A. Staicu). After a short description of the way of penetration of the insecticides through the tegument barrier, the author gives a classification and description of the most important insecticides. Useful information is also presented on synergism, synergizing substances, third generation insecticides and insecticide conditioning. The chapter closes with 10 tables containing data on the insecticides used inside the house and in the natural environment for controlling insects of medico-sanitary importance.

The next chapter is devoted to the principal types of equipment and apparatuses used in the application of insecticides against vector and discomfort-generating insects (Ş. Variu and N. Velehorski).

The chapter entitled "Resistance to insecticides of the vector and discoffort-generating insects" (G. Nicolescu and I Bilbie) shows that at the present stage about 140 species of insects from this group are resistant to insecticides and that their resistance is the result of a complex interaction between intrinsic (genetic, biological) and extrinsic (insecticide-dependent and operational) factors.

In the chapter "Methods for controlling vector and discomfort-generating insects". G. Nicolescu and C. Ceianu present the chemical, genetic, hormonal, biological, ecological methods and the strategy of integrated control of this category of insects. They also specify the advantages and disadvantages of each method.

It results from the chapter "Programme of the integrated control of the mosquitoes" (M. Smolinski) that the chemical method represents yet the basic method in the control actions, although the ecological and biological methods have been more and more frequently used lately.

The peculiarities presented by the control of discomfort-generating insects in sanitary units are described in the last chapter of Part II (I. Gafiţeanu and G. Nicolescu).

Beside the fact that many insects are vectors of very serious diseases, some of them cause dermatosis as a result of their bite or of the development of their larvae within the skin. This subject is treated in Part III (9 pages) consisting of a single chapter: "Dermatoses provoked by insects" (D. Mureşan).

The book is addressed in the first place to specialists from the medico-sanitary network and other fields, which are directly or indirectly involved in the actions of supervision and control of vector insects and/or discomfort-generating insects. It is also useful to all categories of investigators from the field of entomology, generally to biologists and physicians. Through some data of its content, the book also presents interest for students in biology and medicine.

PANTE GHERGHEL

ÎNTREPRINDEREA POLIGRAFICĂ CLUJ, Mun. Cluj-Napoca c-da nr. 60/1987

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