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SOMMAIRE

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, N° 2, P. 79—158, BUCAREST, 1988

PARASITOID ICHNEUMONIDS *(HYMENOPTERA, ICHNEUMONIDAE)* RECOVERED FROM THE DEFOLIATOR MOTHS *LYMANTRIA DISPAR* L. AND *MALACOSOMA NEUSTRIA* L. IN ROMANIA

RAOUL CONSTANTINEANU and IRINEL CONSTANTINEANU

Nine species of Ichneumonidae recovered from two defoliator moths in 11 oak woods in Southern Romania are recorded : *Phobocampe unicincta* (Grav.), *Hyposoter tricoloripes* (Vier.) (from *Lymantria dispar* larvae in their second to fourth stage), *Ephialtes compunctor* (L.), *Itoplectis enslini* Ulbr., *Euceros superbus* Kriechb. (from *L. dispar* pupae), *Gregopimpla malacosomae* Seyr., *G. inquisitor* (Scop.), *Theronia atalantae* (Poda) and *Coccygomimus instigator* (F.) (from *Malacosoma neustria* (L.) pupae).
Phobocampe unicincta (Grav.) and *Hyposoter tricoloripes* (Vier.) are new for the fauna of Romania. These two parasitoid species parasitize only young gypsy moth larvae. *Lymantria dispar* is a new host in science for *Itoplectis enslini* Ulbr.

In Romania, especially in the Southern part, many oak woods, particularly those which contain *Quercus cerris* L., *Q. frainetto* Ten. and *Q. pubescens* Wild., are often heavily infested by the following defoliator moths : *Lymantria dispar* L., *Tortrix viridana* L., *Operophtera brumata* (L.), *Erannis defoliaria* Cl., *Malacosoma neustria* (L.), *Thaumetopoea processionea* (L.), etc.

In our previous papers we have studied the parasite complex of gypsy moth *(Lymantria dispar* L.) (1, 2, 4) and of tent caterpillar *Malacosoma neustria* (L.) (2) in oak woods in Southern Romania.

In this paper the authors complete the list of parasitoid ichneumonids of these two defoliator moths.

MATERIALS AND METHODS

During 1974—1985 period we did our studies in 11 woods in Southern Romania. Thus, during 1977—1985 period we collected larvae and pupae of *L. dispar* from nine woods : eight from Giurgiu county : Băneasa—Giurgiu commune, Arbori, Bulbucata commune, Nebuna and Bălășcuța, Clejani commune, Blaj, Gostinari commune, Cîlnișteа and Islaz, Comana commune, Babarada, Mihai Bravu commune and Vîrvor wood, Perișor commune, Dolj county. In 1974 we collected pupae of *M. neustria* from Perișor and Vîrvor woods, Perișor commune, Dolj county and Riioasa wood, Răcari commune, Dîmbovița county.

The larvae of *L. dispar* in their second to fourth stage were collected during May 15—May 27 period, while pupae of *L. dispar* and *M. neustria* between May 30—July 11.

Each larva and pupa was put in a vial of 20 cm^3, corked with cotton-wool and kept in the laboratory at room temperature, avoiding dryness. The parasitoid hatching was watched daily.

RESULTS AND DISCUSSIONS

We recovered the following nine species of Ichneumonidae by rearing of immature stages of *L. dispar* and *M. neustria*: *Ephialtes compunctor* (L.), *Itoplectis enslini* Ulbr., *Gregopimpla malacosomae* Seyr., *G. inquisitor* Scop., *Coccygomimus instigator* (F.), *Theronia atalantae* (Poda) (Ephialtinae), *Euceros superbus* Kriechb. (Eucerotinae), *Phobocampe unicincta* (Grav.) and *Hyposoter tricoloripes* (Vier.) (Porizontinae) (Table 1).

P. unicincta and *H. tricoloripes* are parasitizing larvae of gypsy moth in their second to fourth stage; *E. compunctor*, *I. enslini* and *E. superbus* were recovered from gypsy moth pupae. *C. instigator*, *T. atalantae*, *G. inquisitor* and *G. malacosomae* are parasitizing *M. neustria* pupae.

We remarked in our studies that ichneumonids represent a group of natural enemies with a less importance in limiting the defoliator moth populations, as compared with the parasitic Diptera. Thus, ichneumonids, recovered from *L. dispar* (Table 1), have achieved a very low parasitization degree, some of them being recorded in a single speeimen (*H. tricoloripes* and *I. enslini*). Pschorn—Walcher, in Gupta (5), reported *P. unicincta* and *H. tricoloripes* as a dominant parasitoid of gypsy moth in Würzburg, West Germany. In our country we record that this species has achieved a parasitization degree between 0.1—5.9% for gypsy moth in oak woods in Southern Romania.

In this paper we record the ichneumonids *P. unicincta* and *H. tricoloripes* for the first time in the fauna of Romania. *L. dispar* is a new host in science for *Itoplectis enslini*. *E. superbus*, a rare species in the fauna of Romania, was recorded by us for the first time in science from *L. dispar*.

Among Ichneumonidae, *Coccygomimus instigator* and *Theronia atalantae* have the most important role in the parasitization of *M. neustria* populations. We recorded these species also as parasitoids of gypsy moth young instar larvae of gypsy moth was between May 5—July 3, while parasitoids of the defoliator pupae emerged during June 11—August 11 period (Table 1). The knowledge of this fact will permit the possibility of protection of these parasitoid species in accordance with their hatching period.

We present a short description for the two ichneumonid species, new for the fauna of Romania.

Phobocampe unicincta (Gravenhorst) 1829, ♀♂

4 ♀♀ and 5 ♂♂ collected from the following woods in Giurgiu county: Nebuna (Clejani commune), Băneasa (Băneasa—Giurgiu commune), Blaj (Gostinari commune), Islaz, Cîlnișlea (Comana commune) and Babarada (Mihai Bravu commune).

♀♂. Head is transverse, little narrowing behind. Antennae are little shorter than the body. Antennal flagellum is 28 segmented. Face is strongly punctate. Apical margin of clypeus is straight. Propodeum is carinate, generally with strong carinae. Area superomedia is flat, opened behind. Petiolar area is a little concave. Propodeal spiracles are oval. Areolet is small, short petiolate. Spiracles of the first tergite are situated at the anterior part of postpetiole.

Table 1

Parasitoid ichneumonids recovered from *Lymantria dispar* and *Malacosoma neustria*

Host	Immature stage of host	Parasitoid species	Number of individuals	Date of host collection	Collection site	Emerging date of parasite from host	Observations
1	2	3	4	5	6	7	8
Lymantria dispar	II—IV instar larvae	*Phobocampe unicincta*	1	May 18, 1981	Nebuna	May 22, 1981	New species for the fauna of Romania. *L. dispar* is a new host in Romania for this parasitoid
			2	May 19, 1981	Băneasa	June 2, 1981	
			1	May 20, 1983	Islaz	June 3, 1983	
			2	May 27, 1984	Islaz	June 5, 1984	
			3	May 20, 1984	Blaj	June 1, 1984	
			1	May 18, 1984	Babarada	May 29, 1984	
			1	May 15, 1985	Islaz	May 30, 1985	
			2	May 26, 1985	Cîlnișlea	June 5, 1985	
		Hyposoter tricoloripes	1	May 20, 1983	Islaz	July 3, 1983	”
	pupae	*Ephialtes compunctor*	1	July 11, 1980	Arbori	August 5, 1980	
			2	July 11, 1980	Bălășcuța	August 2, 1980	
			1	June 23, 1980	Cîlnișlea	August 1, 1980	
		Itoplectis enslini	1	June 26, 1974	Vîrvor	August 5, 1974	*L. dispar* is a new host in science for this parasitoid
Lymantria dispar	pupae	*Euceros superbus*	1	June 14, 1977	Băneasa	July 29, 1977	
			1	June 11, 1980	Arbori	August 11, 1980	
			2	June 25, 1982	Blaj	July 27, 1982	
Malacosoma neustria	pupae	*Coccygomimus instigator*	41	June 3—4, 1974	Vîrvor	July 23—28, 1974	We recovered this species from *L. dispar*, too
			1	May 30, 1974	Rîioasa	June 22, 1974	
		Theronia atalantae	3	June 4, 1974	Vîrvor	June 26, 1974	”
		Gregopimpla inquisitor	3*	June 4, 1974	Perișor	June 20, 1974	
			5**	May 30, 1974	Rîioasa	June 23—30,1974	
		Gregopimpla malacosomae	22***	June 3, 1974	Perișor	June 11—14,1974	

* The 3 individuals of *G. inquisitor* were recovered from a *M. neustria* pupa
** The 5 individuals of *G. inquisitor* were recovered from a *M. neustria* pupa
*** The 22 individuals of *G. malacosomae* were recovered from 4 pupae of *M. neustria*

Abdomen is mat. Ovipositor is a little longer than the length of the abdomen and slightly upcurved.

Black. Mandibles, palpi, ventral sides of scapus and pedicellus, tegulae, anterior and middle coxae, all trochanters are yellow. Tibiae are yellow; the hind ones with black apex. Apex of petiole, thyridia and apex of second tergite are reddish-yellow. In males the first tergite is entirely black, while the apex of the second is reddish.

Length : 6 to 6.5 mm.

Hosts : *Acronicta alni* L., *A. pisi* L., *Craniophora ligustri* Schiff. (Lepidoptera, Noctuidae), *Chloroclystis rectangulata* L., *Gonodontis bidentata* Cl., *Lomaspilis marginata* L., *Operophtera brumata* L. (Lep., Geometridae), *Nymphalis polychloros* L., *N. urticae* L. (Lep., Nymphalidae), *Alucita galactodactila* Hb. (Lep., Pterophoridae), *Dasychira pudibunda* L. and *Lymantria dispar* L. (Lep., Lymantriidae) [5, 6].

Distribution : Great Britain, Austria, Italy, France, Yugoslavia, Bulgaria, Hungary, Czechoslovakia, USSR, Iran, Japan and U.S.A. (introduced).

Hyposoter tricoloripes (Viereck) 1911, ♀

1 ♀ emerged on July 3,1983 from a gypsy moth larva in its fourth stage, collected on May 20,1983 from Islaz wood, Comana commune, Giurgiu county.

♀♂. Body is punctate. Head is transverse, little narrowing behind. Antennae are a little shorter than half of the body. Antennal flagellum presents 32 articles. Ocelli are large. Petiolar area is rugose. Area superomedia with strong carinae. Costula is not evident. Gastroceli are oval, deeply impressed.

Black. Mandibles, palpi, tegulae, anterior and middle trochanters are yellow. Anterior femorae are yellowish-brown, while the middle ones are reddish-brown. Tibiae, anterior and middle tarsi are yellowish-red. Hind legs are brown, tibiae basis is light-yellow.

Length : 8 mm.

Host : *Lymantria dispar* L. (Lep., Lymantriidae) [5].

Distribution : Austria, France, Poland. It was introduced in U.S.A., but this parasitoid has never become established despite repeated releases [3].

CONCLUSIONS

1. In this paper we record nine species of parasitoid ichneumonids of the defoliator moths *L. dispar* and *M. neustria*.

2. *Phobocampe unicincta* (Grav.) and *Hyposoter tricoloripes* (Vier.) (Ichneumonidae, Porizontinae), parasitoids of the larvae of gypsy moth in their second to fourth stages, are new for the fauna of Romania.

3. *Lymantria dispar* is a new host in science for *Itoplectis enslini*.

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THE BREATHING INSTINCT OF DOLPHINS AND THE STATISTICAL-MATHEMATICAL REPRESENTATION OF ITS COMPONENTS

M. I. MIHAI

By the statistical mathematical methods and electronic calculation techniques, we have represented the two components of the breathing instinct : the variable appetitive act and the constant consummatory act. The resulted numerical parameters permit a best estimation of what this breathing instinct means for the dolphins'aquatic life adaptation, for their captive life adaptation, for the evaluation of their purposed (forced) respiratory responses, etc.

In his work that has become classic, Racoviță was among the first to call attention on the importance of the breathing instinct in cetaceans. "A cetacean — he writes — seeks for food in the water nevertheless it must breathe air. From these two facts results the entire, very special, biology of these beings and many traits of their organisation" (5). As aquatic mammals, dolphins belong to the large family of cetaceans, their lives being completely independent of the original terrestrial life. It is their pulmonary breathing that testifies best their terrestrial origin ; in fact their whole aquatic life unfolds between two breathings. The breathing act in itself as well as the interval between breathings are extremely important for their functional adaptation, and representing them requires the most perfect means.

The breathing act performed in a very short interval in dolphins — 0.3—0.5 s (6) — as well as the great variability of the intervals between breathings, reminds of what Craig discovered (Craig — 1918) to be defining for two fundamental components of an instinctive act : *the consummatory act* in breathing consists of a rapid succession, an expiration followed by an inspiration, a trait acquired phylogenetically "in order to be least exposed to the penetration of water into the event (5) ; *the appetitive act* consists of an intentionalized "forced" oxygenation of the lungs either anticipatory — when the animal prepares for a sounding dive, or consecutive — when certain reasons at the bottom of the sea retain the animal there, prolonging its immersion.

Interruption of breathing under the spur of a stimulus is a typical reaction of the orientation reflex, called by Pavlov "what's the matter" (4), with the receptors directed that way, with other activities interrupted etc. The continuity of the cetaceans breathing is broken off by a most specific stimulus without which, in spite of their terrestrial origin, they could not possibly live : it is the aquatic environment itself. Therefore, the adequate stimulus for evoking their breathing instinct, called by Tomilin"the going out reflex" is the lower pressure of the air, about which they get informed by means of the receptors placed in the event area. The reflex is also unconditionally evoked in a dolphin that has been put out of water on a mattress, whenever water is thrown over it.

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, N° 2, P. 87—94, BUCAREST, 1988

Interruption of breathing could also be of a configurational nature, when a stimulating event changes something in the perceptive field of the animal. This "something" can be a new source of food, an unexpected gesture of the sexually opposed partner or perhaps a very cold stream, etc. All these stimuli are destined to break the order of an equidistanced breathing succession, characteristic of a "rhythmical", phylogenetically-based breathing activity. The great inequality of the intervals between breathings recorded in all water mammals, as well as the variable intensity of the consumption act, is the expression of the second component of the instinctive act, i.e. the expression, ultimately, of a "superior" sense of adaptation to the environment.

Of all aquatic animals not only mammals (cetaceans, pinipeds, etc.) have to rise to the surface in order to breath. Actually, for the simplest graph to have distinguished between the two basic components of the breathing instinct, we are indebted to Tinbergen (8),who studied this behavior in the *Anabantidae* fish, with labyrinthic aerial breathing organs beside the bronchial ones, for which the oxygen in the air is also indispensable for living. Though having totally different breathing organs, the route and the moment of breathing proper are analogous (Fig. 1). To complete it, we only have to add the one we owe to Racoviță (Fig. 2), which represents the same breathing behavior in the whales, with the two components being also distinct. All leave the water surface in search for food, for the partner, etc.; yet all will have to return there to breath, ready to overcome various obstacles, some of a physical nature — like the variable swimming depth, the extension of a glacier, etc.; others of an inter or intraspecific nature, such as an enemy, a stronger rival, etc.

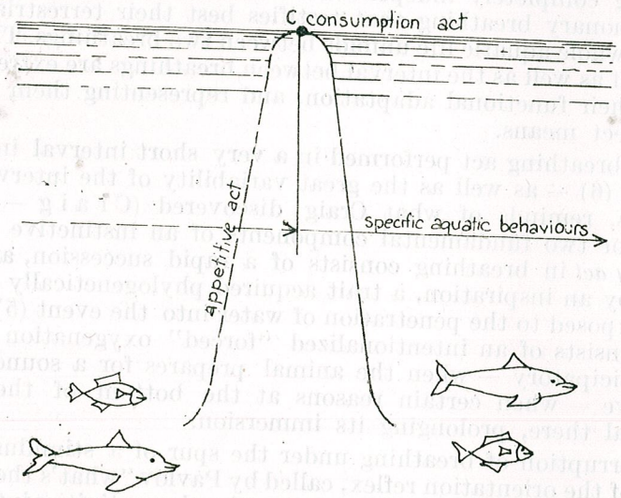

Fig. 1. — The analogue spatio-temporal evolution of breathing instinct in the *Anabantidae* fishes and dolphins.

Despite the fact that the graphs in Figs. 1 and 2 are quite explicit, they fail to comprise the variable time-interval between breathings. The interval between breathings is measured chronometrically. The duration of a breathing being less than one second it is not longer taken into account.

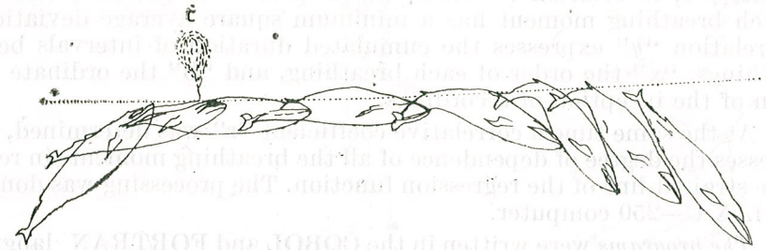

Fig. 2. — The component of the breathing instinct in whales (after Racoviță).

The proposed statistical-mathematical method has in view to offer a comprehensive numerical representation, especially for the appetitive component of this instinct. It is a perfectionated means that allows the quantification of a succession of breathing responses not of the order of tens (as in Fig. 4, or as in the work of Watson and Gaskin — 1983), but of the order of tens of thousands, being able to quantify the dynamics between these breathing moments characteristic for various states, such as migration, sounding, chasing, playing, sleeping, etc.

The processing and representation on a table of the data was done with the help of an electronic computer.Displayed by the computer in the form of a "TABLE OF BREATHING RHYTHMICITY" it contains the following columns (Table 1):

Table 1

Table of breathing rhythmicity

Delf. code	Rec. date	Tot. durt.	Nr. resp.	Avrg. (sec)	Max.	Min.	C_{cor}	$y = ax + b$
03	25.11.73	363	22	16.50	45	4	0.99191	$y = 16.27x + 9.7$
31	24.07.74	440	22	20.00	70	3	0.99442	$y = 21.40x + 14$
61	28.12.73	1543	44	35.06	84	4	0.99799	$y = 33.33x + 54$

— DOLPHIN CODE — code given to recorded dolphin;
— REC. DATE — date of recording;
— DURATION — duration of recording;
— NR. BREATH. — number of breathings, recorded in the respective length of time;
— AVERAGE — average of intervals between breathings;
— CORREL. COEFF. — coefficient of correlation, indicative of the dynamics of intervals between breathings;
— REGR. CURVE — regressive function of the respective succession of recorded breathings.

If needed, the computer can also print the actual graph of this regression function.

In order to cover the wide distribution of the chronometrically recorded data, indicating the intervals between consecutive breathings, laws of the linear regression type were used. This consists in determining for the variable succession of intervals between breathings a function of the type $y = ax + b$, in relation to which the dispersion of the indicator points of each breathing moment has a minimum square average deviation. In this relation "y" expresses the cumulated duration of intervals between breathings, "x" the order of each breathing, and "b" the ordinate at the origin of the inception of recordings.

At the same time a correlative coefficient "r" was determined, which expresses the degree of dependence of all the breathing moments in relation to the straight line of the regression function. The processing was done into a FELIX C—250 computer.

The programs were written in the COBOL and FORTRAN languages. The work has implied establishing processing algorithms and selecting the form of print for the results. Five programs were elaborated, three of which for creating the data card index and two for the processing and printing the results (Fig. 3).

The first program CREFIS includes a card index. The primary information is inscribed in printed forms and punched into the cards. A card has the following structure :

The first row contains the dolphins code. This code takes up values ranging from 01 to 99, and is divided arbitrarily into three groups :

— from 01 to 30 for *Phocaena ph.* specimens
— from 31 to 60 for *Delphinus d.* specimens
— from 61 to 90 for *Tursiops tr.* specimens

The second row contains the type of card indicated from 1 to 9. This row is necessary in the case the recorded state contains more information than can go into one card, i.e. more than 23 successive measurements.

The third row includes the date of recording (day, month, year).

The last row contains the data to be processed — the intervals between breathings. Such an interval covers 3 characters (1 to 999 sec), one card holding at most 23 intervals (Time *i*), where "*i*" takes values from 1 to 23.

The CREFIND program takes over the information from the card, checks it up, displays it, removes eventual numerical errors and makes up an index card on a magnetic disc with the following structure : dolphin code ; recording date ; number of breathings ; time-length of intervals. One recording can hold a maximum of 350 data, indicating the successive intervals between breathings, which the program can cover in one processing.

The SORTCOD program sorts the recordings in items with two sorting keys : in increasing order of dolphin code values, and within the same code in increasing order of data.

The SORTCOD program does confer to the card index a new manner of organizing a so-called sequential indexing and signals possible items in the same key. Each item must be determined in a unique way by a row called "key" which, in our case, is a grouped row made up of the dolphin

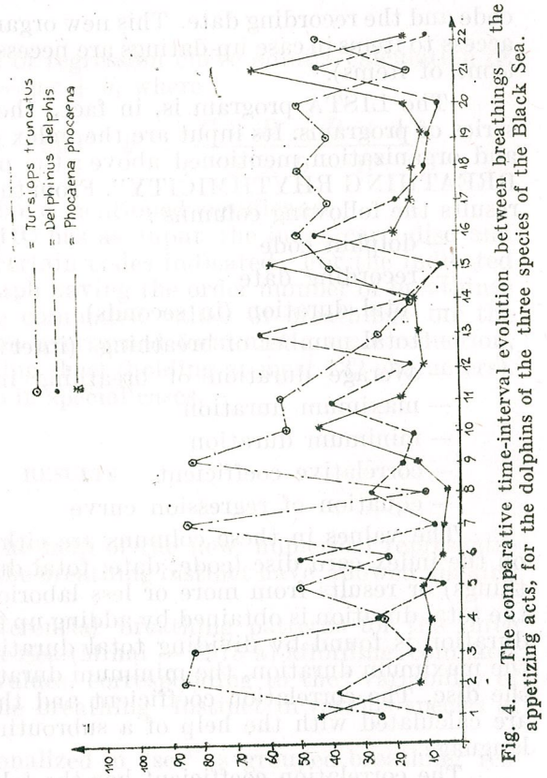

Fig. 4. — The comparative time-interval evolution between breathings — the appetizing acts, for the dolphins of the three species of the Black Sea.

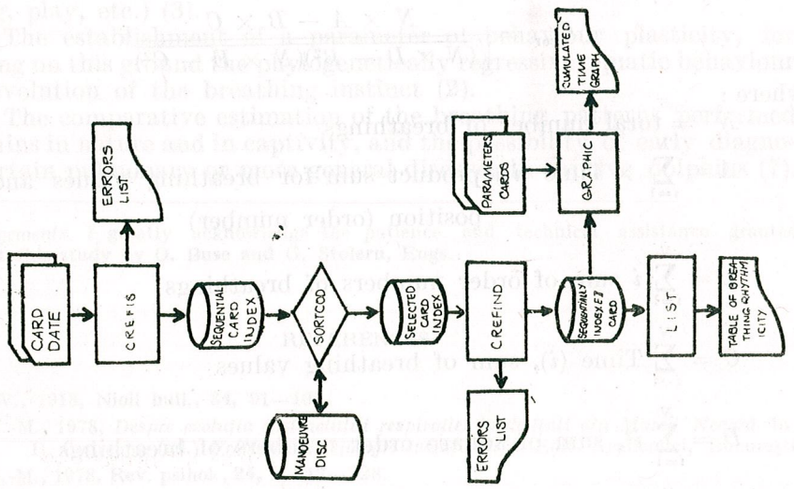

Fig. 3. — Program flowchart.

code and the recording date. This new organizing manner permits an easier access to items in case up-datings are necessary (additions, erasings, corrections of items).

The LISTA program is, in fact, the most important in the whole series of programs. Its input are the index card data, having the structure and organization mentioned above ; the output being the "TABLE OF BREATHING RHYTHMICITY". From the processing of each item there results the following columns :

— dolphin code
— recording date
— total duration (in seconds)
— total number of breathings (interval between them)
— average duration of breathing intervals
— maximum duration
— minimum duration
— correlative coefficient
— equation of regression curve

The values in these columns are either taken over from the items on the index card disc (code, date, total duration, total number of breathings) or results from more or less laborious calculations. For example, the total duration is obtained by adding up all breathing data ; the average duration is found by dividing total duration by number of breathings ; the maximum duration, the minimum duration are directly collected from the disc. The correlation coefficient and the regression curve coefficients are calculated with the help of a subroutine written in the FORTRAN language.

The correlation coefficient has the following formula :

$$C_{cor} = \frac{N \times A - B \times C}{\sqrt{(N \times D - B^2)(N \times E - C^2)}}$$

where :

N = total number of breathings

$A = \sum_{i=1}^{N} \times$ Time (i), product sum for breathing values and their position (order number)

$B = \sum_{i=1}^{N} i$ sum of order numbers of breathings

$C = \sum_{i-1}^{N}$ Time (i), sum of breathing values

$D = \sum_{i=1}^{N} i^2$, sum of square order numbers of breathings

$E = \sum_{i=1}^{N}$ Time $(i)^2$/sum of square values of breathings

The last column, equation of regression curve entails calculation of coefficients of the equation : $y = ax + b$, where

$$a = \frac{N \times A - B \times C}{N \times D - B^2} \quad \text{and} \quad b = \frac{C \times D - B \times A}{N \times D - B^2}$$

N, A, B, C and D having the above mentioned significance.

The last program GRAPHIC has as input the index card disc and a set of parameter cards with certain codes indicated. For the indicated codes, the program realizes a graph having the order number of breathings (order) on its ordinate and the cumulated values of breathings on the abscissa. The graph will have an overturned form and a scale reduction, being constrained by the recording sheet (holding at most 132 characters). This program is only resorted to in special cases.

RESULTS

The data obtained with the help of the new numerical representation of the basic components of the breathing instinct have allowed making some contribution to :

— The description of differential breathing patterns for the three species of dolphins in the Black Sea (Mihai — 1978 a) affording comparative estimations of numerical values, corresponding to the variability of the appetitive component of the breathing instinct in various species of aquatic mammals.

— The manner of intentionalized in "set" s grouped breathing responses, subordinate to various water behaviours of dolphins (migration, sounding, play, etc.) (3).

— The establishment of a parameter of behaviour plasticity, for estimating on this ground the phylogenetically regressive aquatic behaviour of the evolution of the breathing instinct (2).

— The comparative estimation of the breathing patterns performed by dolphins in nature and in captivity, and the possibility of early diagnosis of certain pulmonary or more general diseases in captive dolphins (7).

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CHANGES INDUCED BY THE NITROGEN LASER IRRADIATION ON THE SUPEROXIDE DISMUTASE PURIFIED FROM *E. COLI*

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The kinetic parameters of the partially purified superoxide dismutase from *E. coli*, before and after pulsed nitrogen laser irradiation (3371 Å), have been investigated. The laser irradiation produces a 2.3 fold increase in the enzyme activity and changes its optimum parameters which would suggest that the nitrogen laser alters in some way the conformation of the molecule bringing about the above observed changes.

The use of laser radiations in biology and medicine have been hampered for many years by the lack of systematic studies on the interaction of these physical agents with living matter. In biomedical applications the thermal effects are those which determine the complex denaturation of macroscopic systems. The presence of such effects is studied by following the activity of certain subsystems such as the enzymes. The distribution of the absorbed energy by an irradiated tissue depends on the dissipation properties of the tissue as well as on its absorption characteristics at the wavelength used for irradiation. As for the proteins, the laser radiations may photodissociate the disulphide bridges, may breake some physical interactions (hydrogen bonds, Van der Waals forces), saline interactions (2, 3, 8, 12). The UV lasers affect aromatic and heterocyclic amino acids as well as thiol amino acids (cysteine, cystine). These interactions result in a change of the three-dimensional structures of proteins while the enzymes exhibit an increase or a decrease of their activity depending on the degree to which the active site has been altered.

In isolated cells and microorganisms it was demonstrated that UV lasers induce alterations of the genetic material especially through the dimerization of two adjacent tymine residues on the same DNA chain. Thus, the spatial structure of the DNA is altered and the ability to transcribe the genetic information is impaired (4, 9, 10, 11).

As a biochemical marker of the changes induced by the UV lasers we chose the superoxide dismutase (EC 1.15.1.1) that catalyzes the reaction

$$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow H_2O_2 + O_2$$

It was assumed that following the nitrogen laser irradiation, superoxide radicals are formed in the cell. These reactive species of oxygen may stimulate the biosynthesis of this enzyme and/or the laser irradiation may induce conformational changes in the quaternary structure of the enzyme.

In the previous paper (6) we have investigated the effects of the laser irradiation of an *E. coli* culture on the superoxide dismutase activity. It was shown that the enzyme was activated following laser irradiation, some kinetic parameters were changed and detection of enzyme activity after polyacrylamide gel electrophoresis demonstrated the presence of

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, N° 2, P. 95—101, BUCAREST, 1988

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a new molecular form of superoxide dismutase having a net positive electric charge.

In order to get a better insight into the activatory effect of laser irradiation and to distinguish between changes occurring in the enzyme molecule and those taking place within the cell, some physicochemical properties of the purified superoxide dismutase from *E. coli*, before and after laser irradiation, have been investigated.

MATERIALS AND METHODS

The *E. coli* strain 029 culture was obtained from the "Dr. I. Cantacuzino" Institute. The cells were harvested by centrifugation at 10,000 r.p.m. for 20 min, then washed three times with cold 0.05 M potassium phosphate buffer pH 7.8, 0.4 mM EDTA. The pellet was suspended in a small volume of buffer and sonicated for 15 min in a Sonomatic 150 apparatus. The homogenate was clarified by centrifugation at 10,000 r.p.m for 30 min. The clear supernatant was used as starting material for the purification of superoxide dismutase.

The enzyme was purified generally according to Keele et al. (5) adapted to *E. coli* strain used by us and included heat precipitation, ammonium sulphate fractionation, dialysis, CM- and DEAE- cellulose chromatography. The purified enzyme solution was irradiated at the Central Institute of Physics with a nitrogen pulsed laser (3371 Å, 30 kW/pulse, 30 ns/pulse, 15 Hz) for 10 min.

Protein concentration was determined according to Lowry et al. (6) using bovine serum albumin as standard.

The superoxide dismutase activity was assayed as described by Beauchamp and Fridovich (1) and modified by Winterbourn et al. (13). The superoxide radicals generated by the illumination of riboflavin reduce the tetrazolium salt (nitro BT) to formazan. The enzyme activity is assayed by the inhibition of the photoreduction of the tetrazolium salt, the superoxide radicals being decomposed by the superoxide dismutase. The assay mixture contained 0.15 μmoles nitro BT, 6 nmoles riboflavin, 20 μmoles EDTA, 0.1 ml enzyme solution (40 μg/ml) in a final volume of 3 ml buffered with 0.036 M potassium phosphate buffer pH 7.8. The assay and the control — without the enzyme, were illuminated for 5 min., then the absorbance at 560 nm was read against distilled water. In the presence of light, the riboflavin is oxidized and superoxide radicals are formed. The extent of inhibition produced by the control was considered as 0% and the percentage inhibition achieved by the sample is calculated by the following equation :

$$\% \text{ Inhibition} = 100 - \frac{A_{560nm} \text{ (sample)}}{A_{560nm} \text{ (control)}} \times 100$$

One enzyme unit is defined as the amount of enzyme that causes a 50% inhibition in the reduction of the tetrazolium salt. Superoxide dismutase activity is expressed in units/mg proteins.

RESULTS AND DISCUSSION

Enzyme purification. The crude protein fraction was heated at 40°C for 10 min., then the precipitated proteins were removed by centrifugation. From 240 mgs proteins (100 ml — 2.4 mg/ml) 81.6 mg were thermoprecipitated, remaining 158.4 in the supernatant. The superoxide dismutase is thermostable, was recovered in the supernatant, which was next subjected to ammonium sulphate fractionation, separating three fractions precipitated in a 0—0.35; 0.35—0.65 and 0.65—0.9 saturation range. The enzyme activity has been detected only at the 0.65—0.9 $(NH_4)_2SO_4$ saturation (purification factor 7.23).

The partially purified preparation was dialysed overnight against distilled water, then applied to a CM-cellulose column (32 × 1.8 cm) equilibrated with 2×10^{-3}M potassium acetate buffer pH 5.5. The adsorbed proteins were eluted with an increasing concentration gradient of acetate buffer (2×10^{-3}M—2×10^{-1} M) pH 5.5 (Fig. 1). The fractions exhibiting

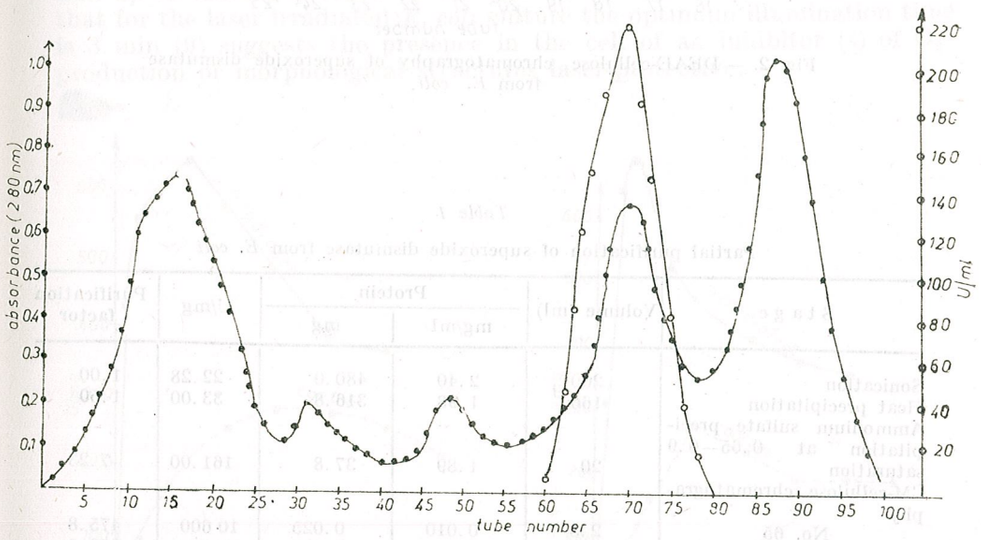

Fig. 1. — Elution curve of proteins and superoxide dismutase from CM-cellulose.

superoxide dismutase activity were pooled and concentrated by ultrafiltration on an Amicon membrane. The enzyme solution was dialysed against distilled water overnight and applied to a DEAE-cellulose column (28 × 1.8 cm) equilibrated with 5×10^{-3}M potassium phosphate buffer pH 7.8. After sample sorption, the column was eluted with the same buffer solution. The enzyme positively electric charged was excluded from anionit, in a concentrated form (Fig. 2). The specific activity of superoxide dismutase after each step of the purification procedure is presented in Table 1.

The kinetic parameters of the superoxide dismutase preparation were comparatively studied before and after the nitrogen laser irradiation.

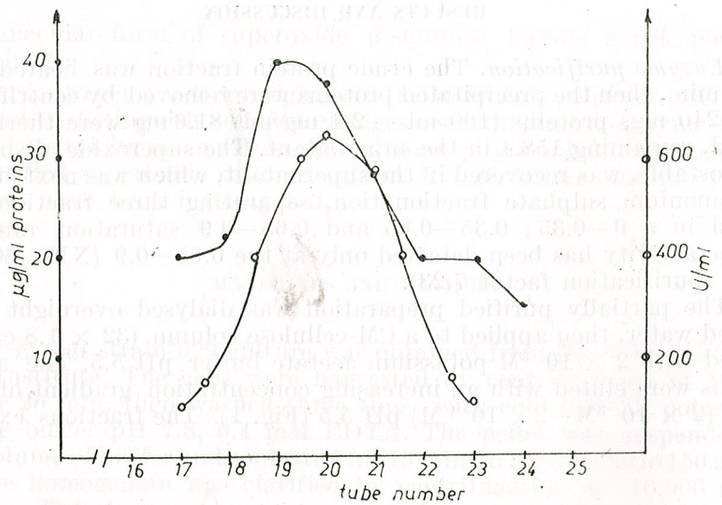

Fig. 2. — DEAE-cellulose chromatography of superoxide dismutase from *E. coli*.

Table 1

Partial purification of superoxide dismutase from *E. coli*

Stage	Volume (ml)	Protein		U/mg	Purification factor
		mg/ml	mg		
Sonication	200	2.40	480.0	22.28	1.00
Heat precipitation	160	1.98	316.8	33.00	1.50
Ammonium sulfate precipitation at 0.65—0.9 saturation	20	1.89	37.8	161.00	7.23
CM-cellulose chromatography					
No. 65	2.5	0.010	0.025	10 600	475.8
No. 66	2.5	0.015	0.037	8 000	359.0
No. 67	2.5	0.018	0.045	9 666	433.9
No. 68	2.5	0.020	0.050	3 889	426.4
No. 69	2.5	0.054	0.135	3 667	174.5
No. 70	2.5	0.060	0.150	3 500	164.6
No. 71	2.5	0.060	0.150	3 791	157.0
No. 72	2.5	0.048	0.120	3 700	170.2
No. 73	2.5	0.040	0.100	3 950	177.3
No. 74	2.5	0.040	0.100	2 650	118.3
No. 75	2.5	0.035	0.087	2 571	115.4
DEAE-cellulose chromatography					
No. 19	2.0	0.040	0.080	13 300	597.0
No. 20	2.0	0.038	0.076	16 974	761.8
No. 21	2.0	0.028	0.056	20 785	932.9
No. 22	2.0	0.020	0.040	11 000	493.7

Riboflavin may be looked at as an indirect substrate for the superoxide dismutase since by reduction it generates superoxide radicals. Fig. 3 shows the effect of riboflavin concentration in the reaction medium on the irradiated and nonirradiated enzyme. The laser irradiation markedly increases the superoxide dismutase activity and decreases the amount of riboflavin required for maximum enzyme activity (from 3.6 nmoles to 1.8 nmoles). This may be explained either by the fact that the laser irradiation induces conformational changes of the dimer enzyme leading to an increased substrate affinity, or that irradiation yields $O_2^{-\cdot}$ in the cell and these radicals add up to those generated by the photoreduction of riboflavin.

In another experiment we tested the effect of the UV illumination time of the reaction mixture on the enzyme activity. As seen in Fig. 4 in the case of the irradiated enzyme, a shorter illumination time — 45 sec. — is needed than for the control experiment — 120 sec. These findings show that the laser irradiation yields $O_2^{-\cdot}$ in the medium. These radicals add up to those generated by the photoreduction of riboflavin. The fact that for the laser irradiated *E. coli* culture the optimum illumination time is 3 min (9) suggests the presence in the cell of an inhibitor (s) of $O_2^{-\cdot}$ production or morphological structures laser-protective.

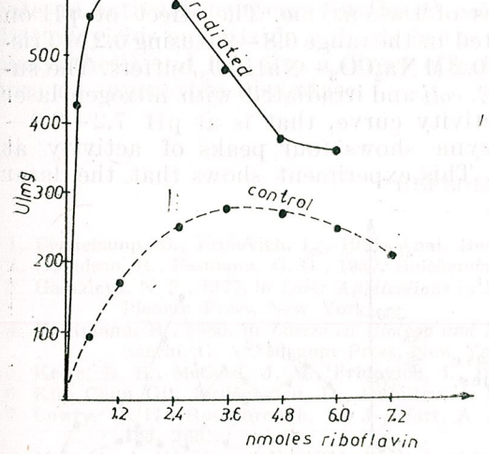

Fig. 3. — The saturation curve in riboflavin of a purified superoxide dismutase preparation, before and after nitrogen laser irradiation.

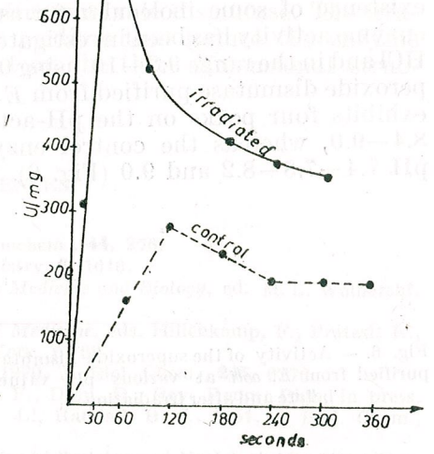

Fig. 4. — The effect of illumination time on the purified superoxide dismutase activity before and after nitrogen laser irradiation.

Fig. 5 shows the effect of temperature on the irradiated and nonirradiated enzyme preparations. The control preparation had its optimum temperature at 37°C, whereas following the laser irradiation the optimum temperature of the superoxide dismutase drops to 30°C. It would appear

that the laser makes the enzyme heat labile. This is more apparent at temperatures above 30°C.

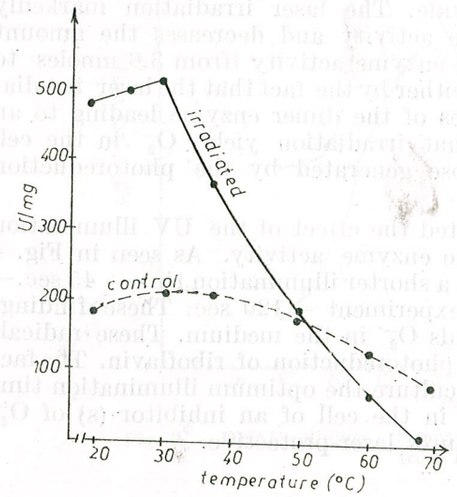

Fig. 5 — The effect of temperature on the superoxide dismutase activity before and after laser irradiation.

The medium pH/catalytic capacity function may offer indications about the nature of the catalytic active site micromedium and the existence of some molecular forms of the enzyme. The effect of pH on enzyme activity has been investigated in the range 6.8—9.0 using 0.2 M Tris-HCl and in the range 9.2—10 using 0.2 M Na_2CO_3—$NaHCO_3$ buffers. The superoxide dismutase purified from *E. coli* and irradiated with nitrogen laser exhibits four peaks on the pH-activity curve, that is at pH 7.2—8.0—8.4—9.0, whereas the control enzyme shows four peaks of activity at pH 7.4—7.8—8.2 and 9.0 (Fig. 6). This experiment shows that the laser

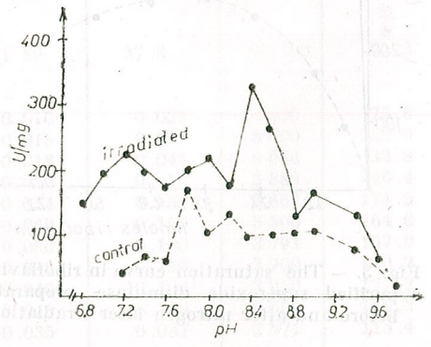

Fig. 6. — Activity of the superoxide dismutase purified from *E. coli* at various pH values, before and after irradiation.

irradiation affects the microenvironment of the active site of the enzyme, likely operating on some interactions which stabilize the enzyme conformation. The laser irradiated enzyme has the highest activity at pH 8.4, whereas the nonirradiated superoxide dismutase has its highest activity at pH 7.8.

The results presented in this paper have shown that the laser irradiation causes conformational changes of the dimeric structure of *E. coli* superoxide dismutase leading to an increased activity, a greater affinity for its substrate and a decrease of its thermal stability. These changes are only observed in the first 24 h from the moment of irradiation after which the physico-chemical properties of the enzyme returned to normal values (Table 2).

Table 2

The activity of purified *E. coli* superoxide dismutase at various times after irradiation

Time after irradiation	U/mg
20 min	9 600
30 min	9 666
60 min	9 580
2 h	9 600
6 h	9 600
24 h	9 600
48 h	3 889
72 h	693

Our studies dealing with the effect of laser irradiation on superoxide dismutase activity in *E. coli* cultures (9) and the results of this paper indicate an activation process for the *E. coli* superoxide dismutase. The irradiation yields superoxide radicals in the medium that induce the enzyme biosynthesis and has also a direct effect on the three-dimensional structure of superoxide dismutase.

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MODIFICATIONS OF THE LIPID METABOLISM IN THE LAYING HEN OVARY, ELICITED BY THE ADMINISTRATION OF SPIRULINA

N. BUCUR and VICTORIA-DOINA SANDU

Fodder supplemented with 5 or 10% Spirulina biomass in laying hens (Roso hybrid) improved both the quality of their eggs and the lipid metabolism of the ovary (total lipids, total cholesterol, non-specific esterase).

Proteins, quantitatively and qualitatively fitted to the necessities of laying hens, are compulsory components of the fodder under the conditions of intensive breeding (3, 7, 8, 10). Proteins are necessary both to cope with the general need of nitrogen, and to supply essential amino acids indispensable for egg production. These requirements justify the efforts of many investigators to find alternative or complementary sources of proteins utilizable in aviculture. Promising results in egg production have been obtained using some algae, especially Spirulina (S. plat., S. max.) biomass added to the food of hens. Due to their high protein content (64—72% of the dry matter) rich in amino acids needed for egg production (6), Spirulina can replace the traditional protein sources; due to its content in carotenoids, it ensures also an intense colour of the yolk in the produced eggs (1, 2).

Investigations reported in the literature on the effects of Spirulina biomass administration concern especially egg production (2, 11). The possible influences upon the metabolism of the ovary — the essential organ in egg genesis — have not been dealt with. That is what we tried to do in this research.

MATERIAL AND METHODS

Investigations were conducted on adult hens (Roso hybrid), aged 37 weeks at the beginning of experiments. The birds were divided in three groups:

—Control group (C), fed with a standard fodder for laying hens;

— Grup I, fed the same fodder supplemented with 5% Spirulina biomass;

— Grup II, fed the same fodder supplemented with 10% S. biomass.

The biomass was of Spirulina platensis and has been produced in our Research Centre. The experimental feeding lasted for 50 days. During this time, the hens were caged individually, care being taken to ensure them the same conditions of light and temperature. Fodder and water were given ad libitum. Fodder consumption and egg production were monitored at each hen.

The hens were sacrificed by exsanguination, at 9—12 h in the morning after about 20 h of fasting. Ovaries were sampled only from those

hens which had an egg in the uterus, i.e. which were at about 20 h after the last oviposition ; thus, all our experimental data refer to one and the same stage of the functional cycle.

Some pieces of the ovary were quickly frozen in liquid nitrogen and sectioned (at 10 μ) on a Slee-type cryotom. On these sections total sudanophil lipids were put into evidence with Sudan Black B, and the activity of the non-specific esterase by the Gömöri method (5). Other fragments were used for the determination of total cholesterol by the method of Engelhardt and Smirnova (4).

RESULTS

The eggs of the Spirulina-fed hens had thicker and more coloured shells than those of the control group. The yolk was more intensely orange-coloured, the intensity of the colour reaching and even overpassing the maximum level of the Roche scale.

The ovaries of the hens of groups I and II had, as compared to those of group C, a larger number of mature and intensely coloured egg cells.

The histochemical study revealed a larger content of sudanophil lipids both in the interstitial tissue and the granular layer of the secondary follicles, in the ovaries of Spirulina fed hens (the order was II > I > C) (Figs. 1 and 2). The non-specific esterase activity was lower in C hens.

Total cholesterol content (Fig. 3) was strongly decreased in both Spirulina-fed groups (−36.6% in I, −43.3% in II, both a $P < 0.001$).

DISCUSSION

It results from the above data that addition of Spirulina biomass to the fodder of laying hens exerts an influence both on the quality of eggs and on the lipid metabolism of ovary tissue.

It seems that alga stimulates the increase and maturation of follicles. The intensified colour of egg cells (and of the yolk of the eggs) shows that the carotenoid pigments of Spirulina are utilized the ovary.

The increased lipid content and the reduced esterase activity indicate a lipid storage in the ovary, resulting from the reduction of catabolic processes. We suggest that this excess of lipids constitutes an energetic reserve of the ovary.

We cannot give a definite explanation for the significant decrease of the cholesterol content. The following putative causes may be referred to :

— a stimulation by Spirulina of the transfer of cholesterol into the yolk, where it represents 1.3% or even more (9) ;

— an alteration of synthesis in the liver (as the main place of cholesterologenesis) and/or of the transfer to the ovary ;

— a stimulation by the alga of the production of ovarian hormones, and thus of the consumption of cholesterol, as a precursor of estrogens.

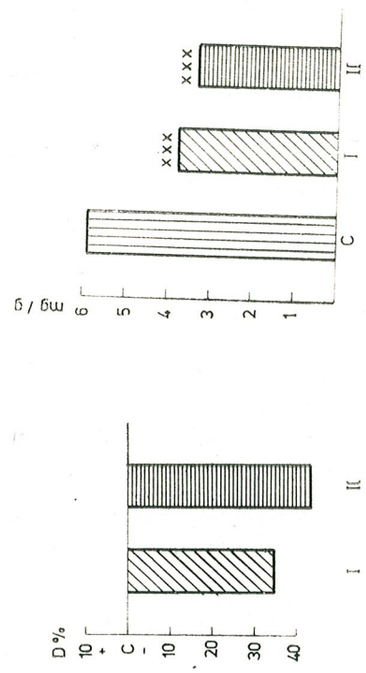

Fig. 3. — Level of total cholesterol in the ovary of hens from groups C, I and II (absolute values and percentage differences in groups I and II as compared to C).

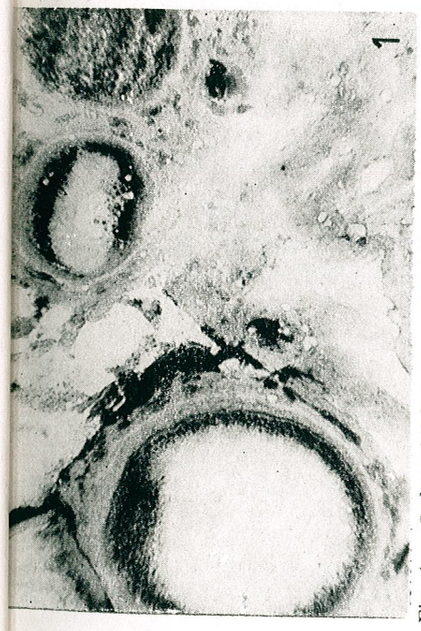

Fig. 1. — Sudanophil lipid contents in the ovary of control hens.

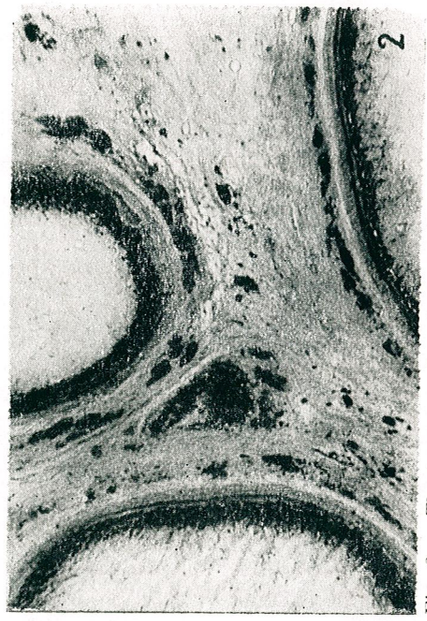

Fig. 2. — The increase of sudanophil lipid contents in the ovary of hens fed on fodder supplemented with 10% Spirulina (group II).

CONCLUSION

1. The alga Spirulina, added to the food of laying hens, has — in our experimental conditions — benefic effects upon the quality of eggs.

2. Spirulina seems to exert a direct effect upon the ovary, stimulating the maturation of follicles and influencing its tissue metabolism especially that of lipids.

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EFFECT OF BETA-ADRENOCEPTOR BLOCKADE WITH PROPRANOLOL IN STRESSED YOUNG RATS UPON GLUCOSE UPTAKE AND INSULIN-SENSITIVITY OF THE ISOLATED DIAPHRAGM

J. MADAR, MARIA GROSU, NINA ŞILDAN and ANA ILONCA

The effect of propranolol administration on the background of formalin-induced stress upon basal and insulin-stimulated glucose uptake by isolated diaphragms was investigated in immature and mature young male Wistar rats. After daily stress induction for 5 days, the basal glucose uptake of hemidiaphragms from Krebs-Henseleit solution in both age-groups was reduced (by 17.7 and 26.7%, respectively), and the insulin promoted glucose uptake was diminished by 68.7 and 20.9, respectively. When stress-induction was associated with s.c. administration of propranolol, the basal glucose uptake of hemidiaphragms remained at normal level both in immature and mature animals, while the muscle insulin resistance was reduced with 75.3 and 15.8%, respectively. It has been concluded that in stress-induced inhibition of glucose uptake and insulin sensitivity of skeletal muscle in young rats the age-related beta-adrenergic activation is mainly involved.

Insulin-stimulated entry of glucose into muscle "in vivo" is a major factor of blood glucose regulation (5), (14), (18), (19). Both impairment of insulin secretion and impairment of peripheral sensitivity to insulin (insulin resistance) may contribute to the hyperglycemia that occurs in white rats under stress conditions (12), (9), (19)—(24).

There is evidence that soleus muscle from rats after hemorrhagic stress is insensitive to insulin "in vitro" (4). We pointed out elsewhere that the hyperglycemic effect and the reducing action of formalin-induced stress, upon basal and insulin-stimulated glucose uptake by isolated diaphragms from male young rats, are age-related (20)—(24).

It is well established that epinephrine excess (6) and beta-adrenoceptor stimulation (1) are involved in human insulin resistance. Recent investigations have identified the beta-adrenoceptors in skeletal muscles (8) and it has been demonstrated that a beta-adrenoceptor adenylate-cyclase-system is present in the sarcolemmal membrane (27) and transversal tubules (3) of striated muscles.

Propranolol is considered as a specific beta-adrenoceptor blocking agent, without sympathomimetic activity (17). Starting from this fact, and on the ground of the above data, the present investigations were undertaken to clarify the relative importance of beta-adrenoceptor blockade with propranolol in stressed immature and mature young rats in the basal and insulin-stimulated glucose uptake by isolated diaphragmatic muscle.

MATERIALS AND METHODS

Groups of normal, stressed, and propranolol-treated stressed immature (35-day-old) and mature (60-day-old) male albino Wistar rats were used as tissue-donors for experiments, from the stockfarm of our labora-

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, Nº 2, P. 107—111, BUCAREST, 1988

tory. They were fed on standard diet and kept at 20—21°C ambient with 12 h of light per day from 07.00 h.

The daily stress-stimulus, for a period of 5 days, was produced between 09.00 h and 10.00 h, by s.c. injection of 0.25 ml formalin 2% ("Chemapol", Czechoslovakia) per 100 g b.w. in the interscapular region.

Commercial propranolol (1-/isopropylamino/-3-/-1-napththyloxy/-2--propanol), "I.M.B.", Bucharest, was administered s.c. in daily doses of 50 micrograms/100 g b.w. for 5 days. Normal and stressed controls were injected with 0.25 ml saline.

After an 18-hour fasting period and 24 hours following the last injections, the animals were sacrificed by decapitation. The diaphragms were quickly excised and immersed for 20 minutes in ice-cold Krebs—Henseleit saline (without glucose, pH = 7.4) and sectioned in approximately equal hemiorgans.

From each animal a hemidiaphragm was used for testing the "in vitro" basal glucose uptake, while on the other half the insulin-stimulated glucose uptake was tested.

The incubation medium was 1 ml Krebs—Henseleit bicarbonate solution (pH = 7.4), containing 16.7 micromoles glucose (p.a. "Merck") and 2 mg calf-skin gelatine (p.a. "Merck"). Recrystallized glucagon-free ox-insulin ("Calbiochem", California, potency 23 I.U./mg, grade B) was used in a final concentration of 10^{-3} I.U. per ml incubation medium.

The incubation of hemiorgans was performed for 2 hours at 37.6°C in an original device (18), with a gas phase of 95% O_2 + 5% CO_2 and a shaking of 90 oscillations per minute and 5 cm amplitude.

The initial and final glucose content of the incubation medium was determined enzymatically using the Test-Combination Glucose Kit ("Boehringer", GmbH, Mannheim, FRG), according to Werner *et al.* (29). The colour intensity of the samples was measured spctrophotometrically at 610 nm, using a "Specol" apparatus (Carl Zeiss, Jena, GDR).

The basal glucose uptake (BAS) as well as the global glucose uptake in the presence of insulin (INS) by the hemidiaphragms were calculated in micromoles/100 mg fresh tissue per 2 hours. The insulin-sensitivity of diaphragmatic muscle was evaluated by calculating the insulin-stimulated net glucose uptake, i.e. by estimating the values of Δ (INS—BAS).

The results were statistically checked for the homogeneity of the means using Chauvenet's criterion. Mean values were compared according to Student's t test, $P = 0.05$ being accepted as the limit of significance.

RESULTS AND DISCUSSIONS

From the results summarized in Table 1 and Figure 1 it is obvious that the basal and insulin-stimulated "in vitro" glucose uptake by isolated diaphragms from immature normal rats are significantly higher than in the case of mature normal group. This observation is in good agreement with our previous data which indicate that the age of young rats is an important conditioning factor both in glucose uptake and in insulin effect upon this process in isolated diaphragms (20), (21), (23). Other authors

also concluded that the age influences the glucose metabolism and the response to insulin of the skeletal muscle in white rats (10), (11).

Table 1

Basal glucose uptake (BAS), global glucose uptake in the presence of insulin (INS) and insulin-stimulated net glucose uptake (ΔINS—BAS) by isolated hemidiaphragms from normal (N), stressed (S) and propranolol-treated stressed (PS) groups of immature and mature young Wistar rats

Groups	micromole glucose uptake/100 mg fresh tissue per 2 hrs		
	BAS	INS	Δ(INS—BAS)
	35-day-old animals		
N	4.9055±0.394 (8)	7.1123±0.531 (8)	2.2068±0.121 (8)
S	4.0345±0.176 (8)	4.7258±0.130 (8)	0.6913±0.034 (8)
PS	4.2450±0.127 (8)	5.4575±0.158 (8)	1.2123±0.09 (8)
	60-day-old animals		
N	3.0780±0.252* (8)	4.8275±0.196* (8)	1.7495±0.105* (8)
S	2.2549±0.107* (8)	3.6372±0.229* (8)	1.3823±0.042* (8)
PS	2.7340±0.114* (8)	4.3249±0.147* (8)	1.5909±0.07* (8)

(The values represent means ± S.E. The number of experiments is given in brackets. Asterisks show statistically significant differences vs. the corresponding values obtained in 35-day-old N groups).

24 hours after cessation of repeated stress-induction with formalin, the basal glucose uptake by the hemidiaphragms of 35-day-old animals decreases with 17.7%, while the insulin-promoted glucose uptake of the diaphragms is reduced with 68.7%, as compared to the corresponding normal values. In the case of 60-day-old group formalin stress elicits a decrease with 26.7% of muscular basal glucose uptake and reduces with 20.9% the insulin-stimulated glucose uptake by hemidiaphragms.

We have shown elsewhere that after repeated acute formalin stress in young rats the reduction of insulin release during intravenous glucose tolerance test is associated with the impairment of peripheral glucose utilization, and that in this response the insulin resistance of skeletal muscle plays an important causative role (19). On the other hand, it has been concluded that the acute impairment of tissue glucose utilization in injured rats is strongly related to the peripheral insulin resistance (4), (9), (12).

As it results from the data referring to the association of formalin stres-stimulus with propranolol administration, in the case of immature

animals the basal glucose uptake of isolated hemidiaphragms remains at normal range, while the insulin resistance of diaphragmatic muscle is reduced with 75.3 in comparison with that found in stressed controls. In the case of mature stressed group the basal glucose uptake of diaphragmatic muscle is nearly normal, while the resistance of muscle to insulin is reduced only with 15.8% versus the values of stressed controls.

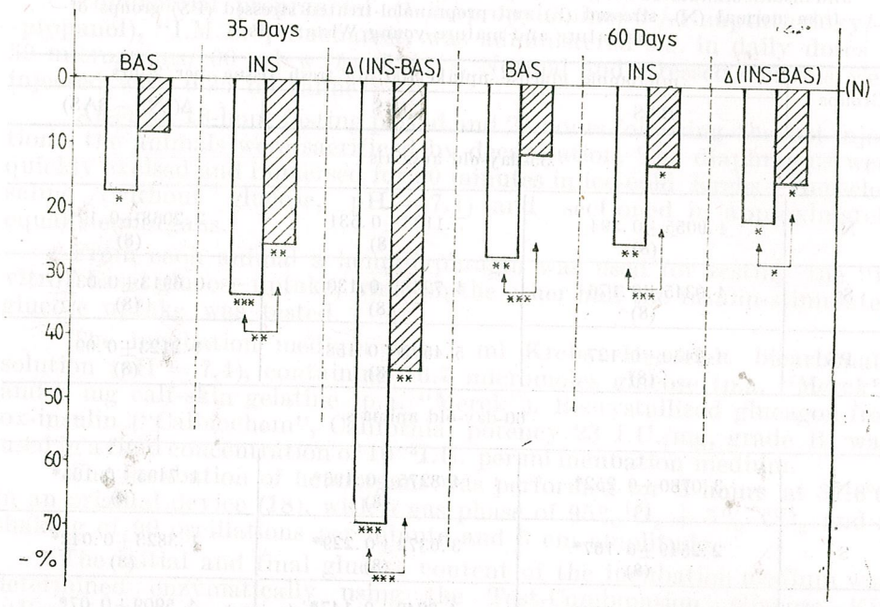

Fig. 1. — Percent differences vs. the corresponding normal values (N) of "in vitro" basal glucose uptake (BAS), global glucose uptake in the presence of insulin (INS), and insulin-stimulated net glucose uptake (ΔINS—BAS) by the hemidiaphragms isolated from 35- and 60-day-old rats. (White columns = stressed groups; shaded columns = propranolol-treated stressed groups; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The arrows indicate significant modifications between stressed and propranolol-treated stressed groups).

It is well established that in stressed rats the adrenergic function and the catecholamine release are strongly activated (7), (15), and there is clinical evidence that epinephrine administration elicits insulin resistance (6) and beta-adrenoceptor stimulation is involved in diabetic insulin resistance (1). On this basis one can assume that in age-related anti-insulin effect of the repeated formalin stress the activation of muscular beta-adrenoceptors by excessively released catecholamines is mainly involved.

There is evidence that the concentration and functionality of beta-adrenoceptors in tissues of some target organs of white rats present age-related modifications (2), (13), (25), (26), (28) and adrenergic mediated stress-responses are age-related (16). On the other hand, investigations in our laboratory have pointed out that propranolol administered in white rats exerts age-dependent actions upon some parameters of carbohydrate metabolism in isolated skeletal and heart muscles (30), (31).

In conclusion, our results obtained in stressed and in propranolol-treated stressed young rats suggest that beta-adrenoceptor stimulation participates in age-related decrease of glucose uptake and insulin sensitivity of diaphragmatic muscle.

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EFFECTS OF THIOUREA ADMINISTRATION ON THE INTESTINAL ABSORPTION OF GLUCOSE IN CHICKENS

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Cornish-Rock chickens were administered thiourea, 20 mg per kg body weight per day, in the 5th and 6th days of life. Intestinal glucose absorption was measured one day after the second administration, and at the ages of 13, 27 and 63 days. A late and reversible decrease of the process was put into evidence, due probably to the inhibition of thyroid function by the drug.

Carbohydrate absorption at the level of the small intestine is similar for all types of sugars (17), (19), and its mechanism is the same in any intestinal segment where it takes place (19). The absorption depends on the age of animals (2), (15), on the concentration of carbohydrates and proteins in the food (9), (18), on the ionic conditions in the intestine (1), (8), and on the concentrations of various hormones in the blood (4), (7), (13), (14). Thus, factors influencing the hormonal pattern may be expected to affect also intestinal absorption.

Pursuing our research in this direction (5), (6), we investigated the effect of thiourea administration in the early ontogenesis of the chicken upon the absorption of glucose from the jejunum. It is well known that thiourea reversibly blocks the thyroid function (11).

MATERIAL AND METHODS

Experiments were conducted on Cornish—Rock chickens, purchased one day after hatching and reared in our laboratory, in normal zoohygienic conditions. Age-fitted concentrated fodder and water were given *ad libitum*.

Two doses of *pro analysi* thiourea, 20 mg per kg body weight each, were given in the fodder, on the 5th and 6th posteclosional days. The effects were investigated at the ages of 6 (the day after the second administration), 13, 27 and 63 days, on groups of 10 chickens each, paired by control groups of the same age and the same number of individuals.

The chickens were sacrificed by decapitation, after an 18-hours fasting. A portion of the jejunum was immediately sampled and made an "everted sac" (14), (20), about 4 cm long.The sac was filled with Krebs—Henseleit saline, (phosphate buffered to pH 7.4, at a final phosphate concentration of 10 mM), and containing 5 mM glucose. The tied sac was introduced in a flask containing the same saline with 10 mM glucose. This "external" fluid was made radioactive (4.10^5 disintegrations per minute (DPM) per ml) with (U—^{14}C)-D-glucose. The labelled substance, having a specific activity of 5.52 mCi/mmol and a radiochemical purity of 98%, was a product of the Institute for Physics and Nuclear Engineering, Bucharest. After one hour of incubation in a shaking water-bath thermosta-

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, Nº 2, P. 113—115, BUCAREST, 1988

ted at 40 ± 0.2°C, the sacs were rinsed with distilled water, blotted on filter paper, and opened. The volume of the fluid content of each sac was measured, and its radioactivity determined in a Betaszint BF—5000/300 liquid scintillation spectrometer (Berthold, F. R. of Germany) using Bray's solution. A correction was applied to eliminate the effect of the "quench" introduced by the glass of the scintillation flasks. The empty sacs were weighed. Results were expressed either in DPM per gram jejunum, or in percentage partition coefficients.

Statistical processing of the data involved the use of Chauvenet's criterion for the elimination of aberrant values, and of Student's "t" test for comparisons between the mean values of paired groups. Significance was considered when $P \leqslant 0.05$.

RESULTS AND DISCUSSIONS

The data obtained (Table 1) show an inhibitory effect of thiourea upon the jejunal glucose absorption, the effect occurring at a late stage after the drug administration. It can be due to an inhibition of the thyroid function, as it is known that thyroid hormones enhance the intestinal carbohydrate absorption (13). The same effect was obtained in a previous investigation, when we used a larger dose of thiourea and administered it at a still earlier age. Similar results were also obtained by other authors with antithyroidian drugs (10).

Table 1

Glucose absorption (DPM) and partition coefficient (%) in the chicken small intestine after administration of thiourea

Days:		6		13		27		63	
Groups:		C	T	C	T	C	T	C	T
DPM	$\bar{x}$	1471.3±	1288.1±	753.6±	1051±	447.6±	214.4±	12.6±	10.2±
	±SE	523.8	379.0	109.0	264.0	94.1	59.0	1.5	2.5
	D%	—	−12.4	—	39.4	—	−52.1	—	−18.5
	p	—	—	—	—	—	<0.05	—	—
%	$\bar{x}$	79.3±	74.8±	39.9±	39.8±	30.7±	15.6±	—	—
	±SE	8.9	8.4	5.2	3.7	5.4	0.8	—	—
	D%	—	−5.6	—	−0.2	—	−49.1	—	—
	p	—	—	—	—	—	<0.02	—	—

$\bar{x}$ = mean values; ±SE = standard error; D% = percentage differences versus control; C = control; T = treated. Other explanations see in text.

The intimate mechanism of action of thyroid hormones at the level of the intestine is not known. Their action may be exerted upon the glucose or upon the ionic conditions at the level of the absorbant epithelium. The glucose carrier is Na^+-dependent (12), and a reduction of the sodium content in the fodder leads to a pronounced decrease of glucose absorption (16),

The latter result was obtained on the caecum, but it might be true for the jejunum too, as the glucose transport proceeds in the same manner in both intestinal segments of the birds (3). Other possible targets of the hormone action could be some enzymes involved in the intestinal cell membranes permeability, such as the alkaline phosphatase, modifications of which under the action of the thyroid hormones have been put into evidence (11), (13).

Our data clearly show that the thyroid inhibition due to thiourea is reversible: in the last age-group the values are normal again.

In conclusion, thiourea administered to young chickens elicits a retarded and reversible decrease of the jejunal glucose absorption, probably due to a thyroid inhibition.

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CORRELATIONS BETWEEN DIETARY PROTEIN DEFICIENCY AND HEPATIC GLYCOGEN METABOLISM IN DUCKS

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Ducks fed on normal and protein deficient diets were compared. The effects of the slight but long term protein deficiency were : 1) a decreased glycogen breakdown 2) a decreased glycolysis 3) an increased glucose production in the liver. These results are probably related to the protein deficiency-induced hypoinsulinemia, noticed in the literature.

Fasting lowers plasma insulin levels (3, 6). Some literature data (4, 8, 10, 12, 15, 18, 19) and conclusions which derive from some experimental data (11) indicate that dietary protein deficiency has similar effects on insulinemia with those of fasting, at least in mammals.

Although the importance of insulin in birds was questioned in some papers (13), others (7, 16) hold true the idea that insulin has an important role in the regulation of tissue carbohydrate metabolism also in birds.

Correlations existing between dietary protein deficiency and carbohydrate metabolism were observed in our laboratory in a previous experiment made on broiler chickens (manuscript in preparation).

Taking into account the above-mentioned observations, this study attempts to search correlations between protein deficiency and glycogen metabolism in the liver.

MATERIALS AND METHODS

The experiments were performed on 50-day old ducks, divided into two groups formed by 6 individuals each. Groups 18 and 14 were fed on 18% and 14% protein containing diets, respectively. The ducks were Romanian four line hybrids for fowl. The composition, raw/protein content, and metabolizable energy content of diets are given in Table 1.

The ducks were sacrificed by decapitation. Liver was immediately sampled on ice. 30 mg liver was homogenized on ice with 1 ml Krebs—Henseleit phosphate buffer (pH = 7.4); 0.2 ml glycogen solution (10 mg per ml) as well as 0.05 ml toluene (to avoid bacterial infection) were added. The homogenates were incubated at 37°C for three hours.

Glycogen content of the liver, as well as glycogen content of homogenates (with the glycogen solution added) before and after incubation were determined according to (9). Glucose and glucose-6-phosphate were chromatographically separated (silicagel thin layer chromatography)and quantitatively determined according to (5). The test solutions for the standard curves were submitted to similar procedures to experimental samples.

Statistic analysis of the results was made according to the paired Student's t test.

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, N° 2, P. 117—120, BUCAREST, 1988

Table 1

The composition, raw protein content and metabolizable energy content of the experimental diets

Components	Experimental groups	
	18	14
Corn	66.1	72.8
Soya-bean	22.0	13.5
Sunflower-bean	55.0	3.0
Fish flour	1.0	1.0
Meat flour	1.0	1.0
Methionine premix	0.7	2.2
$CaCO_3$	1.0	1.4
Ca_2PO_3	1.0	2.6
Salt	0.2	0.5
Zoofort (vitamine-mineralpremix)	1.0	1.0
Choline premix	1.0	1.0
Raw protein content	18.12	14.25
Metabolizable energy	2826.35	2829.80

Values are given in %, except for the metabolizable energy content, which is given in kJ/kg.

RESULTS AND DISCUSSION

The results of the experiment are sumarized in Table 2. The glycogen content (G) of the liver was elevated in group 14, as compared to group 18. Glycogen breakdown (ΔG, measured as a difference between initial and final quantities of glycogen in the homogenate), was quicker in group 18, while glucose production rate was lower in this group (Δg). The amount of glycolytically degraded glucose-6-phosphate was calculated based on the following formula :

$$dg = \Delta G - \Delta g - \Delta g6P$$

where ΔG is the amount of consumed glycogen, Δg and Δg6P are the resulting amounts of glucose and glucose-6-phosphate. The liver of group 14 has a significantly lower value of dg. The increase in glucose-6-phosphate content of the homogenate shows no significant differences between the experimental groups.

Since Claude Bernard (1) noticed that liver releases glucose to the blood stream, liver carbohydrate metabolism is considered to have a unique position in the carbohydrate metabolism of the whole organism. The liver is able both to produce and to consume glucose. The glucose output in the liver derives mainly from glycogenolysis, and only to a smaller extent from gluconeogenesis (17).

The general patterns of insulin action on the liver in birds are similar to the well-known effects of this hormone in mammals (7, 16), although the insulin sensitivity of tissues is lower. It is worth mentioning that insulin concentrations in portal venous blood are 3—10 fold higher as compared to peripheral veins (2).

Table 2

The effect of dietary protein deficiency on the glycogen breakdown in the liver

Parameters	Experimental groups	
	18	14
Glycogen content	147.9±22.8 (6)	252.8±32.7* (6)
Glycogen degradation	−120.2±2.8 (6)	−86.7±7.9*** (6)
Glucose production	+35.2±3.6 (6)	+43.9±1.6** (6)
Glucose-6-phosphate production	+8.1±3.7 (6)	+13.3±6.5 (6)
Dg	−79.9±5.8 (6)	−29.5±3.4**** (6)

Values (means±SEM) are given in μM/g tissue for glycogen content, and in μM/g tissue/hour for glycogen degradation, glucose production, glucose-6-phosphate production, and Dg. The formula for the calculation of Dg is given in text.
* — significant difference at $p < 0.05$; ** — significant difference at $p < 0.02$; *** — significant difference at $p < 0.01$; *** — significant difference at $p < 0.001$. The number of determinations is given in the brackets.

Our results are in good agreement with the dietary protein deficiency-induced hypoinsulinemia noticed in the literature. Glucose-6-phosphate degradation was directioned mainly to glucose production, while in normally fed animals a larger amount of glucose-6-phosphate was degraded glycolytically (the pentose-phosphate pathway in mature birds is almost inactive (7, 13)). The reduction of glycogenolysis (ΔG) as well as the reduction of glycolysis (dg) could be due to an enhanced lipolysis. It is considered that the antilipolytic effect of insulin is present in birds (16), thus, in hypoinsulinemia, carbohydrates degradation could be substituted by lipids degradation. The increased glycogen levels in group 14 could be due to the diet. It is known that tissular glycogen levels are strongly related to dietary carbohydrate intake (14). In our experiment, the carbohydrate content of the protein deficient diet was higher.

It seems that dietary protein deficiency increases hepatic glucose production, while carbohydrates are less utilized as fuel in the liver. These effects could be results of the lowered plasma insulin levels noticed in the literature.

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VARIATION OF SERUM NATRIUM, POTASSIUM AND CALCIUM IONS IN TREATMENTS WITH SOME NEW HYPOCHOLESTEROLEMIANTS

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Oral administration, to Chinchilla rabbits subjected to an atherogenic regimen, of a new biosynthesis antibiotic A. 12.3 (400 u/kg body/day), as powder, associated with ergosterol (0.5 mg/kg body/day) or with the original vasoactive product rutacyl (4.5 mg/kg body/day), evidences, besides hypolipemiant and hypocholesterolemiant properties, a positive effect, too, upon the modification of the concentration of serum natrium, potassium and calcium ions, similar to that induced by the chlofibrate drug (25 mg/kg body/day).

Certain investigations carried upon pathological dislipidemia, as well as on the administration of cholesterol to experimental animals have evidenced some modifications of the ion concentration (1), (3), (5), (6), (7), besides the increase of serum lipids and cholesterol.

Various polyene antibiotics exhibit significant hypolipemiant and hypocholesterolemiant effects (1—4), (17), (19) due to their interaction with sterols from the structure of cell membranes (10), (11), (12), (16), (20) which have been already mentioned in our previous investigations (1—4). They also have positive effects upon the modification of serum ion concentration, as we have stated in a paper (5) dealing with the effects of nystatin.

On the other hand, the positive effects of ergosterol on atherosclerosis (8), (18) and on improving the action of hypocholesterolemiant agents (2) have been studied concomitantly with the pharmacological properties of rutacyl, an original derivative of the rutosid flavonoid (13), (15) having implications in cardiovascular affections and also specific interactions with the ions of Na^+, K^+ and Ca^{2+} (14).

Starting from all these observations, the present paper studies the effects of the original biosynthesis antibiotic A.12.3, associated with ergosterol or rutacyl, upon the modifications of serum natrium, potassium and calcium concentration, with rabbits subjected to an atherogenic regimen.

MATERIALS AND METHODS

Experiments have been carried on 4 groups of Chinchilla rabbits — of 10 individuals — each of them of about 2.0 kg weight; they have been subjected to a uniform atherogenic regimen, by oral administration of a cholesterol dose of 0.125 g/kg body/day.

Group I (control group) was given only cholesterol on the whole duration of experiments. The other ones also received only cholesterol in the first stage of the experiment (from T_0 to T_1), then from T_1 to T_3 they have been treated differently. Thus group II has been orally given A.12.3, as powder (400 u/kg body/day), associated with ergosterol

(0.5 mg/kg body/day), group III received again orally, the same amount of A.12.3 associated with rutacyl (4.5 mg/kg body/day), while group IV has been orally administered chlofibrate (25 mg/kg body/day), considered as reference drug (7).

Analyses of concentration of serum Na^+, K^+ and Ca^{2+} ions have been performed, by flame-photometry, at the beginning of the experiment (T_0), after a fortnight of atherogenic regimen (T_1), after two weeks (T_2) and four weeks of treatment (T_3).

RESULTS

On the whole duration of the treatment, a series of variations of the concentration of serum Na^+, K^+ and Ca^{2+} ions is to be observed with all animal groups.

The normal initial values of serum concentration of Na^+, with all four groups, ranged between 265.33—299.66 mg/100 ml serum. In the first stage of atherogenic regimen, serum Na^+ increased with all groups, showing values ranging between 326.33—357.44 mg/100 ml serum. In the case of the control group, Na^+ maintains itself at concentrations higher than the normal value, on the whole duration of the treatment, yet lower than that recorded at T_1 (Fig. 1).

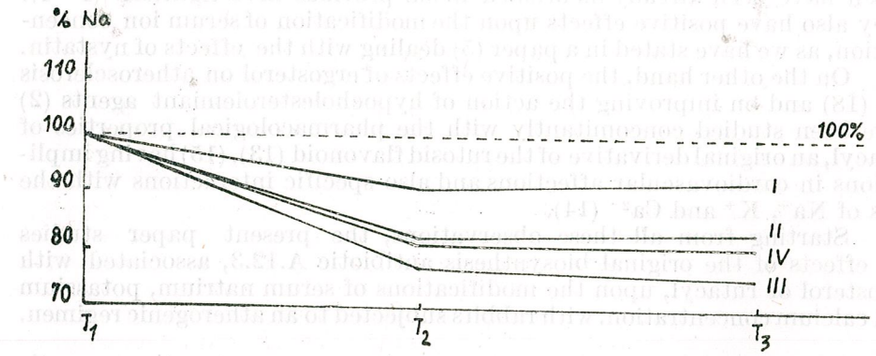

Fig. 1. — Variation (expressed in %, versus the value at the beginning of the treatment) of serum Na^+ concentration with rabbits subject to an atherogenic regimen, untreated (I), treated with A. 12.3 and ergosterol (II), A. 12.3 and rutacyl (III) or with chlofibrate (IV). T_1—T_2—treatment phases.

In the case of all groups treated with various agents, serum natrium shows lower values than those recorded with the control group, on the whole duration of the treatment, the final values, as compared with those at T_1 (100%), being of 83.13% with group II, 75.66% with group III and 80.90% with group IV.

The concentration of serum K^+ ions had normal initial values ranging between 17.25 and 26.04 mg/100 ml serum. In the first stage of atherogenic regimen, a non-uniform variation of K^+ has been registered with groups I and II, the concentration of this ion being lower than the normal

one, while this value was higher with groups III and IV. With the control group, the concentration of serum K^+ registered, on the whole duration of the atherogenic regimen, slightly lower values than the initial one, which nevertheless maintained themselves higher than that at T_1 (Fig. 2).

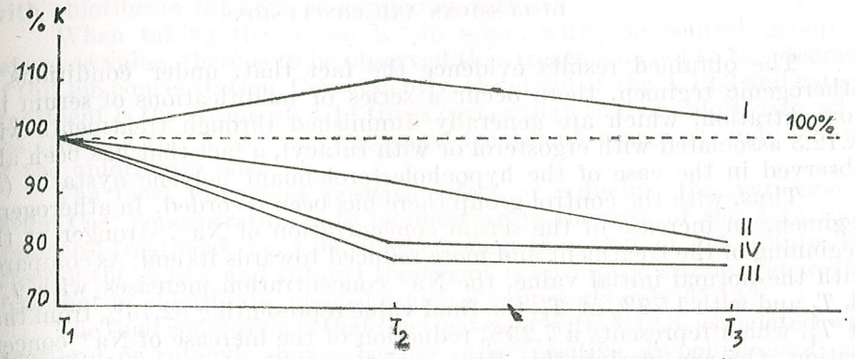

Fig. 2. — Variation of serum K^+ concentration with rabbits. Other explanations similar to those in Fig. 1.

With all treated groups, the serum K^+ values generally decreased as compared with those at T_1 (100%) the final ones being of 82.24% with group II, 78.55% with group III and 81.74% with group IV.

The normal initial values of serum Ca^{2+} ions ranged between 10.43—13.50 mg/100 serum, generally decreasing with all groups, in the first stage of atherogenic regimen (T_1), up to values ranging between 9.40—11.63 mg/100 ml serum.

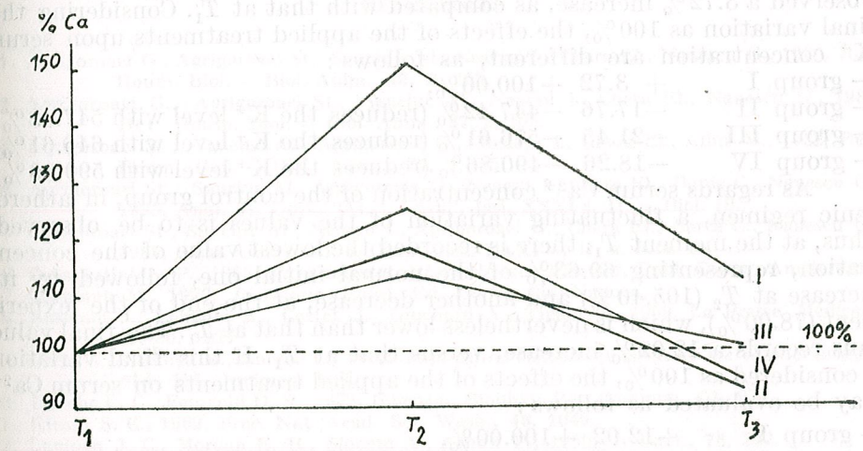

Fig. 3. — Variation of serum Ca^{2+} concentration with rabbits. Other explanations similar to those in Fig. 1.

In the following stages, the control group registers a slight increase of the Ca^{2+} concentration versus the T_1 one, the final value representing 112.02% as compared with it (Fig. 3). In the case of the treated groups,

such values recorded a slight increase at T_2, as against those at T_1, whereas the final ones are generally lower, representing 92.79% with group II, 100.27% with group III and 93.98% with group IV.

DISCUSSIONS AND CONCLUSIONS

The obtained results evidence the fact that, under conditions of atherogenic regimen, there occur a series of modifications of serum ion concentration, which are generally diminished through treatments with A.12.3 associated with ergosterol or with rutacyl, a fact that has been also observed in the case of the hypocholesterolemiant polyene nystatin (5).

Thus, with the control group there has been recorded, in atherogenic regimen, an increase of the serum concentration of Na^+, stronger at the beginning of the treatment and more reduced towards its end. As compared with the normal initial value, the Na^+ concentration increases with 9.75 at T_1 and with 1.79% at T_3, the final value representing 92.75% from that at T_1, which represents a 7.25% reduction of the increase of Na^+ concentration. On considering this final variation with the control group as 100%, the applied treatments have the following effects upon serum Na^+:

— group I — 7.25—100.00%,
— group II —16.87 —232.69% (reduces the Na^+ level with 132.69%),
— group III —24.54 —338.48% (reduces the Na^+ level with 238.48%),
— group IV —19.10 —263.45% (reduces the Na^+ level with 163.45%).

The serum K^+ concentration of the control group, under atherogenic conditions, is low on the whole duration of experiments, as compared with the normal initial concentration. The lowest value is registered at T_1, when it represents 86.18% of the initial one, finally being nevertheless observed a 3.72% increase, as compared with that at T_1. Considering this final variation as 100%, the effects of the applied treatments upon serum K^+ concentration are different, as follows:

— group I + 3.72 +100.00%,
— group II —17.76 —447.42% (reduces the K^+ level with 547.42%),
— group III —21.45 —576.61% (reduces the K^+ level with 646.61%),
— group IV —18.26 —490.86% (reduces the K^+ level with 590.86%).

As regards serum Ca^{2+} concentration of the control group, in atherogenic regimen, a fluctuating variation of the values is to be observed. Thus, at the moment T_1, there is recorded the lowest value of the concentration, representing 69.63% of the normal initial one, followed by its increase at T_2 (105.40%) and another decrease, at the end of the experiment (78.00%), which is nevertheless lower than that at T_1. The final value thus records a 12.02% increase, versus that at T_1. If this final variation is considered as 100%, the effects of the applied treatments on serum Ca^{2+} may be evaluated as follows:

— group I +12.02 +100.00%,
— group II — 7.21 —59.98% (reduces the Ca^{2+} level with 159.98%),
— group III + 0.27 + 2.25% (reduces the Ca^{2+} level with 97.75%),
— group IV — 6.02 —50.08% (reduces the Ca^{2+} level with 50.08%).

Accordingly, there is to be noted the fact that all applied treatments have positive effects on recovering the concentration of Na^+, K^+ and Ca^{2+}

ions, modified under atherogenic conditions. Thus, having in view the increase of Na^+ concentration, in an atherogenic regimen with the control group, the most efficient treatment for the Na^+ reduction is that with A.12.3 and rutacyl, 1.45 times stronger than the effect of chlofibrate. The A.12.3 and ergosterol treatment is also very efficient, yet weaker than that with chlofibrate (81.18% as compared with it).

When taking the serum K^+ increase, with the control group, as reference value, there is to be observed that treatments led to the decrease of this ion concentration. Consequently, the effect of the A.12.3 and rutacyl treatment is the strongest, 1.14 times higher than that of chlofibrate, while the effect of the A.12.3 and ergosterol treatment represents only 92.64% of the chlofibrate one.

The most important positive effect of reducing the variation of serum Ca^{2+} concentration is to be observed in the case of the A.12.3 and ergosterol treatment, i.e. 1.06 times stronger than that of chlofibrate. The effect of the A.12.3 and rutacyl treatment is also quite strong, yet weaker than that of chlofibrate, against which it represents only 65.13%.

The final conclusion is that the treatment with A.12.3, associated with ergosterol or rutacyl, shows, besides other positive (hypolipemiant and hypocholesterolemiant) effects, an effect of normalizing serum ion concentrations, too, which are being modified under atherogenic conditions, similar to that of the polyene antibiotic nystatin (5).

The association of A.12.3 with rutacyl induces the intensification of its action generally stronger than in the association with ergosterol, which is due to a specific interaction of the flavonoid with the ions of Na^+, K^+ and Ca^{2+}, discussed in other papers (14), too.

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ON THE HYPOCHOLESTEROLEMIANT ACTION OF SOME ORIGINAL ROMANIAN PREPARATIONS

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The treatment with an original biosynthesis antibiotic, A. 12.3 (400 u/kg body/day), in the form of powder, associated with ergosterol (0.5 mg/kg body/day), or with the original vasoactive product rutacyl (4.5 mg/kg body/day), orally administered to Chinchilla rabbits subjected to atherogenic regimen, induces the reduction of the total, free and esterified serum cholesterol level. Generally, the action of such products is similar with or stronger than that of the chlofibrate drug (25 mg/kg body/day), which evidences their hypocholesterolemiant properties and the possibility of their application in therapeutics.

Many authors have drawn the attention upon the importance of experimental investigations on laboratory animals, with a view to establishing new treatments in cardiovascular diseases (14), (15). Rabbits are considered as most suitable in such experiments, due to certain physiological properties which, under atherogenic conditions, induce a rapid perturbation of lipidic metabolism and, consequently, installation of atherosclerosis (5), (6), (9).

On the other hand, polyene antibiotics have been discovered as possessing hypolipemiant and hypocholesterolemiant action (17), (18), due to their specific interaction with sterols from the structure of cell membranes (7), (11), (12), (19). Actually, such properties have been demonstrated in our previous papers, devoted to polyene antibiotics nystatin and A.20.5 (1—4).

The present paper discusses the results regarding the hypocholesterolemiant action of A.12.3, a new original biosynthesis antibiotic, associated with other products with similar effects. Thus, having in view the ergosterol capacity of opposing itself to modifications characterizing atherosclerosis (8), (16), and of intensifying the effect of hypolipemiant and hypocholesterolemiant agents [2], as well as of the vasoactive properties and those of improving vascular trophicity of rutacyl, an original, diacetylated derivative of the rutosid flavonoid [13], in treatments applied to rabbits subjected to an atherogenic regimen, we have associated A.12.3 with ergosterol or with rutacyl.

MATERIALS AND METHODS

Experiments were performed on Chinchilla rabbits with an average body weight of 2.0 kg, divided on 4 batches of 10 individuals each. All animals have been subjected to a uniform atherogenic regimen, by oral administering of a 0.125 g/kg body/day cholesterol dose to each individual on the whole duration of the treatment.

REV. ROUM. BIOL. — BIOL. ANIM., TOME 33, N° 2, P. 127—132, BUCAREST, 1988

In the first stage of the atherogenic regimen ($T_0 - T_1$) no treatments were applied. Batch I (the control one) was given only cholesterol on the whole duration of the treatment. After the first stage (T_1), batch II was orally treated with A.12.3 as powder, (400 μ/kg/day) associated with ergosterol (0.5 mg/kg body/day), batch III was also orally given the same dose of A.12.3, associated with rutacyl (4.5 mg/kg body/day), while batch IV was treated, again orally, with chlofibrate (25 mg/kg body/day), considered as reference drug (6).

The effect of the atherogenic regimen and of the treatments applied upon the level of the total (TC) free (FC) and esterified (EC) serum cholesterol has been followed by analyses performed in the beginning of the experiment (T_0), a fortnight after the beginning of the atherogenic regimen (T_1), after a two week (T_2) and four week treatment (T_3), by spectrophotometric methods (10).

RESULTS

At all animal batches, serum total cholesterol showed normal initial values, ranging between 113.00 and 232.00 mg/100 ml serum.

After a two week atherogenic regimen, these values increased with all batches, ranging between 171.00 and 400.00 mg/100 ml serum. In the case of the control batch, total cholesterol increased ceaselessly, reaching at the end of the experiment a value representing 211.20% as compared with the initial one, and 122.50% as compared with that recorded at the moment T_1 (Fig. 1). With the treated groups, serum total cholesterol decreased to various degrees, at the end of the treatment being registered values which, compared with those of the T_1 moment, represent only 54.85% at batch II, 68.71% at batch III and 32.50% at batch IV.

Serum free cholesterol had normal initial values, ranging between 36.80 and 71.80 mg/100 ml serum while, after a two week atherogenic regimen, these values increased, with all groups up to 44.80—102.60 mg/100 ml serum. At the control batch, the free cholesterol evidences a continuous increase, the final concentration representing 220.05% as compared with the initial one, and 153.99% versus that recorded at T_1 (Fig. 2). In the case of the treated batches, these values decrease, depending on the applied treatment, representing at the end of the treatment, as compared with those recorded at T_1, only 80.46% at batch II, 76.78% at batch III and 105.27% at batch IV.

Serum esterified cholesterol registered normal initial values ranging between 76.20 and 150.20mg/100 ml serum. After two weeks of a therogenic regimen these values increased up to 126.20—297.40 mg/100 ml serum. With the control group, the esterified cholesterol concentration increases ceaselessly, the final value being of 332.00 mg/100 ml serum, which represents 221.03% as compared with the initial one and 111.63%

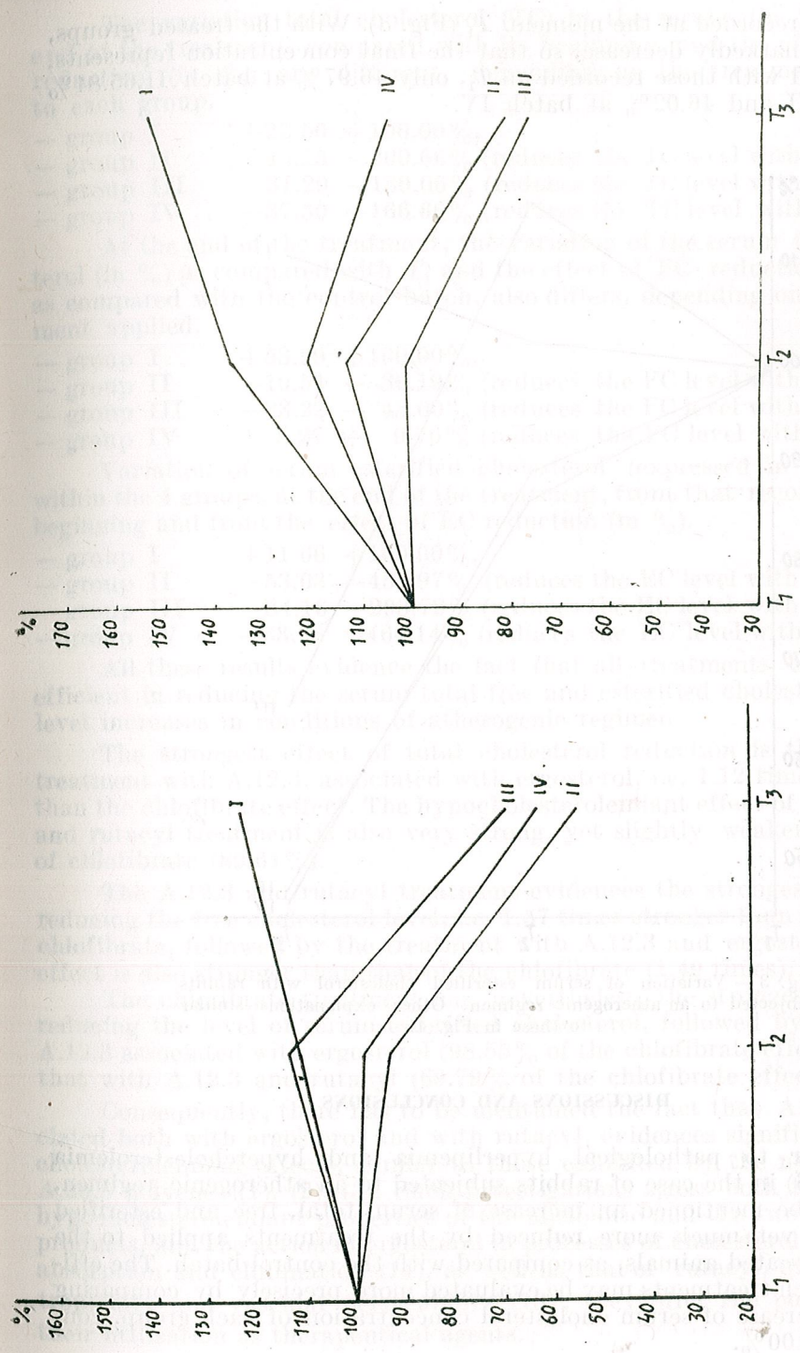

Fig. 2. — Variation of serum free cholesterol, with rabbits subjected to an atherogenic regimen. Other explanations similar to those in Fig. 1.

Fig. 1.— Variation of serum total cholesterol with, rabbits subjected to an atherogenic regimen, untreated (I), treated with A. 12.3 and ergosterol (II), A. 12.3 and rutacyl (III) or with chlofibrate (IV), expressed in % versus the value registered at the beginning of the treatment. T_1, T_2, T_3 — stages of the treatment.

versus that recorded at the moment T_1 (Fig. 3). With the treated groups, the values markedly decrease, so that the final concentration represents, as compared with those recorded at T_1, only 46.97% at batch II,65.84% at batch III and 46.02% at batch IV.

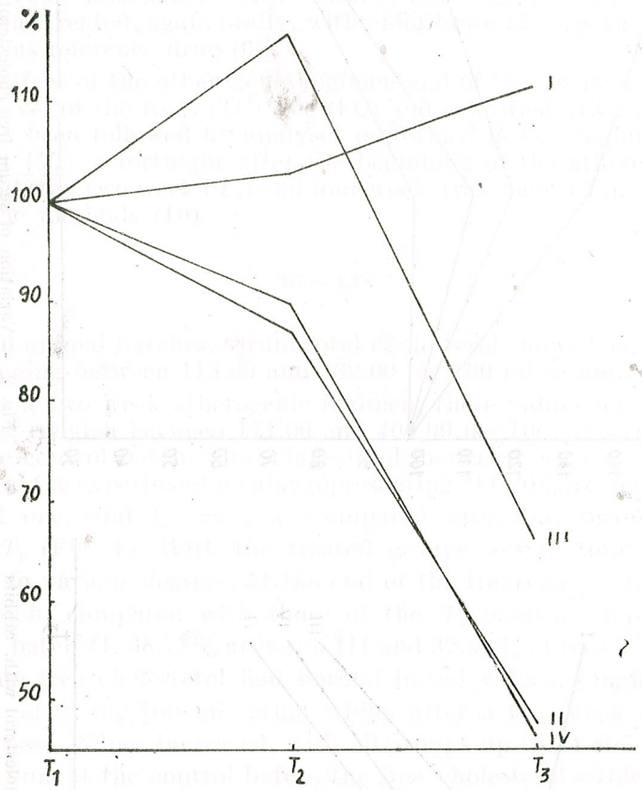

Fig. 3 — Variation of serum esterified cholesterol with rabbits subjected to an atherogenic regimen. Other explanations similar to those in Fig. 1.

DISCUSSIONS AND CONCLUSIONS

Similar to pathological hyperlipemia and hypercholesterolemia (6), (14), (16) in the case of rabbits subjected to an atherogenic regimen, there is to be mentioned an increase of serum total, free and esterified cholesterol, yet much more reduced by the treatments applied to the batches of treated animals, as compared with the control batch. The efficiency of such treatments may be evaluated more precisely by comparing the final increase of serum cholesterol concentration of each group, considered as 100%.

The variation total cholesterol (TC) in the serum (in %), at the end of the treatment, compared with its beginning, and the effect of TC reduction (in %) are different, depending on the treatment applied to each group.

— group I +22.50 +100.00%,
— group II −45.15 −200.66% (reduces the TC level with 300.66%),
— group III −31.29 −139.06% (reduces the TC level with 239.06%),
— group IV −37.50 −166.66% (reduces the TC level with 266.66%)

At the end of the treatment, the variation of the serum free cholesterol (in %) as compared with T_1 and the effect of FC reduction (in %), as compared with the control batch, also differs, depending on the treatment applied.

— group I +53.99 +100.00%,
— group II −19.54 − 36.19% (reduces the FC level with 136.19%),
— group III −23.22 − 43.00% (reduces the FC level with 143.00%).
— group IV + 5.27 + 9.76% (reduces the FC level with 90.84%).

Variation of serum esterified cholesterol (expressed in %) differs within the 4 groups, at the end of the treatment, from that recorded in the beginning and from the effect of EC reduction (in %).

— group I +11.66 +100.00%,
— group II −53.03 −455.97% (reduces the EC level with 555.97%)
— group III −34.16 −293.72% (reduces the EC level with 393.72%)
— group IV −53.98 −464.14% (reduces the EC level with 564.14%)

All these results evidence the fact that all treatments applied are efficient in reducing the serum total free and esterified cholesterol whose level increases in conditions of atherogenic regimen.

The strongest effect of total cholesterol reduction is that of the treatment with A.12.3, associated with ergosterol, i.e. 1.12 times stronger than the chlofibrate effect. The hypocholesterolemiant effect of the A.12.3 and rutacyl treatment is also very strong, yet slightly weaker than that of chlofibrate (89.64%).

The A.12.3 and rutacyl treatment evidences the strongest effect of reducing the free cholesterol level, i.e. 1.57 times stronger than that of the chlofibrate, followed by the treatment with A.12.3 and ergosterol, whose effect is also stronger than that of the chlofibrate (1.49 times).

The chlofibrate treatment has, nevertheless, the strongest effect of reducing the level of serum esterified cholesterol, followed by that with A.12.3 associated with ergosterol (98.55% of the chlofibrate effect) and by that with A.12.3 and rutacyl (69.79% of the chlofibrate effect).

Consequently, there has to be mentioned the fact that A.12.3, associated both with ergosterol and with rutacyl, evidences significant hypocholesterolemiant effects, similar to those exhibited by the nystatin and A.20.5 polyenes (1), (2), (3). Such investigations stress both the specific hypocholesterolemiant properties of the antibiotic and the two associated products, and the action of ergosterol in processes of cholesterol synthesis, absorption and elimination (14), as well as that of rutacyl, of improving trophicity and vascular permeability (13); they offer the possibility of their utilization as therapeutical agents.

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NEW EXPERIMENTAL DATA ON THE CHARACTERIZATION OF THE BIOSYNTHESIS ANTIBIOTIC PREPARATION A 12.3 AS AN ACTIVE CANCEROSTATIC AGENT

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The reproducibility of the antitumoral effect of A 12.3 antibiotic preparation on rats bearing of T—8 Guérin lymphotropic epithelioma, in solid form and its ascitic variant was reconfirmed.
The possibility of optimization of cancerostatic action by therapeutical doses manipulation was also observed.
The comparative analysis of the results obtained by testing of A 12.3 preparation and of Antipholan, Levopholan and Cyclophosphamid cytostatics evidenced the higher or near chimiotherapeutical effectiveness of the new antibiotic with those of the reference agents.

In previous papers we reported the "in vitro" cytotoxic effect on HeLa cell cultures, the "in vivo" antitumoral action of the new biosynthesis antibiotic preparation A 12.3, as well as the reproducibility of its pharmacotherapeutical effect and the existence of a dose-response relationship (7), (8).

The preclinical characterization of a substance as an active cancerostatic agent requires alto the comparison of its antitumoral efficiency with that of a standard cancerostatic (4), (5), (9).

In the present work the results obtained by "in vivo" antitumoral action screening of the A 12.3 antibiotic preparation and of some reference agents (Antipholan, Levopholan, Cyclophosphamid) on rats bearing tumors are exposed.

MATERIALS AND METHODS

White Wistar female rats, weighing 150 g, bearing of T—8 Guérin lymphotropic epithelioma, in solid form and its ascitic variant (6), were used as experimental animals.

24 hours after the transplant the treatment started and lasted for 16 days in the case of solid subcutaneous tumor or until the death of the last control animal for the intraperitoneal ascitic tumor.

In our screening protocol two A 12.3 preparations, isolated in 1982 and 1985 and characterized by different antibiotic activities (A 12.3'82 with 5.600 U/mg and A 12.3'85 with 50.000 U/mg) and three cytostatics of clinical use (Antipholan, Levopholan and Cyclophosphamid) were tested.

The treatment was applied by intraperitoneal administration of the drugs in different doses and at various intervals, which are included in the tables containing the experimental results.

The estimation of the antitumor activity was based on the follow up of the mean tumor weight (MTW) at sacrifice in the case of solid tumor

or of the mean survival time (MST) in the case of ascitic tumor in the treated group as compared with the controls.

The evaluation of the cancerostatic effect was made by the determination of the mean tumor regression (MTR) for solid tumor, by the increase of MST for ascitic tumor and by the calculation of the statistic significance and of the T/C value (where T = mean tumor weight or mean survival time for the treated group and C = mean tumor weight or mean survival time for the controls).

RESULTS

The results obtained in antitumoral activity testing of A 12.3 preparations, administered in different doses to rats with solid Guérin T—8 tumor, are given in table 1.

Table 1

Cancerostatic effect of different doses of A 12.3'85 (1 = 0.2 mg/kg.b.w./at 2 days; 2 = 0.3 mg/kg.b.w./at 2 days; 3 = 0.4 mg/kg.b.w.) and of A 12.3'82 (4 = 0.1 mg/kg.b.w./at 2 days) on solid T—8 Guérin tumor.
Figures in brackets indicate the number of animals.

Group/ Treatment	Mean tumor weight (g)	% tumor regression	T/C value	Statistical significance
CONTROL	14.2±1.5(14)	—	—	—
A 12.3'85(1)	9.6±1.5(9)	32.4	0.67	$p<0.05$
A 12.3'85(2)	8.0±1.0(9)	43.7	0.56	$p<0.01$
A 12.3'85(3)	6.1±0.8(9)	57.5	0.43	$p<0.001$
CONTROL	10.2±1.3(11)	—	—	—
A 12.3'82(4)	5.0±1.6(8)	51.0	0.49	$p<0.05$

The group to which the A 12.3'85 antibiotic was given in a dose of 0.2 mg/kg b.w./at 2 day intervals exhibited a significant decrease ($p < 0.05$) of MTW as compared to control animals. The induced antitumoral activity is characterized by a 32.4% MTR correlated with a T/C value of 0.67.

The increase of the A 12.3'85 dose at 0.3 mg/kg. b.w./at 2 day intervals is correlated with an intensification of the antitumoral effect($p < 0.01$) illustrated by a 43.7% MTR and a 0.56 T/C value.

The administration of the maximum dose (0.4 mg/kg. b.w./at 2 day intervals) induced a higher cancerostatic action ($p < 0.001$) appreciated by a 57.1% MTR and a 0.43 T/C value.

The antitumoral treatment with A 12.3'82 at a dose of 0.1 mg/kg b.w./at 2 day intervals induced a significant decrease ($p < 0.05$) of MTW as compared to the controls. Thus, the 51% MTR and 0.43 T/C values reveal a strong antineoplastic activity.

The results of the antitumoral effect investigation of the standard cancerostatics on rats bearing the same tumoral line are listed in table 2.

It can be seen, in comparison with the control group, that the daily administration of the Antipholan was associated with a significant decrease ($p < 0.001$) of MTW. The strong induced cancerostatic effect is reflected by the MTR (76.3%) and T/C ratio (0.24) values.

Table 2

Antipholan (0.15 mg/kg.b.w./daily), Levopholan (0.15 mg/kg.b.w./daily) and Cyclophosphamid (0.8 mg/kg.b.w./daily) antitumoral activity on solid T—8 Guérin tumor
Figures in brackets indicate the number of animals

Group/ Treatment	Mean tumor weight (g)	% tumor regression	T/C value	Statistical significance
CONTROL	13.5±1.7(13)	—	—	—
Antipholan	3.2±1.8(8)	76.3	0.24	$p<0.001$
Levopholan	11.1±2.7(9)	17.8	0.82	N.S.
Cyclophosphamid	11.6±2.2(9)	14.1	0.86	N.S.

The daily i.p. treatment with Levopholan induced a nonsignificant decrease of MTW which allows us to estimate a 17.8% MTR and a 0.82 T/C value.

A low antitumoral activity was also registered to the group submitted to Cyclophosphamid treatment. In this case a 14.1% MTR and a 0.86 T/C value were calculated.

The comparative investigation of the cancerostatic activities of the A 12.3 antibiotic and of the standard agents was extended on ascitic tumoral line (Table 3).

Table 3

Antitumor activity of A 12.3 antibiotic preparations and of the reference agents on the ascitic rats treated with the same doses as in the other experiments.
Figures in brackets indicate the number of animals.

Group/ Treatment	Mean survival time (days)	% increase MST	T/C value	Statistical significance	% tumor undevelopment
CONTROL	20.1±1.6(13)	—	—	—	—
A 12.3'85(1)	38.4±3.5(5)	91.0	1.91	$p<0.001$	50
A 12.3'82(4)	40.2±1.9 (7)	100.0	2.00	$p<0.001$	30
Antipholan	27.5±5.8(6)	36.8%	1.36	N.S.	40
Levopholan	51.9±1.7(10)	158.2	2.58	$p<0.001$	—
Cyclophosphamid	15.9±0.6(10)	−20.9	0.79	$p<0.01$	—

The groups treated with A 12.3 preparations were characterized by significant increases ($p < 0.001$) of MST as compared to the control group. The corresponding antitumoral activities are illustrated by the MST prolongation values of 91% (A 12.3'85) and 100% (A 12.3'82), respectively, which are correlated with the T/C values of 1.91 (A 12.3'85) and of 2.00 (A 12.3'82), respectively. Five (A 12.3'85) and thre (A 12.3'82) cases of tumor undevelopment were also registered.

It is observed that antitumoral effects were also induced by Antipholan and Levopholan treatments on rats bearing ascitic tumors, as compared with the controls.

Thus, Antipholan antitumoral activity is evidenced by a 36.8% MST prolongation, by a 1.36 T/C value and by 40% tumoral undevelopments. The significant ($p < 0.001$) cancerostatic effect induced by Levopholan is characterized by a MST increase of 158.2% and by a T/C value of 2.58.

On the contrary, as compared with the control group and also with those treated with Antipholan and Levopholan in the case of Cyclophosphamid treatment a significant decrease ($p < 0.01$) of MST was observed.

DISCUSSION

The preclinical characterization of a drug as an active cancerostatic agent is based on :

— the evidence of its antitumoral effect on various tumoral systems ;

— the demonstration of the reproducibility of its pharmacotherapeutical activity ;

— the existence of a dose-response relationship ;

— the comparative analysis of its antineoplastic effectiveness with that of a reference agent ;

— the investigation of its action mechanisms (1—5), (9).

The comparative analysis of the antitumoral therapeutic efficiency of the two A 12.3 antibiotic preparations with those of Antipholan, Levopholan and Cyclophosphamid, used as standard cancerostatics, was the purpose of the present paper.

The antitumoral activity screening of the new A 12.3 antibiotic on rats bearing T—8 Guérin lymphotropic epithelioma, in solid form and its ascitic variant, revealed the following observations :

— the antitumoral treatments with A 12.3 induced significant tumoral regressions and survival time increases, the last correlated with a high percentage of tumoral undevelopments ;

— the intensity of the antitumoral activity is dependent on the therapeutic dose, existing the possibility of antitumoral effect optimization by therapeutic dose manipulations ;

— the A 12.3'82 preparation, with a low antibiotic activity (5.600 U/mg), is characterized by a higher cancerostatic potential.

It can be emphasized, once again, that the results reconfirmed the reproducibility of the antitumoral effects of A 12.3 antibiotic as well as the existence of a dose-response relationship (7), (8).

The testing of the standard cancerostatic agents on the same tumoral lines, in laboratory conditions, showed that :

— Antipholan (0.15 mg/kg b.w./daily/i.p.) induced a significant tumoral regression and a moderate increase of survival time, associated with 40% tumoral undevelopments ;

— a moderate tumoral regression and a strong MST prolongation were obtained by Levopholan treatment (0.15 mg/kg b.w./daily/i.p.) ;

— Cyclophosphamid administration (0.8 mg/kg b.w./daily/i.p.) was correlated with a small tumoral regression and a significant decrease of the survival time.

As compared with these results it can be appreciated that the antitumoral effectiveness of the A 12.3 treatment is near or higher than those of the reference agents.

The positive answer to this question of the screening programs allows us to appreciate the new A 12.3 antibiotic as an antitumoral agent.

However, the final preclinical characterization of the A 12.3 preparate requires further investigations on antineoplastic activity on another tumoral lines in order to establish the cancerostatic spectrum of the A 12.3 antibiotic. The elucidation of the relationship between the antibiotic activity degree and the antitumoral effect intensity is also necessary in order to obtain the A 12.3 preparation with the maximum cancerostatic efficiency.

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ASPECTS DE LA RÉPARTITION QUANTITATIVE DU PHYTOPLANCTON, EN CONDITIONS DE DÉVELOPPEMENT MASSIF, DANS LES EAUX DU LITTORAL ROUMAIN DE LA MER NOIRE

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This paper presents the quantitative distribution of the phytoplankton during the stages of its mass development and the areas with maximum concentration of the phytoplanktonic algae at the Romanian Black Sea littoral.

Par le processus d'eutrophisation de la partie nord-ouest de la mer Noire, les stocks de nutrients augmentent (7, 8, 9), ce qui détermine un taux accru des quantités de phytoplancton dans toutes les zones du littoral roumain. A présent on trouve fréquemment des biomasses phytoplanctoniques au-dessus de 1000 $mg \cdot m^{-3}$ sur toute l'étendue du secteur roumain de la mer, tandis que pendant la période des années '60, antérieure au renforcement de l'eutrophisation, de telles valeurs — ainsi qu'on peut constater sur les cartes de répartition publiées (2, 11) —, représentaient des exceptions, rencontrées pour des surfaces restreintes des eaux de notre littoral.

Visant la connaissance des modalités de distribution dans le secteur roumain de la mer des quantités de phytoplancton, au cours des périodes de son développement massif, et fournissant, en même temps des renseignements sur les zones d'agglomération maximale des algues planctoniques dans les conditions du déroulement, ces dernières années, du processus d'eutrophisation, notre étude ouvre une voie d'approche du problème de l'identification, à notre littoral, des foyers d'initiation des amples phénomènes de floraison, spécifiques à ce processus *.

À la base de ce travail il y a des informations fournies par des données choisies surtout de l'analyse d'environ 500 échantillons quantitatifs de phytoplancton des années 1983, 1984 et 1986, prélevés d'un réseau de stations de surveillance des phénomènes de floraison, réseau qui couvre pratiquement tout l'espace aquatique de la côte roumaine jusqu'à 30 milles marins au large (4). Les échantillons, en volumes d'eau de 1 l, ont été prélevés à l'aide de la bouteille Nansen, et leur analyse a suivi la méthode de la sédimentation.

Bien que, ainsi que nous avons déjà mentionné, les quantités de phytoplancton au long du littoral roumain, dans l'étape actuelle de déroulement du processus d'eutrophisation, soient grandes dans toutes les zones étudiées, la cartographie de celles-ci met en évidence, tout comme avant (2),

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un caractère non-uniforme dans leur distribution, les valeurs de densité et biomasse algales étant supérieures en endroits où se produit soit un contact modéré entre les eaux marines et celles douces, continentales, soit un intense impact anthropique direct sur le milieu pontique (fig. 1—2).

Un intérêt particulier revient aux données sur la répartition du phytoplancton dans l'espace du littoral roumain durant la période immédiatement antérieure au développement d'un phénomène de floraison dans les eaux de Constanța, où la dynamique de ces processus est soumise à nos observations permanentes. On a surpris une telle période au cours de la cartographie des quantités de phytoplancton entre 7 et 11 juillet 1984, quand on a relevé comme espèces dominantes *Exuviaella cordata* Ostf., *Cerataulina bergonii* Perag., *Sceletonema costatum* Grun. et *Nitzschia delicatissima* Cl., dont les deux premières espèces ont développé ultérieurement, le long de tout le littoral roumain, un phénomène de floraison de l'eau. Les cartes qui présentent la distribution pendant la période donnée (fig. 1B, 2B) mettent en relief la présence, dans les secteurs Portița et Chituc, et particulièrement à une distance d'environ 15 milles marins de l'embouchure Portița, des quantités plus grandes d'algues que dans les autres secteurs. A cet endroit du large du secteur Portița les valeurs de densité et de biomasse de tout le phytoplancton au jour du 10 juillet 1984 étaient nettement supérieures à celles enregistrées le même jour dans les eaux de Constanța, et les espèces florissantes *E. cordata* et *C. bergonii* réalisaient des valeurs maximales pour la période respective au même endroit (tableau 1). Après à peine trois jours pour *Exuviaella* et quatre jours pour *Cerataulina*, les valeurs de densité enregistrées le 10 juillet dans le secteur Portița étaient dépassées dans les eaux de Constanța, le développement des espèces mentionnées prenant, ici aussi, l'aspect de la floraison.

Les données respectives suggèrent que le foyer (ou l'un des foyers) de début de la floraison du mois de juillet 1984 a été dans l'espace soumis à un adoucissement modéré devant Portița, où la différence entre les salinités à la surface (diminuées à 10—11 $S^0/_{00}$) et dans les couches inférieures (presque 18 S $^0/_{00}$ à la profondeur de 10—20 m dans le cas exemplifié) a un effet de choc stimulateur dans le développement algal. De cette zone soumise à l'influence du Danube — exercée surtout par le courant nord-sud, prédominant au littoral roumain, à laquelle s'ajoutent les écoulements du complexe lagunaire Razelm-Sinoe —, sous l'influence des vents dominants du nord et du nord-est, les masses d'eaux adoucies et chaudes, où *Exuviaella* et *Cerataulina* se développaient déjà abondamment, ont été poussées dans la direction sud et sud-ouest vers la côte de Constanța, au voisinage de laquelle les populations algales se sont développées en excès sur le compte du stock riche de nutrients qui y existait.

D'ailleurs, la partie centrale du littoral roumain, qui englobe les secteurs Portița et Chituc, apparaît dans beaucoup de cartes de la répartition du phytoplancton sur notre littoral comme l'une des zones les plus chargées d'algues planctoniques (fig. 1B, 1 D—F, 2 A—E).

Ainsi que nous avons constaté à l'occasion d'autres recherches (6), les espèces marines et saumâtricoles de masse non seulement qu'elles végètent normalement dans les secteurs affectés par l'adoucissement, mais les réductions de salinité stimulent même leur développement intense, ce fait étant observé aussi par d'autres chercheurs en d'autres zones adoucies des

Tableau 1

Répartition des quantités du phytoplancton total et des principales espèces de masse des eaux de surface de quelques stations, pendant la période de floraison produite par *Exuviaella cordata* et *Cerataulina bergonii* au cours de l'année 1984

Date	Secteur	*Phytoplancton*		*Espèces de masse*		T°C	S‰
		milles cell.l^{-1}	mg · m^{-3}	Nom de l'espèce	milles cell.l^{-1}		
07.07	Sulina	2.424	4.313	*E. cordata* *S. costatum*	4 1.040	21,0	4,8
	Mila 9	1.336	20.038	*E. cordata* *C. bergonii*	70 860	20,0	10,9
	Sf. Gheorghe	3.010	16.581	*E. cordata* *C. bergonii* *S. costatum* *N. delicatissima*	144 504 261 464	20,0	11,7
10.07	Portița côte	5.671	84.548	*E. cordata* *C. bergonii*	250 3.390	20,3	11,7
	Portița large	13.445	113.515	*E. cordata* *C. bergonii* *S. costatum*	1.270 4.500 1.135	19,3	10,3
	Periboina	3.300	47.745	*E. cordata* *C. bergonii*	552 1.952	18,7	11,4
	Chituc-Midia	7.500	83.582	*E. cordata* *C. bergonii*	1.035 3.450	19,0	12,8
	Constanța	410	2.327	*E. cordata* *S. costatum*	10 330	18,0	16,4
13.07	Constanța	3.910	36.910	*E. cordata* *C. bergonii*	2.080 1.500	20,8	13,3
14.07	Constanța	46.560	166.834	*E. cordata* *C. bergonii*	35.280 5,160	22,5	12,5

mers (1, 10). Dans le cas des espèces qui vivent dans la mer Noire, cette caractéristique a été également due aux processus successifs de sélection et adaptation aux conditions locales de salinité réduite (par rapport à celles de l'Océan Planétaire), auxquelles ont été soumises les algues planctoniques dès le moment de leur pénétration dans le bassin pontique depuis la destruction du seuil bosphorique jusqu'à présent. Ce sont justement les espèces d'algues planctoniques qui ont évolué en s'adaptant aux salinités basses qui sont devenues des espèces de masse dans la mer Noire et, par conséquant, elles réalisent des développements massifs dans les zones soumises à l'influence des eaux douces, les diminutions brusques de la salinité ayant un effet stimulateur sur ces développements (3, 5, 6).

En même temps, la couche d'eau supérieure, avec la salinité réduite, est en été plus chaude que celle sous-jacente, les températures élevées de cette couche adoucie ayant, elles-aussi, un effet stimulateur dans le développement, à la surface, des espèces estivales d'algues (6).

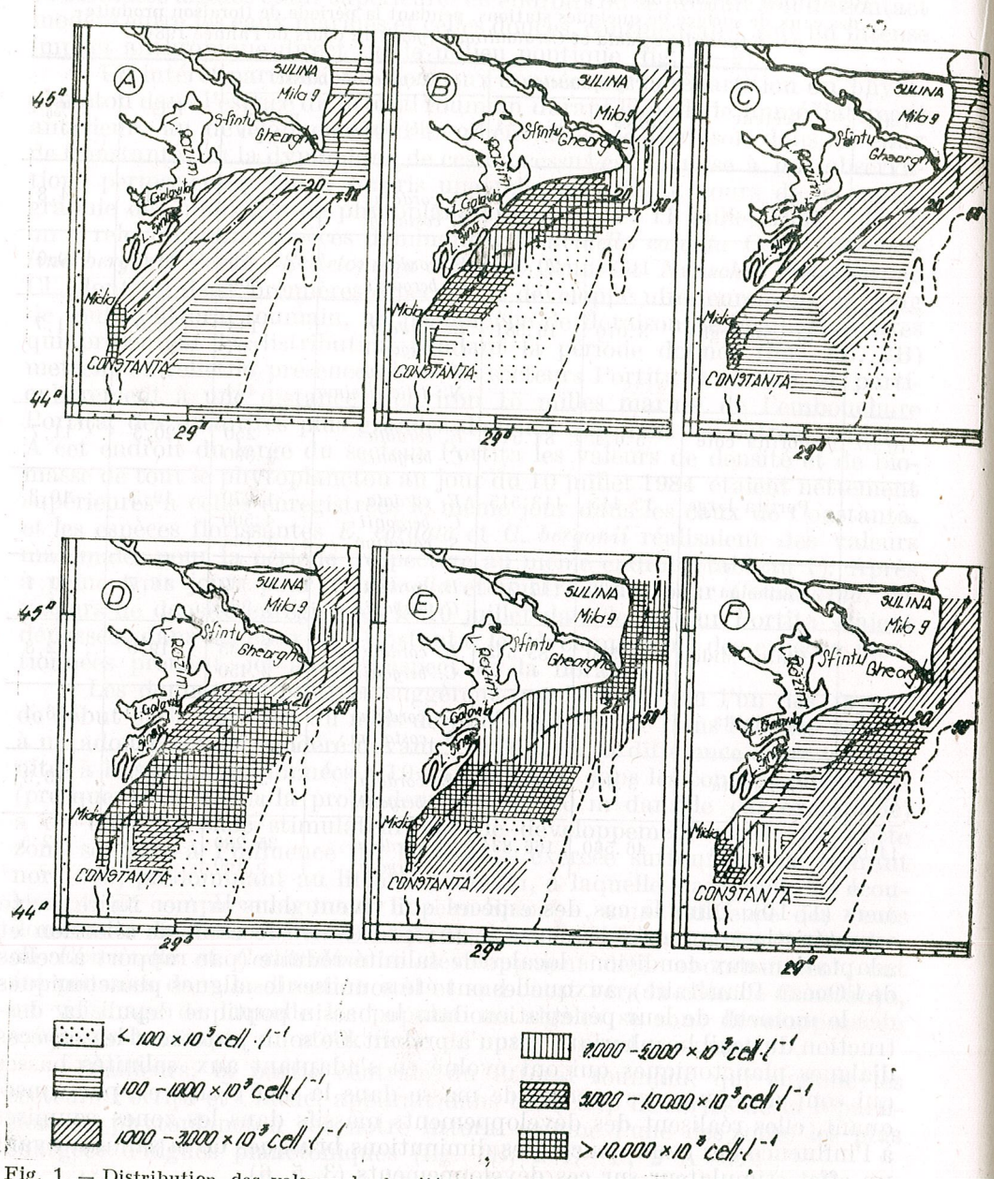

Fig. 1. — Distribution des valeurs de densité numérique du phytoplancton dans les eaux de surface du littoral roumain pendant les périodes 4—20 juin 1984 (A), 3 — 11 juillet 1984 (B), 3 août — 4 septembre 1984 (C), 8 — 19 mai 1986 (D), 4 — 11 uin 1986 (E), 3—8 juillet 1986 (F)

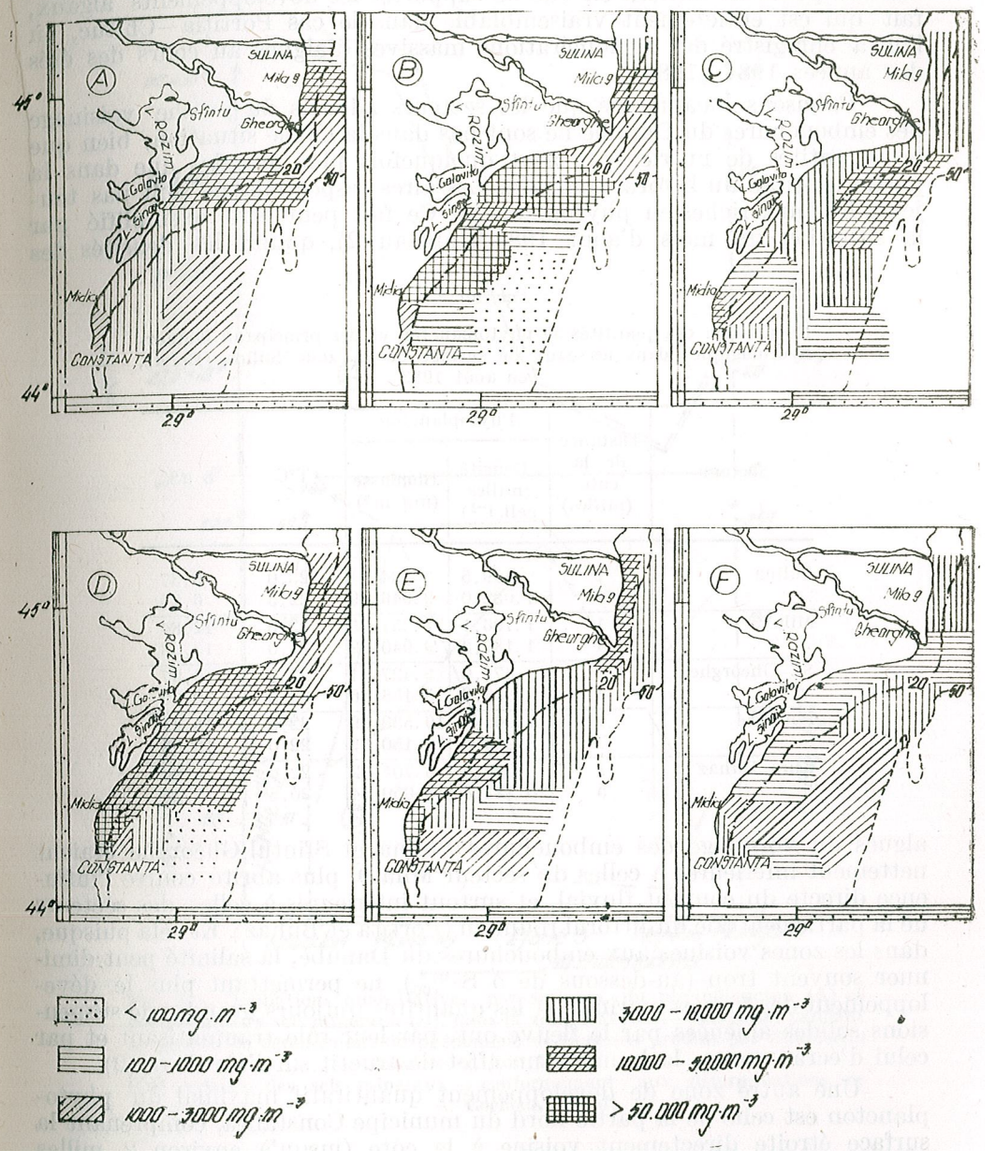

Fig. 2. — Distribution des valeurs de biomasse du phytoplancton dans les eaux de surface du littoral roumain pendant les périodes 4—20 juin 1984 (A), 3—11 juillet 1984 (B), 3 août — 4 septembre 1984 (C), 8—19 mai 1986 (D), 4—11 juin 1986 (E), 3—8 juillet 1986 (F)

Les zones soumises à l'influence des eaux douces sont aussi celles qui reçoivent directement l'apport continental de nutrients, disposant donc de tout un potentiel nutritif en vue de supporter les développements algaux, fait qui est entièrement vraisemblable dans le cas Portiţia—Chituc, où l'on a enregistré des agglomérations massives d'algues au cours des étés des années 1984—1986.

Précisons néanmoins que les secteurs adoucis du proche voisinage des embouchures du Danube ne sont pas dans la même situation, bien que les quantités de nutrients y sont quelquefois plus grandes que dans la partie centrale du littoral roumain. Les aires respectives ne sont pas toujours les plus riches en phytoplancton. Ce fait peut être exemplifié par la situation du mois d'août 1983 (tableau 2), quand les densités des

Tableau 2

Répartition des quantités de phytoplancton et des principaux facteurs hydrologiques dans les eaux de surface de la zone Sulina—Buhaz, en août 1983

Secteur	Distance de la côte (milles)	Phytoplancton		T°C	S g‰
		Densité (milles cell. l^{-1})	Biomasse (mg. m^3)		
Sulina	1	559,5	3.734,14	23,0	6,37
	4	1.082,0	9.946,89	22,5	6,74
Mila 9	1	1.796,1	21.351,05	22,0	12,84
	4	1.187,8	9.940,92	22,0	13,33
Sf. Gheorghe	1	671,6	9.427,71	22,5	9,97
	4	826,2	5.156,86	22,5	10,10
Portiţa	1	2.064,8	19.532,09	22,0	14,91
	7	8.948,6	12.150,55	22,0	15,04
Gura Buhaz	1	10.656,6	3.704,02	22,0	15,98
	5	20.722,8	18.050,24	20,5	15,28

algues du voisinage des embouchures Sulina et Sfintul Gheorghe étaient nettement inférieures à celles du secteur Mila 9, plus abrité contre l'influence directe du courant fluvial, et surtout inférieures à celles des secteurs de la partie centrale du littoral roumain (Portiţa et Buhaz). Et cela puisque, dans les zones voisines aux embouchures du Danube, la salinité peut diminuer souvent trop (au-dessous de 5 S ‰), ne permettant plus le développement des formes marines, et les quantités toujours grandes de suspensions solides amenées par le fleuve ont, par leur rôle traumatisant et par celui d'écran contre la lumière, un effet destructif sur l'algoflore (2).

Une autre zone de développement quantitatif maximal du phytoplancton est celle de la partie nord du municipe Constanţa, comprenant la surface étroite directement voisine à la côte (jusqu'à environ 2 milles marins vers le large) entre les villes Constanţa et Năvodari, zone soumise à l'intense influence anthropique liée au voisinage urbain, industriel, touristique et portuaire (fig. 1A, 1 C—F, 2 C—E). Par rapport aux quantités de phytoplancton du sud de Constanţa, celles d'ici sont nettement supé-

rieures, fait illustré par les données obtenues au cours de certaines floraisons produites par *Exuviaella cordata* en juin 1976 (fig. 3) et en juillet

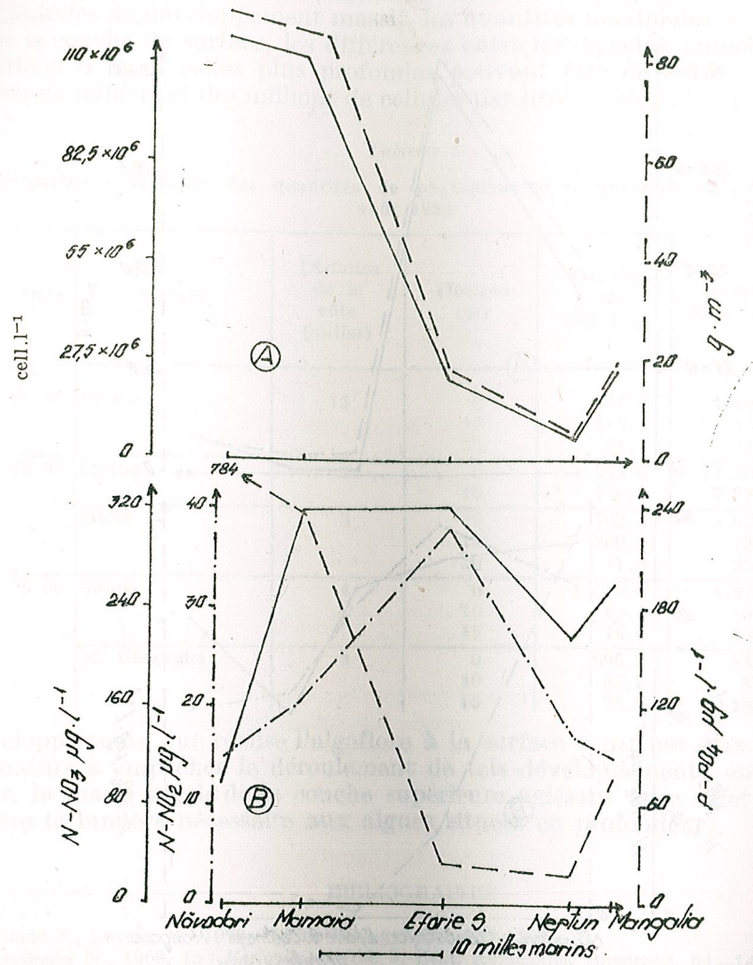

Fig. 3. — Oscillations quantitatives, par secteurs, du phytoplancton (A) et des principaux sels minéraux (B), dans la zone Năvodari—Mangalia, pendant la période de déroulement du phénomène de floraison produit par *Exuviaella cordata* en juin 1976.
(Les valeurs des sels minéraux — conformément aux déterminations de A. Cociaşu, IRCM)

1983 (fig. 4). Les quantités colossales d'algues planctoniques enregistrées alors à Năvodari, Mamaia et Constanţa correspondent à un taux supérieur de nutrients sous forme de phosphates et nitrates par rapport à celles trouvées devant les localités situées au sud — Costineşti, Neptun et Mangalia— où les densités et les biomasses algales ont été comparativement réduites.

Il résulte que dans la répartition en plan horizontal du phytoplancton on met en évidence de grandes quantités sur toute l'étendue du littoral roumain, étant d'habitude supérieures dans la zone Portiţa—Chituc

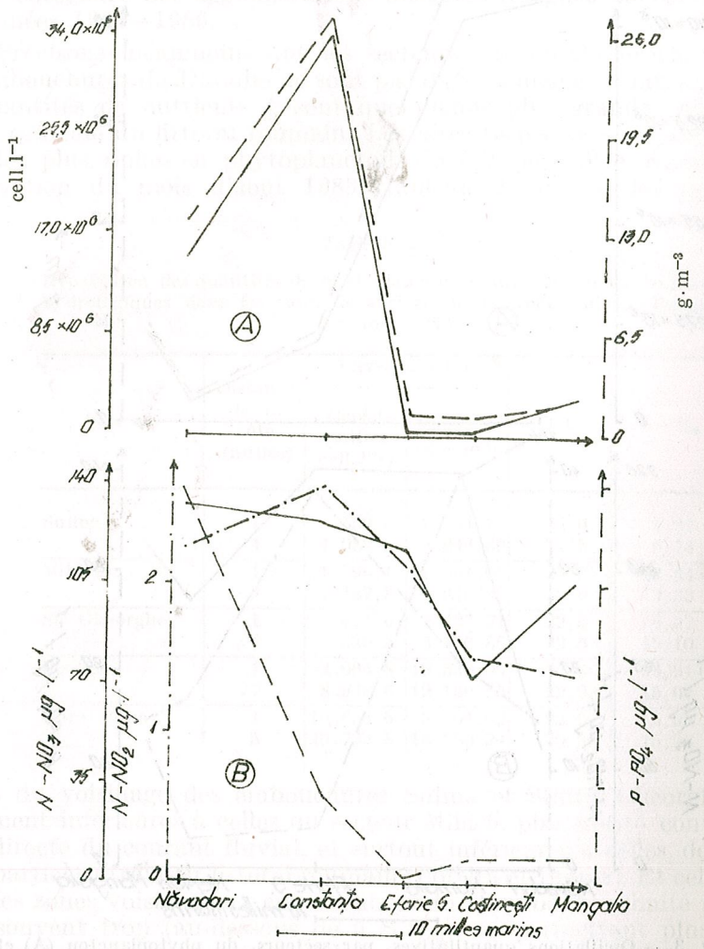

Fig. 4. — Oscillations quantitatives, par secteurs, du phytoplancton (A) et des principaux sels minéraux (B), dans la zone Năvodari—Mangalia, pendant la période de déroulement du phénomène de floraison produit par *Exuviaella cordata* en juin 1983
(Les valeurs des sels minéraux — conformément aux déterminations de I. Bilal, IRCM)

jusqu'à environ 15 milles marins distance de la côte (où l'effet stimulateur des diminutions de la salinité et des croissances de la température est favorisé par le contact modéré entre les eaux pontiques et celles douces, continentales, pénétrées en mer), ainsi que dans la zone Constanţa—Năvodari,

jusqu'à une distance de 2 milles marins de la côte (où l'influence anthropique intense entraîne la présence de grands stocks de nutrients).

En ce qui concerne la distribution verticale du phytoplancton durant les périodes de développement massif, les quantités maximales se trouvent dans la couche de surface, les différences entre les densités numériques de l'horizon 0 m et celles plus profondes pouvant être de l'ordre des centaines de milliers et des millions de cellules par litre (tableau 3). Les grands

Tableau 3

Répartition verticale des quantités de phytoplancton en quelques stations, en août 1985

Date	Secteur	Distance de la côte (milles)	Horizon (m)	Densité (milles cell. l^{-1})	Biomasse (mg. m^{-3})
05.08	Portiţa	15	0	549,6	1.688,5
			10	113,2	736,1
			20	51,4	712,0
06.08	Chituc	1	0	2.243,6	14.306,3
			10	740,6	7.270,2
	Midia	3	0	702,4	3.922,6
			10	200,0	728,0
			20	31,0	254,3
30.08	Sulina	4	0	1.072,0	4.074,5
			10	33,4	107,5
			15	18,6	320,3
	Sf. Gheorghe	4	0	696,0	534,2
			10	85,3	87,6
			15	25,5	188,4

développements que réalise l'algoflore à la surface sont, par eux mêmes, de nature à empêcher le déroulement de tels développements en profondeur, la masse algale de la couche supérieure agissant avec effet d'écran contre la lumière nécessaire aux algues situées en profondeur.

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DIE DYNAMIK DER WICHTIGSTEN NÄHRSTOFFE (N, P) IN EINIGEN SEEN DES DONAU-DELTAS, WELCHE ALS ZEIGER DER TROPHIE GELTEN

ILEANA HURGHIȘIU

The present paper is dealing with the dynamics of total nitrogen and phosphorus, as well as of the organic and mineral matter (soluble and total) in the lakes Puiu-Roșu and Matița-Merhei during the time-lapse 1976—1985, laying stress on the interdependence between these chemical parameters as resulting from their annual and multiannual dynamics.

Während der letzten 10 Jahre waren die ökologischen Studien im Donau-Delta hauptsächlich auf die Bewertung des Trophiegrades der Ökosysteme ausgerichtet. In diesem Sinn wurden Stuckstoff und Phosphor untersucht, die wichtige Bestandteile in der Entwicklung des trophischen Zustandes der Delta-Seen darstellen.

Die vergleichenden vielfältigen Untersuchungen über die Dynamik des Stickstoffs und des Phosphors im Wasser und in den Sedimenten einiger Seen im Meeres-Delta, wie auch im Strom-Delta, waren Gegenstand ständiger Bemühungen. Diese Forschungstätigkeit fand ihren Niederschlag in wissenschaftlichen Arbeiten (2), (5), (6), (7), (8), (10) mit originellen Resultaten. Darin wird hervorgehoben, dass eine Zunahme der Stickstoff- und Phosphorkonzentration stattfindet, als auch der organischen Stoffe, was die fortschreitende trophische Entwicklung der untersuchten Ökosysteme, unter dem Einfluss langsam verlaufender biochemischer Oxydatonen der organischen Stoffe, bzw. eines mangelhaften Mineralisierungs-Vorganges widerspiegelt.

Die vorliegende Arbeit ist gewissermassen die Synthese der im Zeitraum 1976—1985 im Gebiet der Seen Puiu-Roșu im Meeresdelta und Matița-Merhei im Stromdelta durchgeführten Forschungen. Der trophische Zustand dieser Ökosysteme findet seinen Ausdruck hauptsächlich in der Dynamik des Stickstoffs und Phosphors (gesamt, mineralisch und organisch), sowie in der Konzentration der mineralischen und organischen Stoffe (lösliche und partikelförmige) im Wasser und in den Sedimenten der genannten Seen.

MATERIAL UND METHODIK

Die Wasser- und Sedimentproben wurden aus den Seen Puiu, Roșu, Matița und Merhei, aus dem Meeres- und dem Strom-Delta, aufgesammelt; die Probenahmen erfolgten allmonatlich von März-Dezember 1976—1985 u. zw. von 5 verschiedenen Stellen in jedem der Seen.

Der Gesamtstickstoff wurde nach der Methode von Kjeldahl (1) bestimmt; der Mineralstickstoff, der Gesamtphosphor und der mineralische mittels Kolorimetrie (5), (9), die organischen und die Gesamtmineralstoffe nach (4) und die löslichen organischen Stoffe nach (3). Die Ergebnisse sind in g%, mg%, mg/l und O_2 mg/l angegeben.

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DIE ERGEBNISSE UND DEREN DISKUSSION

1) *Die Dynamik des Stickstoffes und Phosphors im Wasser und in den Sedimenten des Puiu-Sees.* Die 1977—1978 im Puiu-See durchgeführten Untersuchungen zeigten eine jahreszetliche Schwankung des *Gesamtstickstoffs* im *Wasser* an und zwar eine höhere Konzentration im März, nämlich 6,0 mg/l, um dann fortlaufend bis zum November auf 1,6 mg/l zu sinken. Da der Stickstoff das wichtigste biogene Element im Wasser darstellt, wurde er von den pflanzlichen Organismen bei Zunahme deren Biomasse aufgenommen. Im Sediment jedoch bleibt die Stickstoffkonzentration im Laufe des ganzen Jahres — von März bis Oktober- auf einem höheren Niveau und schwankt in den Grenzen von 3,6—5,0 mg %. Diese im Vergleich zum Wasser umgekehrte Entwicklung von Sommer bis zum Herbst, mit höheren Stickstoffkonentrationen im Sediment ist entweder auf einen geringeren Stickstoffverbrauch durch die Organismen oder auf mangelhafte Mineralisierung der organischen Stoffe zurückzuführen.

Der *Gesamtsphosphor* im *Wasser*, bei offensichtlich niedrigeren Konzentrationen im Vergleich zum Stickstoff, wies ebenfalls eine quantitative Abnahme vom März bis zum November auf, wobei jedoch die Differenzen gering waren u. zw. 0,20—0,15 mg/l. In den *Sedimenten* bleiben die Gesamtphosphorwerte von März bis Oktober höher als vergleichsweise im Wasser und bewegen sich zwischen 0,30—0,45 mg %. Im allgemeinen beruhen die höheren Konzentrationen an Stickstoff bzw. Gesamtphosphor hauptsächlich auf der Anwesenheit von organischem Stickstoff bzw. Phosphor, welche gegenüber den mineralischen überwiegen, was auf einen mangelhaften Mineralisierungsprozess hindeutet.

Die Konzentration der *löslichen organischen Stoffe* im *Wasser* belief sich auf 3,0—5,0 O_2 mg/l im Jahre 1977 und etwas höher 1978, nämlich 3,0—9,0 O_2 mg/l. Die gesamte *mineralische Substanz* im *Wasser* erreichte 1977 geringere Werte, von 250—310 mg/l und leicht erhöhte 1978, als die Gehalte von 240 bis 330 mg/l schwankten.

1977 zeigten die *gesamten organischen Stoffe* in den *Sedimenten* hohe Konzentrationen, die von 43,0 g % im März bis auf 73,0 g % im November anstiegen. 1978 jedoch findet ein auffälliger Rückgang des Gehaltes an organischen Stoffen in den Sedimenten statt, mit Werten von 19,0 g % im März und 34,0 g % im Oktober.

Die *gesamte mineralische Substanz* wies 1977 niedrigere Konzentrationen auf, die zwischen 28,0 g % und 57,0 g % lagen; 1978 ist ein Zunehmen der Menge mineralischer Stoffe im Sediment zu erkennen, und die Werte schwankten von 66,0 g % bis zu 81,0 g %.

Die vorgelegten Daten bezeugen einen Zusammenhang in der dynamischen Entwicklung der Konzentrationen an mineralischen und organischen Stoffen, an Stickstoff und Phosphor u. zw. bewirkt die quantitative Abnahme der organischen Substanz einen Anstieg der Konzentration mineralischer Substanz bzw. von Stickstoff und Phosphor, also eine stärkere Mineralisierung.

2) *Die Dynamik des Stickstoffs und Phosphors im Wasser und in den Sedimenten des Roşu-Sees.*

Die im Zeitraum 1977—1985 im Roşu-See durchgeführten Untersuchungen haben die jahreszeitliche Dynamik des *Gesamtstickstoffs* im

Wasser aufgedeckt; es ergab sich ein Trend zur Abnahme der Konzentrationen von März mit Werten um 5,8 mg/l bis November mit niedrigen Werten um 1,8 mg/l, was einer jahreszeitlichen dynamischen Entwicklung entspricht, die derjenigen im Puiu-See ganz ähnlich ist.

In den *Sedimenten* ist die Konzentration des Gesamtstickstoffs ausgesprochen höher als im Wasser, bei beträchtlichen Differenzen der Werte von Jahr zu Jahr, von 1978 bis 1985. Aus den im Laufe eines Jahres auftretenden Differenzen geht dieselbe jahreszeitliche Entwicklung hervor, mit Zunahme der Konzentration von Juli bis Dezember. Die Konzentrationsgrenzen schwankten 1978 zwischen 3,2—3,8 mg %, und 4,8—10,6 mg % im Jahre 1985. Die jahreszeitliche dynamische Entwicklung des Stickstoffgehaltes in den Sedimenten ist demzufolge gegenläufig zu der jenigen im Wasser, und es besteht eine ständige Tendenz zur Anhäufung des Stickstoffs. Im allgemeinen ist die Entwicklung mit der im Puiu-See vergleichbar, doch sind die Konzentrationen doppelt so hoch, max. 5,0 mg% im Sediment des Puiu-Sees, bis zu 1,6 mg % im Roşu-See.

Im Roşu-See ist die Konzentration des *Gesamtphosphors* im *Wasser* gering und niedriger als der Stickstoffgehalt. Diese Konzentration zeigt eine ähnliche jahreszeitliche Dynamik wie im Puiu-See, also einen Rückgang der Konzentration von März mit 0,75 mg/l bis November mit 0,15 mg/l.

In den *Sedimenten* nimmt der Gesamtphosphorgehalt von 1978 mit 0,30—0,45 mg % zu bis 1985, wenn die Werte 1,15—3,10 mg % betrugen. Die jährliche jahreszeitliche dynamische Entwicklung weist eine Tendenz zum Absinken der Konzentrationen von August bis Dezember in den Jahren 1978 und 1985 auf, während 1984 die Werte anstiegen.

Die *Gesamtmenge* an *organischer Substanz* im *Wasser* entsprach im Zeitraum 1976—1978 den Konzentrationen von 4,0—9,0 O_2 mg/l und 3,0—10,0 O_2 mg/l 1978. In der betreffenden Periode war die jahreszeitliche Entwicklung normal und die Konzentration wies keine sprunghaften Änderungen auf.

In den *Sedimenten* jedoch war der Gehalt an gesamter organischer Substanz deutlich höher als im Wasser. Es wurden bedeutende jährliche Differenzen im Zeitraum 1977—1985 verzeichnet, mit Grenzwerten der Konzentration von 37,0—76,0 g % 1977 und 30,0—40,0 g % 1985. Die Entwicklung war also zeitlich positiv und endspricht einer Steigerung der Intensität der Mineralisierungsvorgänge im Zeitraum 1978—1985. Die Dynamik der Gesamtmenge organischer Stoffe in den Sedimenten, sowohl die vieljährige als teilweise auch die jahreszeitliche ist in den Seen Roşu und Puiu ähnlich.

Die *Gesamtmineralsubstanz* im *Wasser* des Roşu-Sees wies im Zeitraum 1976—1978 Konzentrationen von 220—1.866 mg/l im Jahre 1976 und 320—360 mg/l 1978 auf. Im Roşu-See ist eine Anreicherung der Gesamtmineralstoffe im Wasser gegenüber dem Puiu-See festzustellen.

In den Sedimenten ist im Zeitraum 1977—1985 der Gehalt an totaler mineralischer Substanz sichtlich höher als im Wasser, und man kann eine Tendenz zu deren Anhäufung im Laufe der Zeit erkennen, was in vieljährigen Differenzierungen zum Ausdruck kommt. So betrugen 1977 die Grenzwerte der Konzentration 24,0—63,0 g % und 1985 3,0—70,0 g%. Die höchsten Konzentrationen an mineralischen Stoffen wurden im Laufe des Jahres 1978 verzeichnet, mit Grenzwerten von 70,0—86,0 g%. Die

zeitliche Anreicherung an mineralischer Substanz in der genannten Periode entspricht einer Intensivierung der Sedimentmineralisierung im Roşu-See, im Vergleich zum Puiu-See.

3) *Die Dynamik des Stick stoffs und Phosphors im Wasser und in den Sedimenten des Matiţa-Sees.*

Im Zeitraum 1980—1982 wies der *Gesamtstickstoff* im *Wasser* eine mehrjährige Entwicklung auf, wobei die Variationsgrenzen 1980 1,6—10,3 mg/l, 1981 2,4—7,6 mg/l und 1982 2,6—4,6 mg/l, betrugen. Es bestand also eine schwache Tendenz zur Zunahme der minimalen Konzentrationen und — umgekehrt — zur Abnahme der maximalen in diesem Zeitraum. Die Entwicklung der jahreszeitlichen Dynamik ist als «normal zu betrachten, mit niedrigen Konzentrationen im Sommer, also Juli—August, und mit hohen Werten im Frühjahr — im April — sowie auch im Herbst gegen den Winter zu — Oktober—Dezember.

In den *Sedimenten* kann von 1980 bis 1985 ein Zunehmen der Gesamtstickstoff-Konzentration vermerkt werden. Die Werte waren höher als im Wasser u. zw. erreichten sie 5,0—12,4 mg % im Jahre 1980 und 8,2—13,4 mg% 1985. Die jahreszeitlizhe Variation ist in den Jahren 1980—1982 im Sommer — Juni bis September — gegenüber derjenigen im Wasser gegenläufig; in dieser Periode sind die Konzentrationen hoch, nämlich 12,4—13,8 mg%.

Der *Gesamtphoshor* im *Wasser*, dessen Konzentrationen unter denjenigen des Stickstoffs liegen, weist trotzdem in den Jahren 1980 und 1981 höhere Werte auf u. zw. 0,20—1,30 mg/l bzw. 0,30—1,4 mg/l, während vergleichsweise 1982 die Werte nur 0,20—0,90 mg/l betrugen. Die maximalen Konzentrationen fielen in den Juli; die dynamische Entwicklung des Phosphorgehaltes vetläuft also im Sommer in umgekehrter Richtung, bezogen auf den Stickstoffgehalt.

In den *Sedimenten* nimmt der Gesamtphosphor im Zeitraum 1980—1985 betächtlich zu. 1982 sind die Konzentrationen niedrig mit 0,55—1,10 mg%, 1985 hingegen hoch mit 1,10—3,45 mg%. Innerhalb dieser vieljährigen Variationsbreite läuft eine normale jahreszeitliche dynamische Entwicklung ab, die durch höhere Werte im Frühjahr gekennzeichnet ist, um dann gegen niedere Werte im Sommer zu tendieren, worauf dann im Herbst zum Winter hin ein neuerliches Ansteigen der Konzentrationen erfolgt.

Die *Gesamtmenge* an *organischer Substanz* im *Wasser*, ausgedrückt als Oxydabilität, entspricht 1980 hohen Konzentrationen mit 5,0—42,0 O_2 mg/l und noch höheren Werten 1981, mit 12,0—67,0 O_2 mg/l; 1982 finden wir dann 7,0—31,0 O_2 mg/l, was eine Verbesserung der ökologischen Entwicklung bedeutet, im Sinne einer verstärkten Mineralisierung.

In den Sedimenten ist die Konzentration an totaler organischer Substanz hoch, wobei eine Tendenz zum Anstieg von 1980 bis 1985 zu verzeichnen ist. 1982 betrugen die Konzentrationsgrenzen 23,0—28,0 g%, und 1985 28,0—46,0 g%. Im Rahmen der vieljährigen Entwicklung ist die jahreszeitliche Dynamik der totalen organischen Substanz in den Sedimenten dutch unbedeutende Schwankungen ausgezeichnet. Die Entwicklung verläuft in umgekehrter Richtung als im Wasser, wo im Laufe der Untersuchungsjahre die Tendenz ansteigend war.

Was die *Gesamtmineralsubstanz* im *Wasser* anbelangt, so ist eine schwache Neigung zur Abnahme der Konzentrationen von 1980 bis 1982 festzustellen. 1981 betrugen die Konzentrationen 290—370 mg/l, 1982 221—340 mg/l.

In den Sedimenten ist jedoch die Konzentration an gesamter mineralischer Substanz hoch. Die mehrjährige Entwicklung zeigt ein Ansteigen der Konzentrationen von 1980 bis 1982 an, um dann 1984—1985 in ein Absinken der Konzentrationen überzugehen. Die höchsten Werte gehören dem Jahre 1982 an, mit Grenzkonzentrationen von 73,0—76,0 g%. Die jahreszeitliche Dynamik zeigt unbedeutende Schwankungen der Gesamtmineralsubstant auf, ohne dass ein Entwicklungsrichtung klar erkennbar wäre.

4) *Die Dynamik des Stickstoffs und Phosphors im Wasser und in den Sedimenten des Merhei-Sees.*

Aus den in den Jahren 1980—1982 durchgeführten Untersuchungen im Merhei-See geht eine normale jahreszeitliche Dynamik des *Gesamtstickstoffgehaltes* im *Wasser* hervor. Belege dafür sind die hohen Konzentrationen in der Periode April—Mai mit Werten bis 9,2 mg/l 1980, 7,0 mg/l 1981 und 4,6 mg/l 1982. Im Juli wurden nur 1,6 mg/l 1980, 2,6 mg/l 1981 und 3,0 mg/l 1982 registriert. Im Zeitraum Oktober-Dezember kommt es dann wieder zur Anreicherung des Stickstoffs im Wasser mit Werten bis 3,2 mg/l 1980, 7,2 mg/l 1981 und 3,4 mg/l 1982. Bei einer normalen jahreszeitlichen Dynamik mit Srickstoffmangel im Sommer, ist eine Tendenz zur Abnahme der Konzentrationen von 1980 bis 1982 zu erkennen, was einer Verbesserung des trophischen Gleichgewichts des Ökosystems gleichkommt.

In den *Sedimenten* sind die Konzentrationen des Gesamtstickstoffs beträchtlich höher als im Wasser. Die jahreszeitliche Dynamik ist nicht ausdrücklich orientiert, während die vieljährige Dynamik eine Neigung zur Anreicherung des Gesamtstickstoffs im Zeitraum 1980—1985 offenbart. Die Grössenordnung geht aus den hohen Konzentrationen hervor, die 1981 mit max. 13,2 mg%, 1982 mit 13,8 mg% und 1985 mit 13,4 mg% registriert wurden.

Hinsichtlich des *Gesamtphosphorgehaltes* im *Wasser* ist eine mehejährige Variation zu erkennen; 1981 waren die Konzentrationen höher, strebten aber niedrigeren Werten im Jahre 1982 zu. So hatten die hohen Juli—Werte 1980—1981 Maxima bis zu 1,25 mg/l und nur 0,35 mg/l im Jahre 1982.

In den *Sedimenten* ist eine Anreicherungs-Tendenz des Gesamtphosphors im Zeiraum 1980—1985 zu beobachten; die vieljährige Entwicklung läuft auf eine stetige Zunahme der Werte hinaus. Die hohen Werte wurden im April—Mai mit 1,4 mg% 1980 und mit 1,55 mg% 1981 verzeichnet; 1985 erreichte das Maximum 3,45 mg%. In diesem Zeitraum wurdeen 1982 die niedrigsten Konzentrationen von nur 0,55 mg% gemessen. Die jahreszeitliche Variation des Gesamtphosphors in den Sedimenten lässt keine bestimmte Entwicklungsrichtung erkennen.

Die *organische Gesamtsubstanz* im *Wasser*, als Oxydabilität ausgedrückt, folgt einer ausgesprochenen jahreszeitlichen Dynamik, wobei die Konzentrationen in den Jahren 1981—1982 gegenüber 1980 ansteigen.

Die höchsten Konzentrationen traten 1981, im Oktober und November auf, als sie 78,0 O_2 mg/l erreichten ; 1980 und 1982 sind sie dagegen gering betragen 10,4 O_2 mg/l und bzw. 32,0 O_2 mg/l. Die vieljährige Entwicklung entspricht einer Zunahme der Konzentration an totaler organischer Substanz im Wasser von 1980 bis 1982, mit Höchstwerten im Laufe des Jahres 1981.

In den *Sedimenten* besitzen die gesamten organischen Stoffe höhere Konzentrationen als im Wasser. Die vieljährige Entwicklung im Zeitraum 1980—1985 entspricht offensichtlich einem Anstieg der Werte. Die höchsten Werte wurden im Juni 1985 gefunden, als sie 46,0 g% erreichten, während sie 1982 nur 25,0 g% betrugen. Die jahreszeitliche Entwicklung war nicht eindeutig ausgerichtet und wies nur unbedeutende Schwankungen auf.

Die *Gesamtmineralsubstanz* im *Wasser* war in den Jahren 1980—1982 durch hohe Konzentrationen gekennzeichnet, besonders 1981 ; in diesen Jahren ist eine klare Tendenz zur Zunahme der Werte sowohl in der vieljährigen als auch in der jahreszeitlichen Entwicklung zu erkennen. Die höchsten Kontentrationen fielen in den Monat November 1981 mit Werten von bis 470 mg/l. In derselben Periode aber u. zw. 1980 gab es niedrigere Werte, nämlich 330 mg/l. Die jahreszeitliche Entwicklung war normal, mit höheren Konzentrationen im Frühjahr, einem Absinken im Sommer und einem Wiederanstieg im Gerbst.

In den *Sedimenten* hatte die *Gesamtmineral substanz* 1980—1985 hohe Konzentrationen im Vergleich zum Wasser aufzuweisen. Es war eine Tendenz zur vieljährigen Variation mit Zunahme der Konzentrationen zu erkennen. In diesem Zeitraum wurden die höchsten Werte im August 1982 gemessen u. zw. bis zu 76,0 g%, was für die vieljährige und auch für die jahreszeitliche Variation gültig ist. In derselben Periode waren 1983 die Werte geringer, mit 70,0 g% (Abb. 1—4).

Wenn man die dynamische Entwicklung der Hauptnährstoffe, Stickstoff und Phosphor in Betracht zieht, wie auch der totalen organischen un mineralischen Stoffe, welche den Trophie-Zustand der Ökosysteme kennzeichnen, so können wir behaupten, dass eine Abhängigkeit zwischen den Gehalten an gesamtorganischer Substanz, gesamtmineralischer Substanz, Stickstoff und Phosphor besteht, und zwar so, dass bei einemVorherrschen der organischen Stoffe auch die Werte für den organischen Stickstoff und Phosphor höher ausfallen und, dass umgekehrt bei hoher Konzentration der Mineralstoffe auch ein Zunehmen der Werte für den mineralischen Stickstoff und Phosphor eintritt.

Angesichts der allgemeinen trophischen Entwicklung dürfen wir eststellen, dass die Seen Puiu und Roşu des Meeresdeltas eine ähnliche Entwicklungs-Richtung zeigen, doch ist für den Roşu-See die Tendenz zur Zunahme der Konzentration trophischer Komponenten klar erkennbar.

Die Seen-Gruppe Matiţa-Merhei aus dem Stromdelta weist ebenfalls ähnliche Richtungen der Trophie-Entwicklung auf, doch ist ein höheres Niveau der Konzentrationen der Hauptnährstoffe, Stickstoff und Phosphor, im Merhei-See zu verzeichnen.

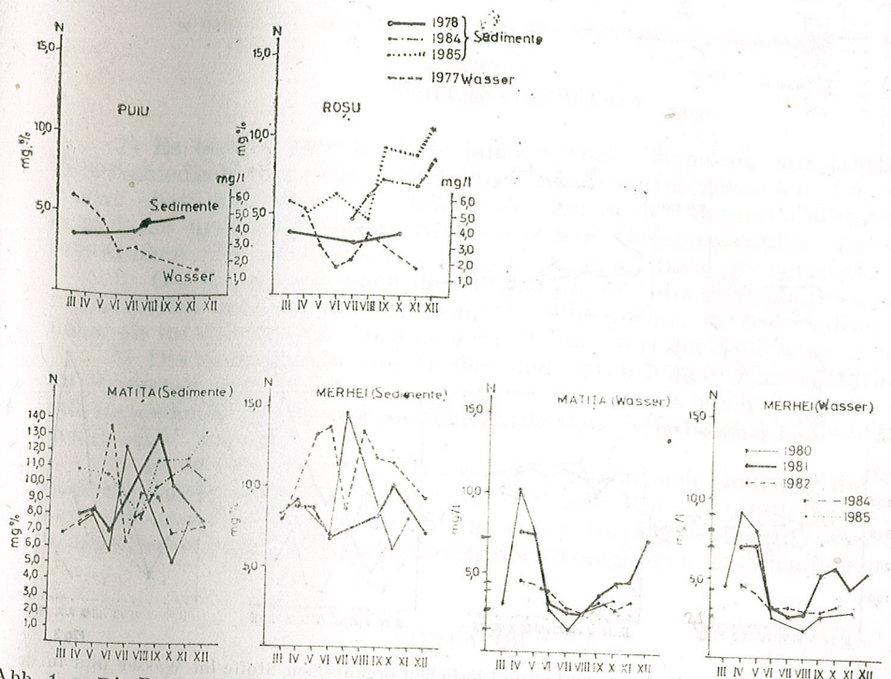

Abb. 1. — Die Dynamik des Gesamtstickstoffs im Wasser und in den Sediment, in d en Se Puiu-Roşu und Matiţa—Merhei, m Zeitraum 1977—1985.

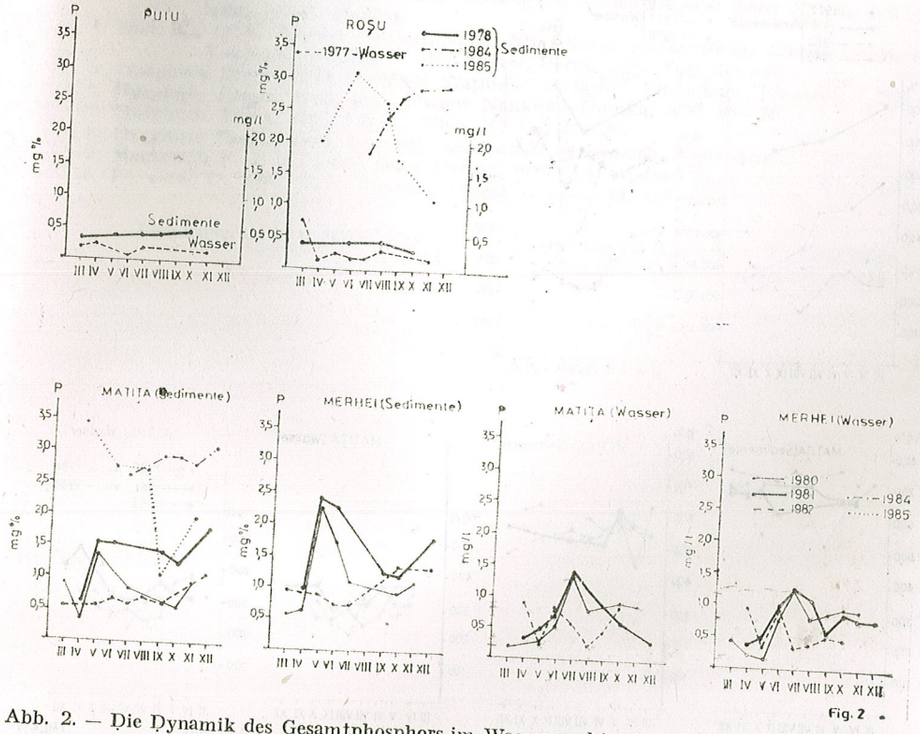

Abb. 2. — Die Dynamik des Gesamtphosphors im Wasser und in den Sedimenten in den Seen Puiu—Roşu und Matiţa—Merhei, im Zeitraum 1977—1985.

Fig. 2

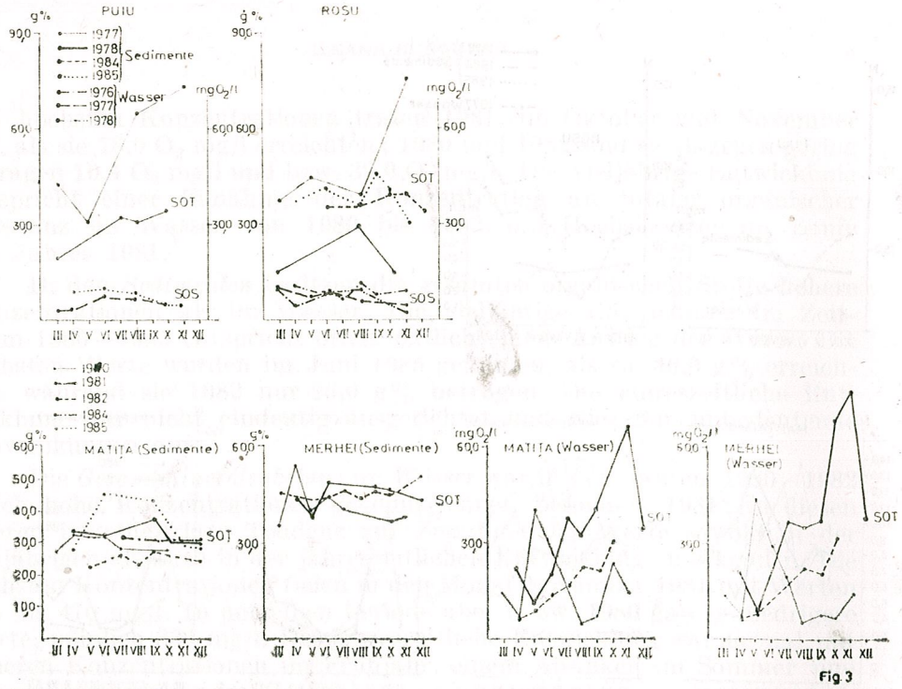

Abb. 3. — Die Dynamik der gesamten und löslichen organischen Stoffe im Wasser und in den Sedimenten in den Seen Puiu—Roşu und Matiţa—Merhei, im Zeitraum 1976—1985.

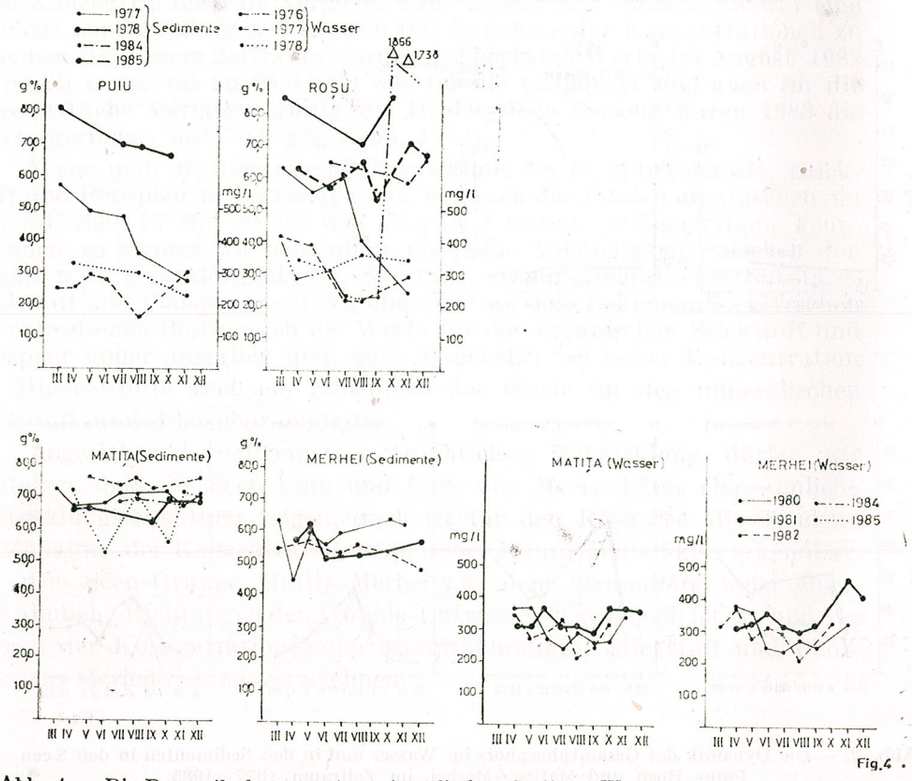

Abb. 4. — Die Dynamik der gesamten mineralischen Stoffe im Wasser und in den Sedimenten in den Seen Puiu—Roşu und Matiţa—Merhei, im Zeitraum 1976—1985.

SCHLUSSFOLGERUNGEN

1) Es besteht eine normale jahreszeitliche Dynamik mit jährlicher Unterschiedlichkeit; diese findet ihren Ausdruck in normalen, für Süsswasser charakteristischen Verhältnissen, indem der Gesamtstickstoff, der organische und mineralische Stickstoff dem Gesamtphosphor und dem organischen und mineralischen gegenüber quantitativ vorherrscht.

2) Die Konzentrationen des Stickstoffs und des Phosphors (totaler, mineralischer und organischer) sind im allgemeinen in den Sedimenten höher als im Wasser, was einen höheren Trophiewert der Sedimente anzeigt.

3) Die Seen Matiţa und Merhei sind durch höhere Konzentrationen an Stickstoff und Phosphor, sowohl im Wasser als auch im Sediment, gekennzeichnet, im Vergleich zu den für den Seen-Komplex Puiu-Roşu gemessenen Werten.

4) In den untersuchten Ökosystemen macht sich eindeutig die Tendenz zur Zunahme der Stickstoff- und Phosphor-Konzentration bemerkbar oder mit anderen Worten zur Zunahme des Trophigrades im Wasser und in den Sedimenten, mit fortschreitender ökologischer Entwicklung.

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