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SOMMAIRE

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THE CONCEPT OF CENTER OF ORIGIN AND ALLOPATRIC SPECIATION

BY

PETRU M. BĂNĂRESCU

The concept of center of origin or of dispersal is not incompatible with allopatric speciation; this center corresponds to the initial range of the last common ancestor of all the species of a higher taxon. Alternative appearances and disappearances of barriers determine successive phenomena of allopatric speciation and reunifications of ranges (sympatry), as well as the extension of the general range of the taxon, finally leading to the concentration of most species towards the center. The "track" obtained by adding the ranges of the species of a lineage corresponds to the range of the ancestor only in the case of recently differentiated species; more usually it includes the entire area within which a lineage evolved, speciated and dispersed.

All biogeographers, either proponents of the permanence of continents, of landbridges or of drift, accepted, until two or three decades ago, that each species and each monophyletic higher taxon originates in a limited area, its "center of origin" or "of dispersal" and later extends its range, or attempts to extend it, in all possible directions, unless barriers prevent this extension. The recent School of "Vicariance Biogeography" claims on the contrary that, speciation being usually or always allopatric, the ancestral species has a wide range and each of its daughter species inherits a smaller range, the concept of a limited "center of dispersal" being hence a nonsense. According to most proponents of the "Vicariance Biogeography" this concept is incompatible with allopatric speciation; the same opinion was expressed even by a "neutral" author, Pielou [9] who recognizes however that the distribution pattern of the component species within many a lineage suggests the reality of the "centers of origin".

A first remark is that one of the adherents of the concept of center of origin is E. Mayr, who also was the most active advocate of allopatric speciation and contributed, more than any other author, to the almost unanimous acceptance of geographical (allopatric) isolation as the most frequent or even the only mechanism of speciation in biparental (amphimictic) organisms. How could the same man be an advocate of allopatric speciation and a proponent of a biogeographical concept incompatible with allopatric speciation?

Accepting that a monophyletic taxon has a center of origin does not in the least mean that all or most of the component species originate in a single locality (or in a very restricted area), i.e. sympatrically, and later some of the species move (all the specimens, like members of

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a nomadic tribe!) each to another area. No modern zoogeographer would consider this possibility. The "center of origin" simply corresponds to the range of the last common ancestor of all recent and extinct species which belong to a given taxon. When barriers appear, the ancestral species splits into two or more daughter species; the later disappearance of the barrier(s) — and it must be mentioned that most barriers are actually short-lasting — allows the range extension of at least one of the daughter species, either over the range of one of its sisters (hence both becoming sympatric) or over some area that did not belong to the range of the mother species. Active centers of origin and of dispersal are restricted or rather wide areas (never single localities) within which barriers successively appear and disappear, determining alternative splittings and extensions of ranges [1, 2].

The palaeontology of mammals [10] furnishes numerous examples of higher taxa, the ancestors of which initially had restricted distributions, then extended their ranges and subsequently split, as a consequence of the appearance of barriers — and barriers always appear within wider ranges — into several daughter species. Later the daughter species extended their ranges, too, split into other species, etc. For example, the oldest camelid, *Poebrodon*, is known from the late Eocene of North America; his descendants extended their range northwards throughout the continent; later, one or a few of them arrived in Eurasia during the Miocene using the Behring landbridge; the two recent species from central and western Asia (one of which was introduced in northern Africa as domesticated form) are the direct offshoots of these Miocene immigrants. Other descendants of the Eocene North American ancestors extended their range into South America in Pliocene times, after the emergence of the Central American landbridge and evolved to the direct ancestors of the recent *Lama*. Hence, the family had a center of origin, North America, from where it extended its range, not by "long-distance jumps" but by normal continental route, after the disappearance of the barriers that initially limited the range of the ancestral form of the family. The later splitting into several genera (at least nine of which became extinct without offshoots in the recent fauna [10]) and species was evidently the result of the numerous barriers which appeared within the enlarged range of the family.

The dispersal history of the tapirs was similar: the oldest fossils are known from the early Eocene of North America which can be considered as representing the center of origin and of the dispersal of the superfamily Tapiroidea. The lineage extended its range already during the Eocene to Europe (by using the still existing North-Atlantic continental connection) and to northern Asia by the North Pacific landroute; it also dispersed to South America during the early Pleistocene, after the emergence of the Central American landbridge [10]. A limited center of origin could be established for most families and other lineages of mammals, for which the fossil record is rich enough; in all cases, this center is smaller than the recent and past range of the lineages. The range extension took place by continental route, except for bats, for the murid rodents from Australia-New Guinea and for the semi-aquatic suids and hippopotamuses from Madagascar. And speciation was in all

cases geographical (allopatric), as a consequence of the numerous barriers which invariably appear in wide areas.

One important fact must be remembered: most barriers which split ranges and determine speciation are not separations of continents and oceans or other major geological changes, but minor changes, such as climatic modifications, local interruptions of mountain chains or of forrest belts, etc. for terrestrial organisms, and river captures or other modifications of the riverine net for freshwater animals. Most of these events, except those that occurred during the youngest periods, remain unknown to geologists.

Since speciation obligatorily implies fragmentation of the range, the center of origin of a taxon can never be a single locality or a spot on the map. But in many a case it is a limited area. The shallow African lake Victoria is a typical center of origin for a number of lineages of the Cichlid fish genus *Haplochromis*, this lake having undergone successive phenomena of fragmentation and reunifications, corresponding to the pluvial and interpluvial periods of the Quaternary, the fragmentations determining splitting of ranges and speciation, the reunifications of the lake dispersal and sympatry of the previously allopatric species [6]. The Mexican Plateau, most of which corresponds to the basin of Rio Lerma, represents another small center of evolution and dispersal (being nevertheless large enough to permit alternative splittings of ranges) for three lineages of freshwater fishes: the family Goodeidae [7, 11], and the genera *Chirostoma* [3, 4] and *Algansea* [8, 5].

Most centers of origin, dispersal and evolution are however much wider areas — East Asia, eastern North America, tropical South America, etc. — within which barriers appear and disappear in a rather frequent sequence and which are separated by long-lasting or almost permanent barriers from other areas.

The two groups of mammals analyzed here in detail (camelids and tapiroids) became extinct from their North American center of origin, surviving in more recently occupied areas. In other taxa however — and probably in most of them, above all in those undergoing an active phenomenon of evolution in recent times — the center is also the present-day center of abundance and of maximum diversification. In ideal cases, the number of species and of other subordinate taxa within a lineage is highest in the central part of the "center", decreasing towards the periphery; Pielou [9] gives suggestive examples in this respect (see his fig. 3.3). Far from being inconsistent with the concept of allopatric speciation as claimed by proponents of the "Vicariance Biogeography", this phenomenon is a normal consequence of allopatric speciation, if one considers that most barriers which split ranges and determine speciation are short-lasting. The ancestral species lived in the center of origin; this center is subdivided by barriers and the species splits into several daughter species. When the barriers disappear, the ranges of the daughter species overlap partially or totally, some of them also extending towards the periphery, as a consequence of the disappearance of other barriers, those which initially limited the range of the ancestral species. The phenomenon recurs, ranges alternatively splitting and extending towards both the center and the periphery and, if no other factors (competition, extinc-

tion, etc.) interfere, the total number of new species gradually becomes higher towards the center.

A viewpoint of the School of Vicariance Biogeography which can be accepted, is that in numerous cases it is not possible to determine the center of origin of a given taxon, but it is possible to determine which was its general range in a remoter past. A number of lineages are presently widely distributed throughout former Gondwanian continents, others only in Africa and tropical South America, or in Australia, New Zealand and South America (above all its temperate areas). It can be asserted that the ancestors lived in Gondwanaland before its breakup, or only in the Africano-Brazilian or the Notogaeic fragment of Gondwanaland. The ancestral species initially had a restricted range within Gondwanaland, from which it dispersed and split (speciated) in pre-drift times. One can not establish which was this range—the center of origin of the entire taxon; one can only assert that the taxon (not its ancestral species!) was "already" widely distributed throughout Gondwanaland or a fragment of it in pre-drift times. The method recommended by Croizat and the School of Vicariance Biogeography for identifying the former range of the taxon by adding the present-day ranges of the component species is actually scientific, while the search for the center of origin (range of the ancestral species) is often illusory, unless palaeontological data made it possible. The impossibility to establish, in many a case, which was the center of origin, does however not mean that the whole taxon did not initially begin its evolution and dispersal from a center of origin — the restricted range of its ancestral species.

A last remark concerns the method used by the Vicariance Biogeography School for estimating the initial range of the ancestral species by adding the ranges of all descendant species (and, in the cases of widely disjunct taxa, also the intermediate areas, where the group is now absent). This method is valid only for recently differentiated species. If for example two closely related sister species (or two conspecific subspecies) live one in Europe, the second in eastern Asia, it can be concluded that the immediate ancestor had a wide distribution from eastern Asia throughout Siberia to Europe, the "vicariant event" that determined the splitting of the range having been the Pleistocene cooling of Siberia; it is however not certain, even in such cases, whether the range of the immediate ancestral species actually included all areas presently occupied by the two daughter species, since the European species may have extended its range westwards, and the East Asian one eastwards, after the disjunction ("vicariant event") took place. In the cases of higher taxa including numerous genera and species and having old ages, the "track" obtained by adding the ranges of all descendant species is always much wider than the initial range of the last common ancestor. This "track" (to use Croizat's terminology) actually includes the entire area within which the lineage has evolved during millions of years, in which successive phenomena of splittings and extensions of ranges took place. Let us consider again the already mentioned camelids and tapiroids. The "track" obtained by adding the ranges of all recent and fossil species includes North America, most of Eurasia, Central America and parts of South America, while the initial range of the ancestral species of both

lineages included only a part of North America and only later did some of the descendant species extend their ranges to Eurasia, others to central America, etc.

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NEW DATA ON THE MYSIDS FROM THE SOUTH-AUSTRALIAN WATERS. THE DESCRIPTION OF *HALEMYSIS AUSTRALIENSIS* GEN. N., SP. N.

BY

MIHAI BĂCESCU and AUREL UDRESCU

L'étude d'une petite collection de Mysidacés des eaux sud-australiennes, envoyée au Muséum « Grigore Antipa » par le regretté Dr. H. Hale, a permis l'identification et la description de *Halemysis* gen. n. La diagnose du nouveau genre est suivie par la description de *Halemysis australiensis* sp.n. On discute les affinités morphologiques de *Halemysis* avec *Mysidium*, genre certainement apparenté phylogéniquement, et l'on fait une série de remarques concernant l'écologie et la morphologie du nouveau Mysidacé.
Ont été encore identifiés : *Siriella australis* Tattersall, 1927, *S. vincenti* Tatt., 1927 et *Paranchialina angusta* (G. O. Sars, 1883), caractéristiques des eaux sud-australiennes, ainsi que *Australerythrops* sp. et des juvéniles de *Gastrosaccus* sp.

In a small collection of Mysids sent to our Museum by the regretted carcinologist Herbert Hale (by that time director of the South-Australian Museum — Adelaide), we were surprised to identify several specimens belonging to a new Mysid genus.

LIST OF STATIONS

Station I — 28.III.1941, Memory Cave, 9.60 m deep, submarine light, weed bottom.
Station II — 5.III.1941, Port Lincoln.
Station III — 5.III.1941, Dangerous Reef, submarine light.
Station IV — 27.V.1941, Port Lincoln.

In this material we found the following species already known of the South-Australian waters : *Siriella australis* Tattersall, 1927 (St. I : 4 ♂♂ A, 19 ♀♀ A ; 25 juv. ; St. II : 1 ♀ juv.) ; *Siriella vincenti* Tattersall, 1927 (St. III : 1 ♀ A) ; *Gastrosaccus* sp. (St. I : 1 ♀ juv. ; St. IV : 1 ♀ juv.) ; *Paranchialina angusta* (G. O. Sars, 1883) (St. II : 6 ♀♀ A, 5 ♂♂ A, 3 juv.) and a new species of *Australerythrops* sp., which will be described in another work, and in St. IV :

Halemysis n. gen.

Diagnosis. This genus, reminding only of *Mysidium* Dana, 1850, is characterized by : large, rounded rostrum ; a special phanerotaxy of antennule (♂, ♀) which forms an immense fan ; the special shape of pleopods IV ♂ with an enormous sympod devoid of endopodite ; the presence of posterior carennae at penis. Type species : *Halemysis australiensis*

We dedicated this genus to the memory of the distinguished cumaceologist Herbert M. Hale.

Halemysis australiensis sp. n.

(Figs. 1, 2)

Description (♂, ♀). Tegument smooth, yellowish. Carapace with short and widely rounded frontal part (Fig. 1 *A*), small antero-lateral prominences, a distinct gastric groove and a slight posterior excavation. Telson small, reaching not even the middle of the uropodal basis; it is perfectly linguiform (Fig. 1 *B*). On 2/3 of its sides, it bears articulate spines and on the perfectly rounded apex, about 27 pointed laminae (Fig. 1 *C*).

Eyes large, brown, short, pyriform, with an immense reniform cornea (seen from above) and with tiny omatidia. Peduncles represent 1/3 of the total volume of the eye.

Antennule massive with proximal segment of the basis shorter than the two terminal ones (♀) and ± equal to them in the ♂. On its distal and outer side (♂♀) is inserted, in caudal direction, an immense phanera as thick as the outer flagellum of antennule and provided with a dense set of very long and fine feathered setae (Fig. 1 *D*; Fig. 2 *A*); near the phanera start, in anterior direction, three (♀) or two (♂) shorter phanerae even more similar to the usual setae but also provided with enormous feathered setae; dispersed, the feathered setae of these phanerae interweave forming a fan; through this and through its irisations, the middle area of antenna seems to show a fine, transparent lamella. The middle segment bears two similar setae. The distal segment bears two large setae on the antero-distal corner and a medio-dorsal apophysis with 3 — 4 short usual setae.

The outer flagellum with the proximal part swollen innerwards; from the latter start 5 — 6 aesthetascs in ♀ (Fig. 1 *D*) and up to 20 in ♂ (Fig. 2 *A*). In addition, the ♂ shows a dentation on the antero-internal corner of the last article and a male long and pointed lobe, with a rich set of sensory fine and short hairs, on the inner side, which do not exceed its tip. Antenna (Fig. 1 *F*; Fig. 2 *G*) provided with an external pointed spine and a peduncle with three short, thick segments usually curved outerwards and tergally. The peduncle is much shorter than the lanceolate triangular scale. The latter shows a clearly separated apical article and the ratio between length and maximum width is 3.5.

Labrum widely triangular, without anterior spine. Labium with many spines and hairs on its lobes.

Maxillula common; maxilla with a small lobe and the apical segment foliaceous, oval, with feathered setae, without spines (Fig. 1 *E*). Mandible (Fig. 1 *G*, *H*) with biarticulate palp; proximal article widened like a lamina and very hairy outerwards, with long hairs on the inner edge and an oval lobe at the basis.

Maxillipedes I and II show a very wide ± circular basis at their exopodite and an epipodite like a lobe ± transparent, of special shape.

Peraeopods of ± equal shape, with a 4-jointed tarsum (carpopropod with three segments), strong sickle-like claws at each articula-

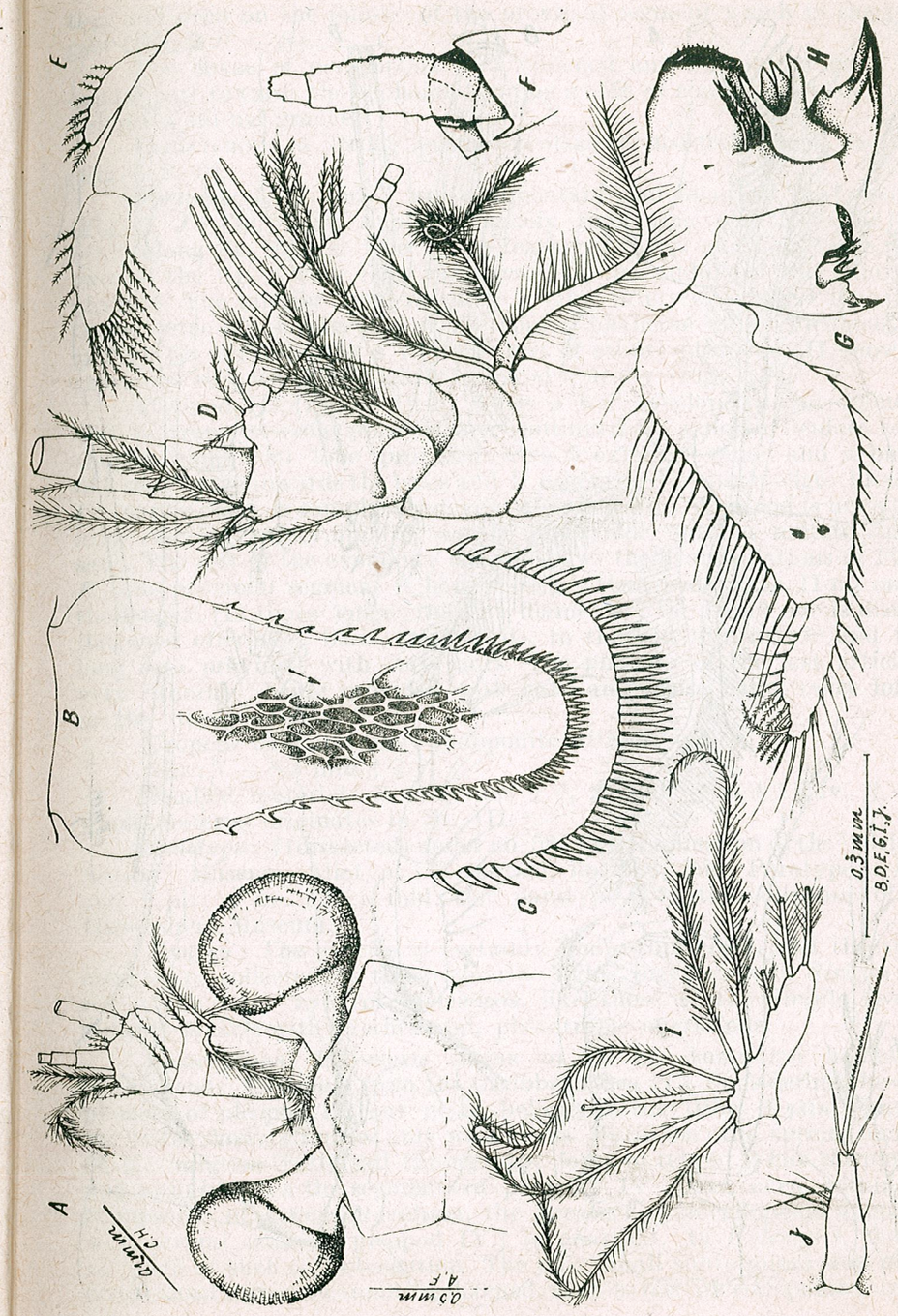

Fig. 1. — *Halemysis australiensis* gen. n., sp.n. ♀ *A*, anterior end; *B*, telson; *C*, the same, apical part; *D*, antennule; *E*, maxilla II; *F*, antenna; *G*, mandible; *H*, the same, masticatory part; *I*, pleopod II; *J*, pleopod III.

Fig. 2. — *Halemysis australiensis* gen. n., sp.n. ♂: *A*, antennule; *B*, penis, lateral view; *C*, the same, seen from above; *D*, pleopod III; *E*, pleopod IV; *F*, pleopod V; *G*, antenna; *H*, peraeopod VI; *I*, the same, apical part; *J*, peraeopod I; *K*, the same, apical part.

tion and even on the middle of the proximal segment which is the longest (Fig. 2 *H* — *K*).

The coxae of peraeopods very widened and imbricated from the caudal part towards the cephalon forming a sort of continuous plates that delimit a sternal groove.

Penis shows a strong postero-ventral, transparent, oval carenna (Fig. 2 *B*, *C*).

Pleopods I, II and V are rudimentary and identical in ♂ and ♀ (Fig. 1 *J*; Fig. 2 *F*). A plate vaguely reminding of a triangle that bears along the inferior face 7 — 9 feathered, large and long setae, and two on the tip, a very long one exceeding the middle of the following pleonite. The inferior setae resemble a crest intersecting their long feathered setae like in the case of the special phanerae of A_1. Pleopods III and IV (♀) do not show a ventral crest of setae; pleopods III show an enormous seta which is slightly serrated apically (Fig. 1 *J*).

Pleopod III (Fig. 2 *D*) in ♂ has a well-developed basal segment, with a strong external musculature, continued by a non-articulate cone-shaped endopodal lobe provided with 5 external setae and a bunch of 3 short setae towards the basis and 2 others on the inner edge. Pleopod IV, paramysoid, is completely devoid of endopodite. Sympod is unusually long showing subterminally, on the inner side only, a minute simple seta. The rest of the exopodite clearly shows the 3 articulations of Fig. 2 *E*. The proximal segment is hardly longer than wider, the II-nd one is the longest (14 times longer than its diameter); the III-rd one is distally flattened offering a basis, innerwards, to the last thin article and to a long seta provided with tiny hairs. The phanera of the last article is very "thorny" being provided with hard and dense hairs, twice longer on the inner side (Fig. 2 *E*).

Uropods long and fine; endopodite without spines.

Size: 7 — 8.5 mm ♂ ♀.

Studied material: 3 ovigerous ♀ ♀, 2 adult ♂ and 1 juv. ♂. The whole material originates in St. III.

Holotype: ♂ (dissected) under no. 585 in the collection of the "Grigore Antipa" Museum. Allotype: ♀ berried, no. 586, ibid. Paratypes: 4 ♀♀ and 1 juv. ♂ no. 586 a ibid. One paratype ♂ in the collection of the Queensland Museum.

Ecology: The species is certainly phototropic (♂♀); all the specimens were collected at the submarine light, together with *Siriella vincenti*. The large eyes of *Halemysis*, like those of the *Siriella* species, indicate Mysids with nycthemeral, phototropic migrations.

Observations. Halemysis seems to be an Australian homologue of the genus *Mysidium* from the Caribbean Sea [1]. Considering the morphology of pleopod IV, our genus belongs to the tribe *Mysini* Hansen; the latter shows affinities only with genus *Mysidium*; the species reminds of *M. integrum* Tattersall through the form of telson. While the number and ratio between the segments of pleopod IV differ even within the framework of genus *Mysidium*, the presence of an endopodal apophysis with several setae on pleopod IV♂ is constant. In *Halemysis*, there is no trace of such a prolongation. The only small subterminal seta of the distal basal segment cannot be likened with a rest of endopodite, taking

into account also its subterminal, not apical insertion. Additionally, the presence of such a long sympod is another characteristic of this pleopod which is for the rest of paramysoid type.

The pleopod III ♂ is also characteristic of the new genus by the ± normal development of the basis, having a slight endopodal cone-shaped prolongation with setae. In *Mysidium* it is only Zimmer who figures a similar pleopod III ♂.

The strange antennular phanerotaxy is a practically unique achievement among the Mysids; the enormous feathered setae on A_1 (♂, ♀) and on the pleopods I—II (♂, ♀), that interweave forming fan-laminae represent another characteristic of the new genus.

Regretfully, the species of the genus *Mysidium* have been very briefly described so that we can compare with the new genus neither the buccal parts, the thoracopods, the penis nor the pleopod III ♂.

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NEW *ZERCONIDAE (ACARI: MESOSTIGMATA)* FROM ROMANIA

BY
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There are described two new species of *Zerconidae: Zercon aniellae* n.sp. and *Prozercon trägårdhisimilis* n.sp. from different biotopes of a coniferous and a mixed wood, in a secular forest of the Eastern Carpathians.

The collecting of a very rich mites material from the Slătioara-Rarău secular forest, a natural reservation in the Eastern Carpathians, between 790 — 1480 m altitude, gave the opportunity to make some studies on the *Zerconidae* family. The samples were taken from a coniferous wood, and a mixed, coniferous and beech forest, from different biotopes : litter, fermentation layer, lapidicolous and terricolous moss synusia, rotting trunks with saproxylicolous and xylicolous layers, during spring, summer and autumn seasons, from 1977 to 1980.

There were identified 18 species of *Zerconidae*, 15 species new for the Romanian fauna [7] and two new species. Some zoocenotical studies on these species were also carried out [3].

The two new species are described in this paper :

Zercon aniellae n.sp.

Female : Length 457 μ, width 403 μ. *Dorsal side* (Fig. 1 *a*).Padonotal setae are smooth, acicular, of about 10—29 μ (i_3 =14 μ, i_6 =29 μ, s_1, s_2=10 μ) except for i_1 finely barbed, of 38 μ. Opistonotal setae : the distinct feature of this species is the presence of two, most often three supplementary uneven setae, between the two rows of the I pairs of setae. All the opistonotal setae are smooth, slender and except for I_1 and Z_1 they have only one barbe on their posterior third (Fig. 1 *b*). Their length increases to the posterior end of the shield. In the I row only I_1 is short, at the same distance from the following pair ; the others, including the supplementary setae, are very long, going farther beyond the insertion of the next ones. The distance between $I_6 - I_6$ =140 — 157 μ, and be-

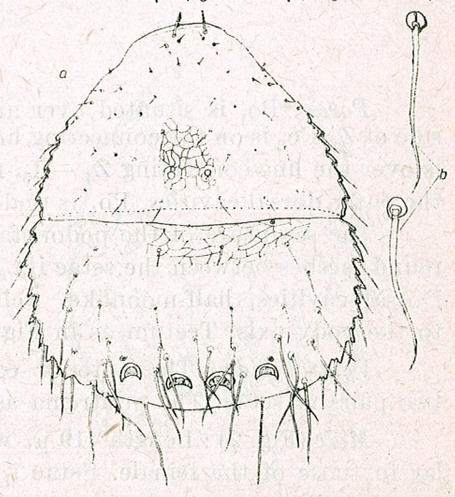

Fig. 1. — *Zercon aniellae* n.sp., female — dorsal side.

tween $Z_5 - I_6 = 36 - 40$ μ. S_2 surpasses by one third the lateral border of the shield and S_3 by more than a half. Marginal setae also smooth and long, go beyond the insertion of the next setae of the row. Marginal edges of the shields are denticulated.

Lengths of opistonotal setae and the distances between setae of the same row are :

$S_1 - 48$ μ | 48 μ | $S_2 - 83$ μ | 45 μ | $S_3 - 96$ μ | 43 μ | $S_4 - 109$ μ

$Z_1 - 36$ μ | 69 μ | $Z_2 - 55$ μ | 43 μ | $Z_3 - 98$ μ | 36 μ | $Z_4 - 114$ μ | 29 μ | $Z_5 - 74$ μ

$I_1 - 36$ μ | 76 μ | $I_2 - 60$ μ | 45 μ | $I_3 - 98$ μ | 29 μ | $I_4 - 98$ μ | 19 μ | $I_5 - 109$ μ | 38 μ | $I_6 - 112 - 119$ μ

$I_{x1,2} = 98$ μ

Pores : Po_1 is situated over and obliquely anteriorly, to the inner side of Z_1. Po_2 is on the connecting line between $Z_2 - S_2$, nearer to Z_2. Po_3 is over the line connecting $Z_4 - I_5$, nearer to Z_4, over the outside edge of the outer dorsal cavities. Po_4 is under the bottom of S_4.

The sculpture of the podonotal shield is network-shaped, with two round meshes between the setae i_6. The opistonotal sculpture is scaly net. Dorsal cavities, half-moonlike, well sclerotized, have the axes parallel to the body axis. Tectum as in Fig. 6 *a*.

Ventral side : The anterior edge of the ventro-anal shield bears two pairs of setae. The peritrema as in Fig. 5.

Male (Fig. 2) : Length 419 μ, width 309 μ. All the features are similar to those of the female. Setae $i_1 = 26$ μ, i_5, $s_7 = 29$ μ ; the marginal setae of the row *r* have very few barbes. The opistonotum with three supplementary setae between the I rows. Distances between setae $Z_5 - I_6 = 33$ μ and $I_6 - I_6 = 129$ μ.

Lengths of the opistonotal setae and the distances between setae of the same row are :

$S_1 - 45$ μ | 48 μ | $S_2 - 71$ μ | 45 μ | $S_3 - 83$ μ | 36 μ | $S_4 - 86$ μ

$Z_1 - 36$ μ | 57 μ | $Z_2 - 60$ μ | 33 μ | $Z_3 - 83$ μ | 39 μ | $Z_4 - 105$ μ | 66 μ | $Z_5 - 71$ μ

$I_1 - 33$ μ | 48 μ | $I_2 - 50$ μ | 31 μ | $I_3 - 95$ μ | 31 μ | $I_4 - 95$ μ | 24 μ | $I_5 - 95$ μ | 60 μ | $I_6 - 105$ μ

$I_{x1-3} = 93 - 95$ μ

$R_1 - 36$ μ

$R_2 - 40$ μ

$R_3 - 50$ μ

$R_4 - 60$ μ

$R_5 - 60$ μ

$R_6 - 60$ μ

$R_7 - 71$ μ

Chelicera as in Fig. 6 *d*.

Deutonymph (Fig. 3) : Length 405 μ, width 310 μ. Its dimensions are close to those of the male, but it is less sclerotized. Lengths of the podonotal setae are 24—26 μ and those of the opistonotal setae and the

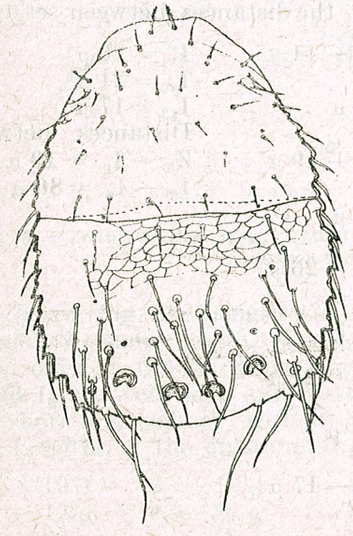

Fig. 2. — *Zercon aniellae* n.sp., male — dorsal side.

Fig. 3. — *Zercon aniellae* n.sp., deutonymph — dorsal side.

2—c. 2433 50

distances between them are :

$S_1 - 38\ \mu$ | $43\ \mu$ | $S_2 - 64\ \mu$ | $43\ \mu$ | $S_3 - 81\ \mu$ | $38\ \mu$ | $S_4 - 95\ \mu$

$Z_1 - 24\ \mu$ | $50\ \mu$ | $Z_2 - 40\ \mu$ | $29\ \mu$ | $Z_3 - 83\ \mu$ | $36\ \mu$ | $Z_4 - 109\mu$ | $74\ \mu$ | $Z_5 - 64\ \mu$

$I_1 - 24\ \mu$ | $45\ \mu$ | $I_2 - 38\ \mu$ | $33\ \mu$ | $I_3 - 95\ \mu$ | $33\ \mu$ | $I_4 - 79\ \mu$ | $17\ \mu$ | $I_5 - 74\ \mu$ | $62\ \mu$ | $I_6 - 105 - 110\mu$

$I_{x1-3} = 71\ \mu$

Distances between

$Z_5 - I_6 = 24\ \mu$
$I_6 - I_6 = 121\ \mu$

Pores : Po_1 is situated over and a little inside the insertion of Z_1 ; Po_2 is under the connecting line between setae $Z_2 - S_1$, nearer to S_1, Po_3 is on the connecting line between $Z_4 - I_5$ and Po_4 under the setae S_4, near to its inside. The scaly net sculpture is more evident on the podonotal shield.

Protonymph (Fig. 4): Length 310 μ, width 214 μ. The podonotal setae are smooth, the setae s_4, s_5, s_6, r_3 and r_6 are longer, the longest being r_3. Lengths of the opistonotal setae and the distances between setae are :

$S_1 - 29\ \mu$ | $31\ \mu$ | $S_2 - 48\ \mu$ | $36\ \mu$ | $S_3 - 55\ \mu$ | $36\ \mu$ | $S_4 - 62\ \mu$

$Z_1 - 12\ \mu$ | $31\ \mu$ | $Z_2 - 12\ \mu$ | $24\ \mu$ | $Z_3 - 50\ \mu$ | $31\ \mu$ | $Z_4 - 69\ \mu$ | $43\ \mu$ | $Z_5 - 43\ \mu$

$I_1 - 14\ \mu$ | $29\ \mu$ | $I_2 - 19\ \mu$ | $33\ \mu$ | $I_3 - 26\ \mu$ | $21\ \mu$ | $I_4 - 21\ \mu$ | $24\ \mu$ | $I_5 - 17\ \mu$ | $36\ \mu$ | $I_6 - 79\ \mu$

$I_{x1} = 26\ \mu$
$I_{x2} = 21\ \mu$
$I_{x3} = 17\ \mu$

Distances between :
$Z_5 - I_6 = 19\ \mu$
$I_6 - I_6 = 86\ \mu$

The middle process of the tectum is deeply bifurcated and denticulated (Fig. 6 *b*). The body and shields are poorly sclerotized, transparent, with smooth borders except for the latero-posterior sides, gently festooned and with a poor network.

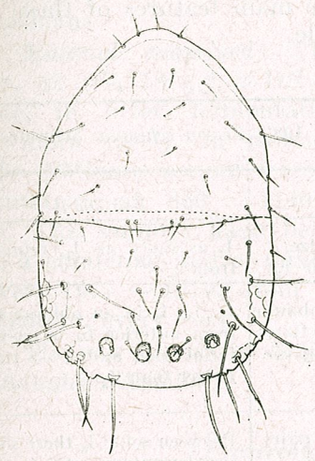

Fig. 4. — *Zercon aniellae* n. sp., protonymph — dorsal side.

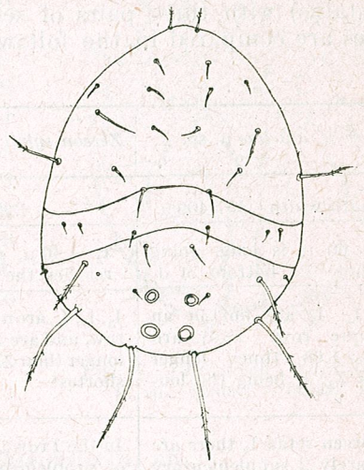

Fig. 5. — *Zercon aniellae* n.sp., larva — dorsal side.

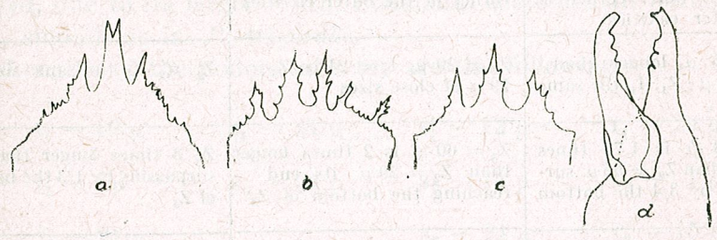

Fig. 6. — *Zercon aniellae* n.sp., tectum : *a*, adults and deutonymph ; *b*, protonymph ; *c*, larva ; *d*, male chelicera.

Larva (Fig. 5) : Length 233 μ, width 200 μ. Podonotal setae smooth, of about the same length, except for s_4, s_5 that are longer ; the only feathered ones on the third end are i_1 and s_5. Of the opistonotal setae, Z_3, Z_4 and I_6 are very long and feathered on their distal half, the others are very short and smooth, without additional setae.

Lengths of the opistonotal setae are the following :

$i_1 - 19\ \mu$ | $i_3 - 12\ \mu$ | $i_4 - 20\ \mu$ | $i_5 - 17\ \mu$ | $i_6 - 2i\ \mu$

$s_2 - 12\ \mu$ | $s_4 - 36\ \mu$ | $s_5 - 43\ \mu$

$I_2 - 10\ \mu$ | $I_3 - 14\ \mu$ | $I_5 - 7\ \mu$ | $I_6 - 83\ \mu$

$Z_2 - 6\ \mu$ $S_2 - 6\ \mu$ | $Z_3 - 46\ \mu$ $S_3 - 3\ \mu$ | $Z_4 - 71\ \mu$ | Distance $I_6 - I_6 - 48\ \mu$

The tectum is represented in Fig. 6 *c*.

Systematic position: Beside this species there are known only two other ones with additional setae in the I row: *Zercon echinatus* Schweizer, 1922 from the Switzerland Alps, with two uneven setae, and *Zercon sylvii* Solomon, 1982 from the Romanian Eastern Carpathians (Călimani mountains) with three pairs of setae. The main features of these three species are compared in the following table.

Zercon aniellae n. sp.	*Zercon sylvii* Solomon, 1982	*Zercon echinatus* Schweizer, 1922
1. Length/width—457/409 μ	438/376 μ	346/268 μ
2. I_2 = 60 μ is long, going beyond the bottom of I_3	I_2 = 40 μ, medium size, reaches the bottom of I_3	I_2 as short as I_1, remote from I_3
3. I_3, I_4, I_5 are not in an oblique row, and are 1.78—1.98 times longer than Z_2, I_5 being the longest	I_3, I_4, I_5 are not in an oblique row, and are 1.55—1.79 times longer than Z_2, I_5 being the shortest	I_3, I_4, I_5 in an oblique row from anterior to outer-posterior one, and 2—3 times longer than Z_2
4. Between setae I_3 there are 3, rarely 2 supplementary setae (I_x).	In the I row there are 3 pairs of supplementary setae	Between setae I_3 there are 2 supplementary setae
5. I_6 = 112 — 119 μ, close in size to I_5 = 109 μ and behind the inner corner of the outer cavities	I_6 = 72 μ, longer than I_5 = 45 μ, behind the inner corner of the outer cavities	I_6 very long, behind the outer corner of the outer cavities
6. Z_2 = 55 μ, longer than I_1 = 36 μ; Z_1, I_1 the same size	Z_2 = 29 μ, I_1 = 24 μ, Z_1 = 19 μ of close sizes	Z_1, Z_2, I_1 the same sizes
7. Z_3 = 98 μ is 1.78 times longer than Z_2 = 55 μ, surpassing by 3/4 the bottom of Z_4	Z_3 = 60 μ is 2 times longer than Z_2 = 29 μ, its end reaching the bottom of Z_4	Z_3 3 times longer than Z_2, surpassing by 1/3 the bottom of Z_4
8. Z_5 remote from I_6, the distance between them = 36 — 40 μ	Z_5 remote from I_6, the distance between them = 22—26 μ	Z_5 tangent to I_6, in its inner side
9. S_2 surpasses by 1/3 the lateral border of the shield and the bottom of S_3	S_2 does not surpass the lateral border of the shield, but reaches the bottom of S_3	S_2 reaches neither the lateral border of the shield, nor the bottom of S_3
10. Po_3 over the line Z_4—I_5	Po_3 over the line Z_4—I_5	Po_3 on the line Z_4—I_5
11. Setae with one barbe on its posterior third	Simple setae	Setae with a wider, more hyaline end

The new species is then closer to *Z. sylvii* Solomon, 1982, by its size, the position of Z_5 — I_6 and of Po_3, but is easily identified by the uneven supplementary setae, the shape and the length of the setae.

Z. echinatus Schweizer, 1922 differs especially by: a smaller size, I_2 short, only 2 supplementary setae and the position of Z_5 — I_6.

All the three species with supplementary setae are specific to mountain forests, in the Alps and Carpathians.

Material examined: 77 specimens, 52 ♀♀, 18 ♂♂, 2 DN, 3PN, 2 larvae, in litter, fermentation layer and more rarely in rotten trunks, in the Slătioara secular forest (Rarău mountains), at 860 — 1140 m altitude. The samples have been taken from a coniferous and a mixed wood, between 1977 — 1979, from April to September, every year.

The new species is dedicated to my daughter Aniella.

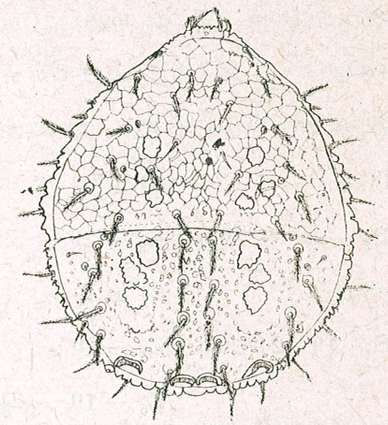

Fig. 7. — *Prozercon trägårdhisimilis* n. sp., female — dorsal side.

Prozercon trägårdhisimilis n. sp.

Female (Fig. 7): Length 333 μ, width 281 μ. The aspect is similar to *P. trägårdhi* (Halbert, 1923), but the shape of the body is oval, more rounded, due to its larger width. Podonotal setae are all barbed, except for i_5 smooth as in *P. trägårdhi*.

Lengths of the podonotal setae are the following:

i_1 — 19 μ	z_1 — 21 μ	s_1 — 12 μ	r_1 — 29 μ
i_2 — 7 μ	z_2 — 24 μ	s_2 — 14 μ	r_2 — 7 μ
i_3 — 21 μ		s_3 — 24 μ	r_3 — 15 μ
i_4 — 21 μ		s_4 — 24 μ	r_4 — 29 μ
i_5 — 24 μ		s_5 — 26 μ	r_5 — 19 μ
i_6 — 29 μ			r_6 — 24 μ
			r_7 — 24 μ

Opistonotal setae are also barbed, with very large rounded bottoms, except for S_1. All the I row setae reach the bottom of the next one (I_1, I_2) or are going beyond it (I_3 — I_5); Z_1 — Z_4 setae reach the insertion of the next one, only Z_4 is going beyond the border of the shield. S_1 is very short, smooth, acicular.

Lengths of opistonotal setae and distances between setae of the same row are :

S_1 — 7 μ | 26 μ | S_2 — 29 μ | 38 μ | S_3 — 24 — 26 μ | 43 μ | S_4 — 19 — 24 μ

Z_1 — 29 — 31 μ | 26 μ | Z_2 — 29 — 31 μ | 31 μ | Z_3 — 29 — 33 μ | 24 μ | Z_4 — 29 μ | Z_5 — 19 — 24 μ

I_1 — 29 — 36 μ | 33 μ | I_2 — 33 — 36 μ | 27 μ | I_3 — 33 — 36 μ | 21 μ | I_4 — 29 — 33 μ | 21 μ | I_5 — 29 μ | 19 μ | I_6 — 26 — 29 μ

Distance between : $I_6 - I_6 = 62$ μ

Pores : po_1 is situated behind setae s_1, po_2 is just above the line connecting setae i_4—s_3, near to the insertion of s_3, po_3 is under the connecting line between z_1—s_4 nearer to and almost behind s_4, po_4 is outer s_5. Po_1 is a little obliquely upwards on the inner side of the insertion of setae Z_1, Po_2 is over and a little outer the insertion of Z_2, Po_3 is immediately under the line between $Z_3 - S_2$ and Po_4 is to the inner side of S_4.

Sculpture : Podonotum with a regular hexagonal network sinuously outlined and with smaller meshes in the middle side. Between the lines

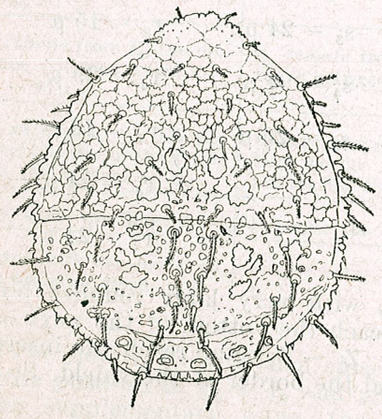

Fig. 8. — *Prozercon trägårdhisimilis* n. sp., male — dorsal side.

$i_5 - z_1$ and $s_3 - z_2$ there are three smooth areas and under z_1 a little half-moon outline. Opistonotum is covered with an evident pitted sculpture and also with three smooth areas between I and Z rows. Dorsal cavities big, well sclerotized, the inner ones nearer to one another, the outer ones more opened to the posterior edge.

Ventral side : A pair of setae are on the anterior border of the ventroanal shield.

Male (Fig. 8) : Length 262 μ, width 228 μ, dorsal shield rounded. All the features are similar to those of the female. Lengths of the opistonotal setae and the distances between setae of the same row are :

S_1 — 12 μ | 26 μ | S_2 — 24 μ | 33 μ | S_3 — 26 μ | 33 μ | S_4 — 24 μ

Z_1 — 21 μ | 24 μ | Z_2 — 19 μ | 21 μ | Z_3 — 21 μ | 19 μ | Z_4 — 21 μ | 29 μ | Z_5 — 14 μ

I_1 — 26 μ | 26 μ | I_2 — 26 μ | 24 μ | I_3 — 24 μ | 14 μ | I_4 — 24 μ | 17 μ | I_5 — 24 μ | 21 μ | I_6 — 24 μ

Distances between : $Z_5 - I_6 = 21$ μ ; $I_6 - I_6 = 52$ μ

Deutonymph (Fig. 9) : Length 243—281 μ, width 200 — 214 μ ; *i* setae are generally short and finely barbed, the shortest being i_2, except for i_1 and i_3 long and barbed, i_3 the longest. Setae z_1, z_2, $s_1 - s_5$ are finely barbed, of equal size. In some specimens the setae seemed to be

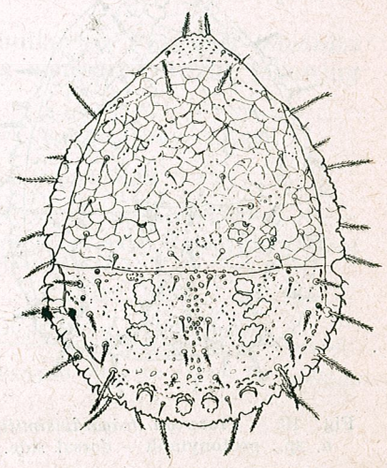

Fig. 9. — *Prozercon trägårdhisimilis* n. sp., deutonymph — dorsal side.

smooth, except for i_1, i_3 even under immersion microscope. The *r* setae are barbed, except for r_2, r_5 spinelike and very short. Opistonotal setae are all barbed, even S_1, in contrast with the adults. S_1 reaches the insertion of S_2; S_2, S_3 the same length, S_4 a little longer. Lengths of the opistonotal setae and the distances between setae of the same row are:

$S_1 - 19\ \mu$ | $17\ \mu$ | $S_2 - 26\ \mu$ | $32\ \mu$ | $S_3 - 26\ \mu$ | $36\ \mu$ | $S_4 - 31\ \mu$

$Z_1 - 10\ \mu$ | $21\ \mu$ | $Z_2 - 12\ \mu$ | $19\ \mu$ | $Z_3 - 17\ \mu$ | $19\ \mu$ | $Z_4 - 31\ \mu$ | $29\ \mu$ | $Z_2 - 14\ \mu$

$I_1 - 10\ \mu$ | $24\ \mu$ | $I_2 - 12\ \mu$ | $21\ \mu$ | $I_3 - 10\ \mu$ | $17\ \mu$ | $I_4 - 10\ \mu$ | $17\ \mu$ | $I_5 - 10\ \mu$ | $12\ \mu$ | $I_6 - 28\ \mu$

Distances between:

$Z_5 - I_6 = 19\ \mu$
$I_6 - I_6 = 38\ \mu$

The position of Po_1—Po_4 is the same as in the adults. The sculpture is also similar to that of the adults, but much less sclerotized, yellowish in color and hardly visible at the middle-posterior side of podonotum. Dorsal cavities are waved in front.

Protonymph (Fig. 10): Length 228 μ, width 180 μ. Podonotal setae are short and smooth, except for i_1, i_3 longer and barbed; *r* setae barbed,

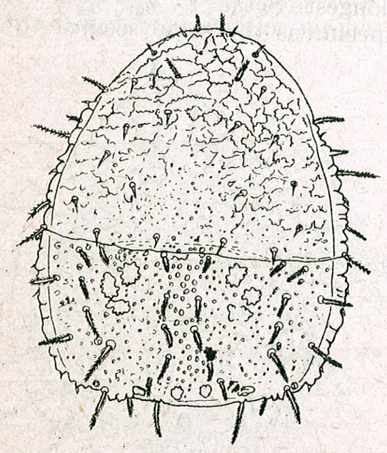

Fig. 10. — *Prozercon trägårdhisimilis* n. sp., protonymph — dorsal side.

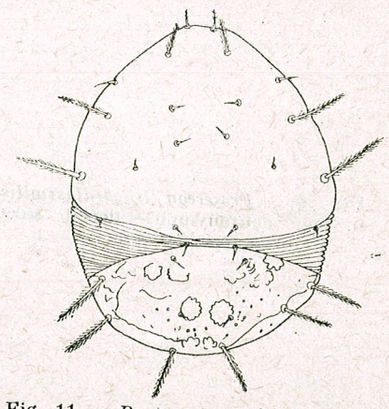

Fig. 11. — *Prozercon trägårdhisimilis* n. sp., larva — dorsal side.

except for r_2, r_5. Opistonotal setae barbed, with the following lengths and distances between setae of the same row:

$S_1 - 17\ \mu$ | $21\ \mu$ | $S_2 - 26\ \mu$ | $28\ \mu$ | $S_3\ 26\ \mu$ | $29\ \mu$ | $S_4 - 28\ \mu$

$Z - 12\ \mu$ | $21\ \mu$ | $Z_2 - 12\ \mu$ | $12\ \mu$ | $Z_3 - 14\ \mu$ | $14\ \mu$ | $Z_4 - 29\ \mu$ | $26\ \mu$ | $Z_5 - 17\ \mu$

$I_1 - 12\ \mu$ | $29\ \mu$ | $I_2 - 19\ \mu$ | $19\ \mu$ | $I_3 - 17\ \mu$ | $14\ \mu$ | $I_4 - 12\ \mu$ | $14\ \mu$ | $I_5 - 12\ \mu$ | $14\ \mu$ | $I_6 - 29\ \mu$

Distances between:

$Z_5 - I_6 = 17\ \mu$
$I_6 - I_6 = 50\ \mu$

Sculpture keeps the same features, but much less sclerotized, and evident on the anterior half of the podonotum; dorsal cavities waved in front.

Larva (Fig. 11): Length 178 μ, width 143 μ. Podonotal setae i_4,i_5, i_6, s_2 and z_1 very short and smooth, the others long and barbed. Opistonotal setae I_2, I_3, I_5 and Z_1 very short and smooth, the others long and richly barbed. Lengths of some of the long setae are:

$s_4 - 29\ \mu$ $S_3 - 29\ \mu$ $Z_4 - 24\ \mu$ $I_6 - 26\ \mu$
$s_5 - 40\ \mu$

Some sculpture is visible only on opistonotum. The superior pair o dorsal cavities bigger than the inferior one.

Systematic position: The species is similar to *Prozercon trägårdhi* (Halbert, 1923), the main differences being compared in the following table:

Prozercon trägårdhisimilis n.sp.	*Prozercon trägårdhi* (Halbert, 1923)
1. Body wider, proportion length/width 1.1—1.3, in all stages	Proportion length/width 1.4—1.5, in all stages
2. S_1 smooth and short in the adults and barbed in the nymphs	S_1 barbed in all stages and longer, reaching the insertion of S_2
3. I_6 (26—29 μ) shorter in the female	I_6 (36—40 μ) longer in the female
4. In the nymphs, podonotal setae of the *i*, *z* and *s* rows are smooth, except for i_1, i_3 and rarely shortly barbed in some deutonymphs	In the nymphs all setae except for i_5 are barbed

Material examined : 1071 specimens 820 ♀♀, 229♂♂, 14 DN, 3 PN, 5 larvae, especially from the litter and fermentation layer, but also from lapidicolous and terricolous moss synusia, as well as from saproxylicolous and xylicolous media, in the Slătioara secular forest (Rarău mountains, Eastern Carpathians) at 860—1140 m altitude. The collecting was done in a coniferous and a mixed wood during 1977—1980, from April to September, every year.

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ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN DER OBERFLÄCHE VON METACERCARIENCYSTEN DES KLEINEN LEBERGELS (*DICROCOELIUM DENDRITICUM* RUDOLPHI, 1818)

VON

DINU PARASCHIVESCU

The electronmicroscopic investigations on the surface of metacercar cysts of *Dicrocoelium dendriticum* from *Formica pratensis* Retz., as intermediary host, have pointed out the presence of some invaginations and ditch-like irregularities (10,000 : 1 magnitude). Differences between the conserved and fresh material do not exist. Under the light microscope the cysts do not show any typical sculpture (300 :1 magnitude).

Die Hülle der meist ovalen Metacercariencysten erscheint lichtmikroskopisch glatt und weiß. Einer KOH-Behandlung widersteht sie nur wenige Minuten. Die sich bewegenden Metacercarien, die S-förmig gekrümmt sind, lassen sich gut erkennen. In vorgegangenen Arbeiten [1], [3—6] wurde bereits über verschiedene Befunde an *Dicrocoelium* berichtet. Bisher existieren REM-Untersuchungen über die Cystenoberfläche nur bei aus Schnecken isolierten Cysten [7]. In der vorliegenden Arbeit sollen diese Untersuchungen und die von Mitlacher [2] durch eigene, an Ameisen erhobene Befunde ergänzt werden.

MATERIAL UND METHODE

Das Material stammt aus einem Weiden-Biotop bei Winterhausen (bei Würzburg). Es wurde aus Ameisen-Arbeiterinnen von *Formica pratenis* (RETZ) in Tetaniephase isoliert und danach entweder (1) in 70 %igem Alkohol ca 6 Monate konserviert, oder (2) als frisches Material weiterverarbeitet. Aus beiden Gruppen standen insgesamt etwa 1500 Cysten zur Verfügung. Das Material (1) wurde stufenweise in 100 %iges Aceton überführt und danach über CO_2 nach dem 'Kritscher-Punkt'-Verfahren (K.P.) getrocknet. Das frische Material (2) wurde über Nacht in 6,25 %igem Glutaraldehyd (pH 7) fixiert, in Tyrode gewaschen und in steigender Acetonreihe entwässert, anschließend wieder über CO_2 K.P. getrocknet. Die REM-Untersuchung erfolgte mit einem ISI-Super III A Rasterelektronenmikroskop (Leihgabe der Deutschen Forschungsgemeinschaft).

ERGEBNISSE UND DISKUSSION

Zwischen konserviertem und frisch fixiertem Material konnten keinerlei Unterschiede festgestellt werden. Abgesehen von kleinen anhaftenden Partikeln, die vermutlich Rückstände der Ameisen-Hämoylmphe darstellen, erscheint die Cystenoberfläche bei geringer Vergrößerung völlig strukturlos (Abb. 1). Bei hoher Vergrößerung (Abb. 2) zeigen sich jedoch in unregelmäßiger Anordnung feine gruben- und rillenartige Vertiefungen,

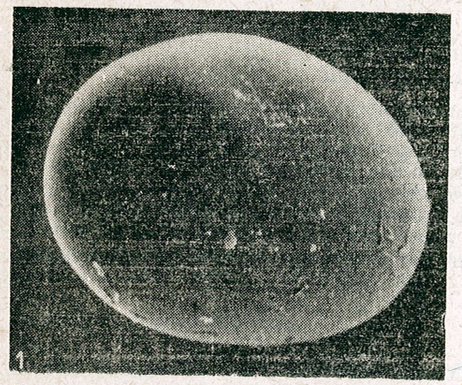

Abb.1. Frisch fixierte Metacercariencyste aus *Formica pratensis*.

Vergrößerung : 300 :1

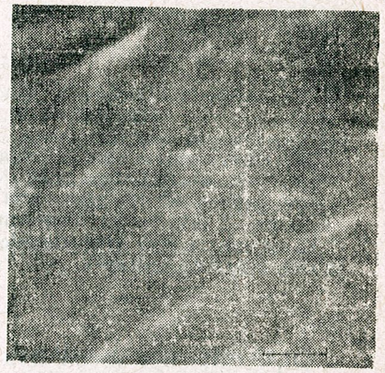

Abb.2. Oberfläche einer Metacercariencyste mit unregelmäßigen gruben-und rillenförmigen Vertiefungen. Maßstab : 1 µm. Vergrößerung : 10.000 : 1

die sicher nicht artifiziell zu deuten sind. Da unsere Kenntnisse über den Feinbau und die chemische Zusammensetzung der Cystenhülle noch lückenhaft sind, wären weitere Untersuchungen mit Ultradünnschnitten und biochemische Analysen notwendig. Dabei wäre auch die Frage zu prüfen, ob die unterschiedlichen Ameisen-Arten, die als Zwischenwirte fungieren, einen Einfluß auf Struktur und/oder chemische Zusammensetzung ausüben.

ZUSAMMENFASSUNG

REM-Untersuchungen an Oberflächen von Metacercariencysten von *Dicrocoelium dendriticum*, die aus dem Zwischenwirt *Formica pratensis* isoliert wurden, bestätigen frühere LM-Untersuchungen, daß die Oberfläche keine typischen Skulpturen aufweist. Bei hoher Vergrößerung lassen sich jedoch gruben- und rillenartige Vertiefungen in unregelmäßiger Anordnung erkennen. Unterschiede zwischen konserviertem und frisch fixiertem Material ergaben sich nicht.

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HÉMOPARASITES *(RICKETTSIALES)* DES PETITS MAMMIFÈRES *(INSECTIVORA* ET *RODENTIA)* HABITANT LES AGROSYSTÈMES DE ROUMANIE

PAR

MARIA SUCIU, DOMNICA TÂCU et MARTIN HAMAR

Studies were carried out on the haemoparasites: *Grahamella muris* (Carini, 1915); *Haemobartonella muris* (Mayer & collab., 1927) and *Eperythrozoon coccoides* (Schilling, 1928), parasitizing the erythrocytes and plasma in the following species of rodents in Romania: *Sorex araneus* (*Insectivora*); *Apodemus flavicollis, A. sylvaticus, Mus musculus spicilegus, Pitymys subterraneus, Microtus arvalis* (*Rodentia*). Among these parasites, *Eperythrozoon coccoides* is reported for the first time in Romania.

Au cours d'une étude complexe concernant l'équilibre biologique dans les agrosystèmes situés sur le terrain de l'Institut de Recherches pour Céréales et Plantes Techniques (ICCPT) de Fundulea, on a cherché a élucider les rapports variés existant entre les petits mammifères et le milieu environnant, parmi lesquels ceux parasitofaunistiques.

La présente note s'occupe des rickettsies — organismes importants pour la pathologie humaine et vétérinaire — qu'on avait identifiées dans le sang des petits mammifères. Il faut remarquer que les rickettsies produisent de graves maladies exanthématiques dans lesquelles les rongeurs jouent un rôle important du point du vue épidémiologique. Les rongeurs constituent le réservoir naturel de ces agents pathogènes tandis que leurs ectoparasites (acariens, siphonaptères) sont les vecteurs transmettant l'agent d'un hôte à l'autre.

En signalant la présence des rickettsies chez les rongeurs habitant les agrosystèmes nous attirons l'attention des épidémiologistes sur un écosystème cultivé.

En ce que concerne les hôtes, Marcheș [5] est le seul auteur roumain qui ait signalé *Grahamella* sp. et *Haemobartonella muris* chez les rongeurs.

Les terrains d'ICCPT Fundulea sont situés sur la plaine de Mostiștea (partie intégrante de la grande Plaine Roumaine), et sont limités à l'est par la rivière de Mostiștea, à l'ouest par la rivière de Dîmbovița, au sud par le Danube et au nord par la plaine de Vlăsia (26°—27° longitude est et 44° — 45° latitude nord). C'est là que se trouve aussi la petite forêt de Lizica, formée surtout de chênes (*Quercus* sp.) au milieu et de mirabelliers (*Prunus cerasifera* Ehrn.) à la lisière.

Du point de vue climatologique, la région — d'ailleurs comme toute la Plaine Roumaine — est caracterisée par une variation thermique appréciable au cours de l'année et des précipitations réduites, qui varient

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d'une année à l'autre. Le système d'irrigations du sud de la Roumanie supplée à ce manque de précipitations.

Les terrains d'ICCPT Fundulea sont cultivés avec une grande variété de plantes : céréales (blé, maïs, etc.), plantes techniques (lin, tournesol, etc.), plantes fourragères (luzerne, etc.).

La faune de petits mamifères comprend des carnivores (*Mustelidae*), des insectivores et des rongeurs, parmi lesquels on remarque le souslik (*Citellus citellus* L.) — élément caractéristique de la steppe.

MATÉRIEL ET MÉTHODE

Au début de la recherche on a capturé 125 exemplaires de petits mammifères appartenant aux espèces suivantes : *Sorex araneus* L. (*Insectivora*), *Apodemus flavicollis* Melchior, *Apodemus sylvaticus* L., *Mus musculus spicilegus* Petényi, *Pitymys subterraneus* de Selys Longchamps et *Microtus arvalis* Pallas (*Rodentia*). Les animaux vivants ont été capturés mensuellement pendant l'année 1967, de mai jusqu'en octobre. On a exécuté plus de 380 préparations de frottis avec le sang prélevé du lobe de l'oreille et du bout de la queue. Coloration de May-Grünwald-Giemsa. Pour la comparaison on a exécuté aussi des frottis de sang prélevé de souris blanches de laboratoire prises comme témoins. Sur les préparations examinées on a trouvé seulement des rickettsies (*Protophyta, Macrotatobiotes*, ord. *Rickettsiales*), à savoir : *Grahamella muris*, *Haemobartonella muris* et *Eperythrozoon coccoides*.

Grahamella muris Carini, 1915

Matériel : identifiée chez 21 individus d'*Apodemus flavicollis* (15 ♂♂ et 6 ♀♀), 3 individus d'*Apodemus sylvaticus* (♂♂), 9 individus de *Mus musculus spicilegus* (7 ♂♂ et 2♀♀), 4 individus de *Pitymys subterraneus* (♀♀) et 4 individus de *Microtus arvalis* (2 ♂♂ et 2 ♀♀).

Grahamella muris est un parasite intracellulaire, à savoir dans les érythrocytes de différents vertébrés. Dans une seule hématie il y a toujours un grand nombre d'individus cocciformes ou bacilliformes intensément colorés. La multiplication se fait par division transversale et par bourgeonnement. En général, on ne leur connaît pas d'action pathogène. Les vecteurs sont des tiques (*Ixodidae*).

Sur nos préparations, *Grahamella* est bacilliforme et en nombre variant de quelques-uns à de très nombreux individus éparpillés ou bien disposés seulement à la périphérie de l'hématie. A ce qu'il paraît, l'intensité de l'infestation varie dans la nature suivant l'espèce et l'âge de l'hôte. Ainsi, *Apodemus flavicollis* est le plus fréquemment infesté par *Grahamella muris* (80,76 %), tandis que *Mus musculus* est le plus rarement infesté (30 %) par cette même espèce. Il faut remarquer quand même qu'on a trouvé *G. muris* chez 53,94 % de tous les individus infestés par des rickettsies. Souvent on a observé *G. muris* associée à *Haemobartonella muris* (31,57 %) et à *Eperythrozoon coccoides* (21,05 %). *Grahamella* sp. est mentionnée [4] chez les insectivores (*Sorex araneus*) et les rongeurs

(*Apodemus* sp., *Clethrionomys glareolus*, *Microtus agrestis* et *Microtus nivalis*) dans les Alpes du Tirol septentrional (Autriche) ; [6] indique *Grahamella musculi* Benoit, 1920 chez *Mus musculus* et *Grahamella muris* chez le rat.

Haemobartonella muris (Mayer et coll., 1927)

Matériel : identifiée chez 1 ♂ *Sorex araneus*, 13 *Apodemus flavicollis* (10 ♂♂ et 3 ♀♀), 13 *Mus musculus spicilegus* (9 ♂♂ et 4 ♀♀), 6 *Apodemus sylvaticus* (4 ♂♂ et 2 ♀♀), 3 *Pitymys subterraneus* (2 ♂♂ et 6 ♀♀).

Haemobartonella muris est un organisme intracellulaire polymorphe ayant une forme sphérique, cocco-bacillaire ou de haltère, anneau, etc. La schizogonie a lieu dans les cellules endothéliales des vaisseaux sanguins de la rate et des ganglions lymphatiques, d'où les parasites passent en grand nombre dans les hématies. La prolifération intense dans l'organisme-hôte, l'homme y inclus, surtout après splénectomie, détermine la symptomatologie d'une maladie (bartonellose) latente jusqu'alors (cryptopathologie).

Sur les préparations examinées on a constaté *H. muris* plus souvent chez *A. sylvaticus* (87,5 %) et en égale mesure chez *Microtus arvalis* (84,61 %), la proportion la plus réduite étant trouvée chez *Pitymys subterraneus* (37,5 %). *Haemobartonella muris* est l'espèce prédominante (61,8 %) chez les petits mammifères infestés par des rickettsies. Souvent on a trouvé *H. muris* associée à *Grahamella muris* ou à *Eperythrozoon coccoides* (32,89 %) et parfois toutes les trois espèces étaient ensemble (18,42 %).

H. muris est un parasite fréquent et commun parmi les rongeurs. D'après Poisson [6], Adier (1930) aurait isolé une race très pathogène pour les souris.

Nous avons rencontré cette espèce chez les rongeurs mais aussi chez un insectivore (*Sorex araneus*). Sur nos préparations *H. muris* a la forme de petits bâtonnets et sphérules situés tant dans l'hématie que dans le plasma, mais dans la plupart des cas elle est concentrée autour de l'hématie. Elle est toujours fortement colorée et dans les infestations massives elle se trouve aussi bien dans les hématies que dans le plasma.

En Roumanie, Marcheş [5] mentionne cette espèce comme *Bartonella muris* chez *Microtus arvalis*.

Eperythrozoon coccoides Schilling, 1928

Matériel : identifié chez 1 ♂ *Sorex araneus*, 11 *A. flavicollis* (6 ♂♂ et 5 ♀♀), 5 *A. sylvaticus* (3 ♂♂ et 2 ♀♀), 6 *Mus musculus spicilegus* (3 ♂♂ et 3 ♀♀), 10 *Pitymys subterraneus*, 5 *Microtus arvalis* (2 ♂♂, 3 ♀♀).

Cette rickettsie de forme sphérique est localisée dans les hématies et dans le plasma. La multiplication se fait par division. On admet que cette espèce, qui est très pathogène, a un mode de transmission directe.

Dans le matériel étudié on a identifié ce parasite chez toutes les espèces-hôtes mais avec une incidence plus basse (38,15 %) en comparaison avec les deux autres rickettsies. L'infestation la plus fréquente a été enregistrée chez *Apodemus sylvaticus* (62,5 %).

Sur nos préparations on a observé toujours un seul élément relativement grand (1,5Ø) et intensément coloré, situé à la périphérie de l'hématie. Chez le même hôte il y avait souvent un grand nombre d'hématies infestées.

Comme on l'a déjà remarqué, *Eperythrozoon coccoides* coexiste parfois avec les deux autres rickettsies. *Eperythrozoon coccoides* n'est pas signalé par Mahnert [4] chez les petits mammifères des zones montagneuses de l'Autriche, ni par Marcheș [5] en Roumanie.

CONCLUSIONS

Parmi les espèces constituant la parasitofaune de petits mammifères habitant les agrosystèmes aux environs d'ICCPT Fundulea (zone de plaine) on a trouvé aussi des rickettsies.

On a examiné du point de vue parasitologique 129 animaux-hôtes appartenant aux espèces suivantes : *Sorex araneus* (*Insectivora*), *Apodemus flavicollis*, *A. sylvaticus*, *Mus musculus spicilegus*, *Pitymys subterraneus*, *Microtus arvalis* (*Rodentia*), chez lesquels on a identifié trois espèces de rickettsies (*Rickettsiales*) : *Grahamella muris*, *Haemobartonella muris* et *Eperythrozoon coccoides*.

Pour la première fois en Roumanie on a précisé l'espèce de *Grahamella*, notamment *G. muris* et on a signalé *Eperythrozoon coccoides*.

A l'exception de *Microtus arvalis*, les autres hôtes sont mentionnés ici comme nouveaux en Roumanie pour ces rickettsies.

L'extension de l'invasion est appréciable, 80 % de tous les animaux examinés étant infestés par ces rickettsies. Quant au sexe, on constate que les mâles sont plus fréquemment infestés que les femelles.

De toutes les rickettsies, *Haemobartonella muris* est l'espèce la plus fréquente (61,84 %) et *Eperythrozoon coccoides*, la plus rare (38,15 %). *Grahamella muris* est rencontrée le plus souvent chez les mâles (65,85 %), et aussi *Haemobartonella muris* (65,95 %) et *Eperythrozoon coccoides* (55,17 %). En général, les mâles sont plus parasités par des rickettsies (61,84 %) (Tableau 1).

L'extension de l'invasion diffère d'une espèce-hôte à l'autre, dans l'ordre suivant : *Sorex araneus* — 100 %, *Apodemus flavicollis* — 89,65 %, *Pitymys subterraneus* — 87,5 %, *Microtus arvalis* —52 %, *A. sylvaticus*— 50 % et *Mus musculus spicilegus* — 43,47 %.

Chez *Apodemus flavicollis* on a trouvé le plus souvent *Grahamella muris* (80,76 %) ; chez *Sorex araneus* et *Apodemus flavicollis* (50 %), *A. sylvaticus* (87,5 %) et *Microtus arvalis* (84,61 %) — *Haemobartonella* ; enfin, chez *A. sylvaticus* on a rencontré le plus souvent *Eperythrozoon coccoides* (62,5 %).

Les trois espèces de rickettsies peuvent coexister de sorte qu'on peut trouver fréquemment *Grahamella muris* associée à *Haemobartonella muris*.

Dans nos recherches on a dépisté les rickettsies de mai jusqu'en octobre.

Tableau 1

Hémoparasites des petits mammifères

Espèces-hôtes	Date des captures	Nombre d'hôtes examinés	Nombre d'hôtes parasités	Sexe	Age	Hémoparasites		
						Grahamella muris (Carini)	*Haemobartonella muris* (Mayer & collab.)	*Eperythrozoon coccoides* (Schilling)
Sorex araneus L.	19.08.1967	2	2	♂♂	ad.		+(1)	+ (1)
Apodemus flavicollis Melchior	17.07.1967	1	1	♀	subad.			+ (1)
	18.07.1967	4	3	♂♂	ad.	+(2)	+(2)	+ (1)
	19.07.1067	2	2	♀♀	subad.	+ (2)		
	17.08.1967	3	2	♂♂♀	ad.	+ (2)	+ (2)	+ (1)
	17.08.1967	2	2	♂♀	ad.	+ (1)	+ (1)	+ (2)
	19.08.1967	4	4	♂♂♂	ad.	+ (4)	+ (2)	+ (3)
	14.09.1967	3	3	♀♀	ad.	+ (3)	+ (1)	+ (1)
	14.09.1967	1	1	♀	subad.		+ (1)	+ (1)
	15.09.1967	4	3	♂♂♀	ad.	+ (3)	+ (2)	
	21.10.1967	5	5	♂♂	ad.	+ (4)	+ (2)	+ (1)
Apodemus sylvaticus L.	11.05.1967	1	—	♀	ad.			
	19.07.1967	2	—	♀♀	ad.			
	17.08.1967	1	1	♀♂	ad.		+ (1)	+ (1)
	19.08.1967	1	—	♂♀				
	19.08.1967	1	1	♂♀	subad.		+ (1)	+ (1)
	14.09.1967	2	1	♂♀	ad.		+ (1)	
	11.10.1967	1	—	♂♂	ad.			
	20.21.10.1967	7	5	♂♂	ad.	+ (3)	+ (3)	+ (3)
Mus musculus spicilegus Petényi	10—11.05.1967	5	—	♂♂	juv.			
	10—11.05.1967	2	—	♀♀	juv.			
	17.08.1967	5	2	♂♂	ad.	+(1)	+ (1)	+ (1)
	17.08.1967	4	—	♀♀	ad.			
	17.08.1967	2	2	♂♂	subad.	+ (2)	+ (1)	
	19.08.1967	1	1	♂	ad.	+ (1)	+ (1)	+ (1)
	19.08.1967	4	4	♀♀	ad.	+ (2)	+ (2)	+ (2)
	14—15.09.1967	4	3	♂♀	ad.		+ (3)	
	14—15.09.1967	1	1	♀	ad.		+ (1)	
	14—15.09.1967	1	1	♂	subad.		+ (1)	
	20—21.10.1967	4	2	♂♂	ad.		+ (2)	+ (1)
	20—21.10.1967	8	3	♂♂	subad.	+ (3)		
	20—21.10.1967	2	1	♀♀	ad.		+ (1)	+ (1)
	20—21.10.1967	3	—	♀♀	subad.			
Pitymys subterraneus de Selys Longchamps	19.07.1967	3	2	♀♀	ad.	+ (2)		
	14—15.09.1967	2	2	♂♂	ad.		+ (2)	+ (1)
	14—15.09.1967	2	2	♀♀	ad.	+ (2)		
	20.10.1967	1	1	♀	subad.		+ (1)	
Microtus arvalis Pallas	15—17.07.1967	7	3	♂♂	ad.		+ (2)	+ (2)
	15—17.07.1967	5	3	♀♀	ad.		+ (2)	+ (3)
	19.08.1967	2	—	♂♂	ad.			
	19.08.1967	2	1	♂	subad.	+ (1)	+ (1)	
	20.08.1967	6	3	♀♀	ad.	+ (1)	+ (3)	
	20.08.1967	1	1	♀	subad.	+ (1)	+ (1)	
	21.08.1967	1	1	♂♂	ad.		+ (1)	
	21.08.1967	1	1	♂	subad.	+ (1)	+ (1)	
Total		125	76 60,8 %			41(53,94 %)	47(61,84 %)	29(38,15 %)

Nous attirons l'attention sur *Eperythrozoon coccoides* qui, à ce qu'il paraît, se transmet aux moutons. En général, les rickettsies doivent être connues par rapport à l'existence des foyers naturels et à la possibilité de leur transmission à l'homme et aux animaux domestiques.

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CHANGES OF SOME KINETIC AND MOLECULAR PARAMETERS OF THE ATP-ASE SYSTEM IN THE GUINEA PIG DIGESTIVE TRACT AS A RESULT OF IMMUNIZATION WITH RIBOSOMAL VACCINES

BY

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The ATP-ase system in the digestive tract of guinea pigs immunized with ribosomes from different pathogenic microorganisms (*S. typhi, S. typhimurium, E. coli* and *Shigella flexneri*) is studied for the possible influence of this treatment upon the transport and absorption processes of different molecules and ions at this level. The comparative studies of the saturation curves in ATP of the ATP-ase system of the ileum of control animals and of immunized guinea pigs show an effect of competitive inhibition as if this type of immunization induced conformational changes at the level of the active catalytic center of the enzyme, affecting its affinity for the substrate. The chromatographic studies carried out on Sephadex G-200 allowed the separation of three protein peaks with enzyme activity from the preparation isolated from the ileum of the control animals. Except for the treatment with ribosomes from *S. typhimurium*, immunization with ribosomal vaccines resulted in the disappearance of the first protein peak with enzyme activity (corresponding to maximum molecular weight) and the maintenance of the other two peaks, that are affected only quantitatively.

$Na^+ + K^+$—ATP-ase has been intensively studied in the intestinal mucosa in relation to the active transport processes at this level and the distribution of this enzyme system in different membrane formations. Thus, Quigley and Gotterer [8] isolated a membrane fraction from the rat intestinal epithelial cells, which contains most of the total cellular $Na^+ + K^+$—ATP-ase. This fraction does not contain the brush border membranes, the nuclear, mitochondrial and microsomal membranes, that represent a part of the lateral or basal plasma membrane [9]. A homogeneous brush border fraction [10] which contains about 15 % of the total cellular $Na^+ + K^+$—ATP-ase was isolated from the same cells. However, there are many uncertainties concerning the localization of the enzyme system in the cells of the intestinal mucosa and its physiological role in the active transport.

Comparing the activities of the $Na^+ + K^+$—ATP-ases in the brush border and plasma membrane of the rat erythrocytes, small differences were found regarding the optimum pH of action, the affinity for the substrate, the optimum concentrations of Na^+, K^+, Mg^{2+}, the Q_{10} value, the inhibition degree with ouabain and phloridzin [10].

The number of enzymes involved in ATP hydrolysis is not known yet, but there are presumably several of them. Thus, a Mg^{2+}—ATP-ase

was demonstrated, but the relationship between the two activities was not established. Experimentally, the ATP-ase activity is determined under two conditions: in the presence of Mg^{2+}, Na^+ and K^+ (total ATP-ase) and only in the presence of Mg^{2+} (Mg^{2+} — ATP-ase). The activity of $Na^+ + K^+$—ATP-ase was calculated by the difference between the two determinations. On the other hand, Mg^{2+} — ATP-ase was measured on the basis of its property to be ouabain-insensitive.

Several studies have been devoted to the effects of free and conjugated bile acids on $Na^+ + K^+$—ATP-ase and Mg^{2+}—ATP-ase of rat intestinal mucosa [1] [3] [6] [7] [11]. Thus, Hepner and Hofman [3] demonstrated that Mg^{2+}—ATP-ase of rat intestine is stimulated by the free dihydroxy bile acids, chenodeoxycholic acid and deoxycholic acid and by the keto derivatives of cholic acid. The glycine or taurine *N*-conjugates of these dihydroxy bile acids inhibit Mg^{2+}—ATP-ase and $Na^+ + K^+$—ATP-ase.

In this paper, the changes of total specific ATP-ase in the segments of the guinea pig digestive tract as a result of immunization with ribosomal vaccines isolated from the four pathogenic microorganisms are presented. A competitive inhibition was detected, set in as a result of this treatment and due probably to the conformational changes at the level of the active catalytic center induced by immunization. The induction of some conformational changes is also inferred from the chromatographic behaviour of the enzyme preparations isolated from the ileum of control animals and immunized guinea pigs.

MATERIAL AND METHODS

The crude ribosomal fraction was prepared according to the procedure described by Kita and Kashiba [4]. In the immunization procedure, gestant guinea pig females (approximately 48 days) were immunized subcutaneously at seven-day intervals, with three doses (2 mg proteins/dose) of ribosomes. Two weeks after birth, the newborn guinea pigs were orally immunized with a single dose. After 7 — 10 days from immunization, they were sacrificed and the digestive tract segments were separated. With a view to extracting the enzyme 1 g of tissue was ground in the presence of 10 ml EDTA Na_2 5×10^{-3}M solution and 0.3 ml Triton X—100 until obtaining a homogenate. After one hour of extraction at 4°C, the homogenate was centrifuged at 7,000 r.p.m. for 10 minutes. The supernatant representing the total protein extract was preserved.

The total ATP-ase activity was assayed by determining with the method of Fiske and Subbarow [2] the orthophosphate released as a result of the enzyme reaction. The reaction mixture content was optimised when testing the ATP-ase activity of the guinea pig ileum. It consisted of 3 μ moles $MgCl_2$, 0.5 μmoles NaCl, 0.5 μmoles KCl, 1.5 μmoles ATP and the enzyme preparation in a final volume of 4 ml, buffered at pH 7 with a buffer solution Tris-HCl 0.01 M. The specific activity was expressed in mU/mg protein/min/37°C. An enzyme unit represents one μmole P_i released/min/37°C.

Protein concentration was assayed according to the method of Lowry et al. [5].

The enzyme preparations chromatography was carried out on a Sephadex G-200 column (1.8 × 30 cm), the gel being imbibed in a 2×10^{-4} M $MgCl_2$ solution. On the column, equal amounts of protein (32 mg) from the enzyme preparations obtained from the ileum of the control guinea pigs and of the animals immunized with ribosomal vaccines were applied and the elution was achieved with the same 2×10^{-4} M $MgCl_2$ solution at a rate of 20 ml/hr. Fractions of 3 ml each were collected and analysed as concerns the protein concentration and enzyme activity.

RESULTS AND DISCUSSION

Fig. 1 presents the specific activities of total ATP-ase in the stomach, jejunum, ileum, cecum and colon of the control and immunized guinea pigs. In the control animals the most intense specific activity of ATP-ase is found in the ileum and the lowest one in the stomach. The high ATP-ase activity in the colon points out the intense active transport and absorption of different molecules and ions. The high absorption capacity of the colon mucosa is well known.

Immunization with the tested ribosomal vaccines was found to produce a strong inhibition of the ATP-ase activity: in the stomach

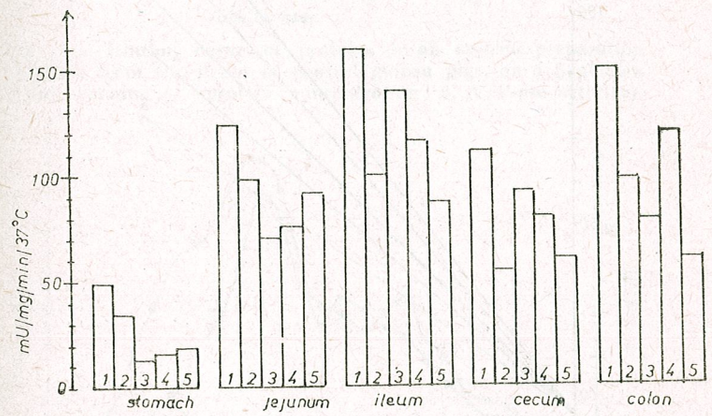

Fig. 1. — Specific activities of total ATP-ase in the stomach, jejunum, ileum, cecum and colon of control animals (1) and of guinea pigs immunized with ribosomal vaccines from *S. typhi* (2), *E. coli* (3), *S. typhimurium* (4) and *Shigella flexneri* (5).

and jejunum the highest values occur after immunization with ribosomal vaccines from *E. coli* and in the ileum and colon — with ribosomal vaccines from *Shigella flexneri*. The lowest activity is found in the cecum of animals immunized with the crude ribosomal fraction from *S. typhi*.

The inhibition of the ATP-ase activity in the different segments of the guinea pig digestive tract may be explained by the action of the treatment with ribosomal vaccines upon the transport processes at this

level. On the other hand, it is possible that the animal organism utilize large quantities of ATP with a view to catabolizing the ribosomes adm nistered orally and to absorbing their splitting products, the enzymat function of hydrolyzing this macroergic compound being thus inhibited

Since the highest specific activity of total ATP-ase was demonstrat ed in the ileum, studies were initiated to elucidate the inhibition ty induced by immunization with ribosomal vaccines. To this end the varia tion with the substrate concentration of the ATP hydrolysis rate, catalys ed by the enzyme preparations isolated from the ileum of normal guine pigs and from those immunized with ribosomal vaccines from *S. typhi S. typhimurium*, *E. coli* and *Sh. flexneri* was investigated. The experi mental results were mathematically processed according to Lineweaver Burk and in Fig. 2 we present the plots which render 1/reaction rat against 1/ATP concentration in the medium. We found a competitiv inhibition type in which the treatment with ribosomal vaccines decrease

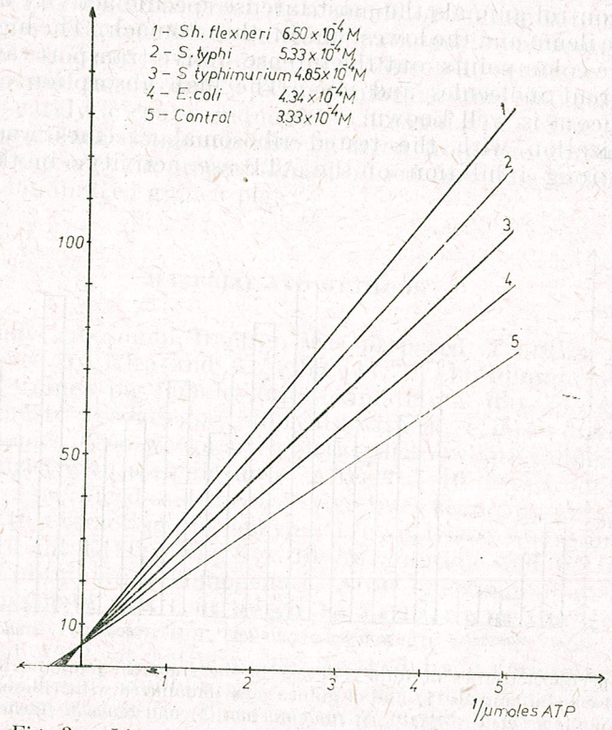

Fig. 2. — Lineweaver-Burk plots of total ATP-ase in the ileum of control guinea pigs (5) and of animals immunized with ribosomal vaccines from *E. coli* (4), *S. typhimurium* (3), *S. typhi* (2) and *Shigella flexneri* (1).

the enzyme affinity for the substrate. K_M of the enzyme in the ileum is affected in the highest degree by vaccination with ribosomes from *Shigella flexneri* and in the smallest degree — by the treatment with the ribosomal fraction from *E. coli*. It is possible that immunization induces

conformational changes at the level of the ATP-ase system in the ileum, followed by the appearance of this inhibition type.

The chromatographic behaviour of the five enzyme preparations isolated from the ileum of the control animals and from the ileum of the guinea pigs immunized with the four ribosomal vaccines was studied in order to detect these possible conformational changes. The chromatographic separation was achieved on a Sephadex G-200 column under the conditions shown above.

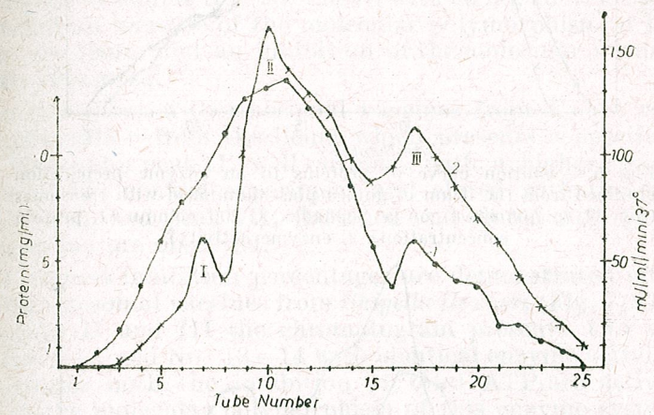

Fig. 3. — Elution curve of proteins in an enzyme preparation obtained from the ileum of control guinea pigs, on a Sephadex G-200 column (*1*, protein concentration; *2*, ATP-ase activity).

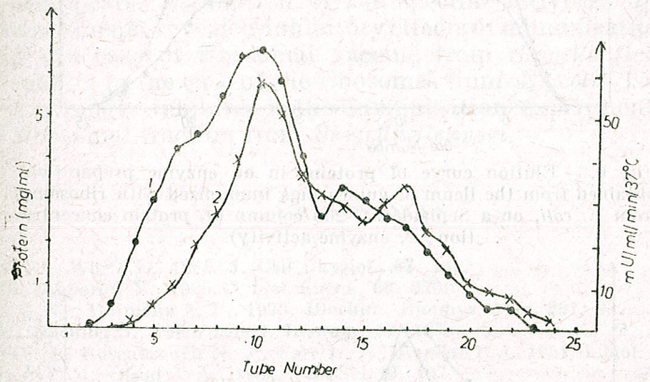

Fig. 4. — Elution curve of proteins in an enzyme preparation obtained from the ileum of guinea pigs immunized with ribosomal vaccines from *S. typhi*, on a Sephadex G-200 column (*1*, protein concentration; *2*, enzyme activity).

In the case of the enzyme preparation isolated from the control, nonimmunized animals, three protein peaks with ATP-ase activity were eluted, with the maxima in the samples No. 7, No. 10 and No. 17, corresponding to elution volumes of 21 ml (I), 30 ml (II) and 51 ml (III) respectively. This chromatogram is presented in Fig. 3.

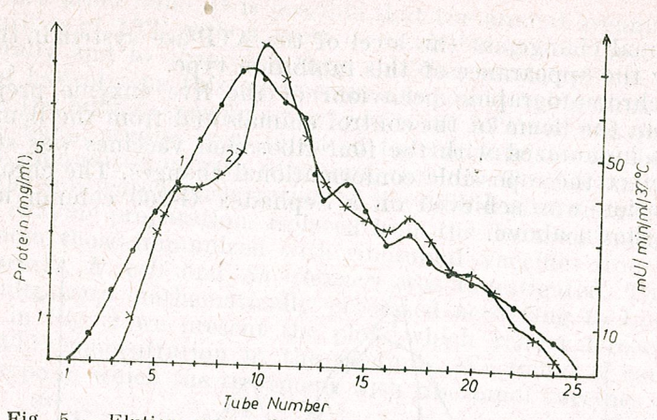

Fig. 5. — Elution curve of proteins in an enzyme preparation obtained from the ileum of guinea pigs immunized with ribosomes from *S. typhimurium*, on a Sephadex G-200 column (*1*, protein concentration; *2*, enzyme activity).

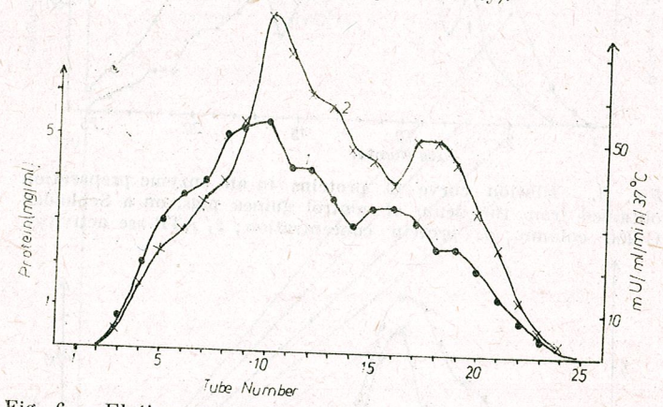

Fig. 6. — Elution curve of proteins in an enzyme preparation obtained from the ileum of guinea pigs immunized with ribosomes from *E. coli*, on a Sephadex G-200 column (*1*, protein concentration; *2*, enzyme activity).

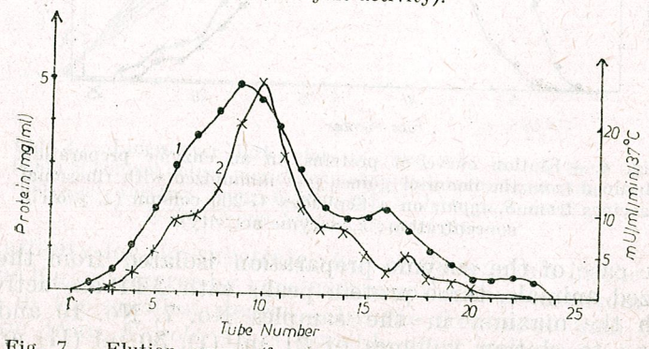

Fig. 7. — Elution curve of proteins in an enzyme preparation obtained from the ileum of guinea pigs immunized with ribosomes from *Shigella flexneri*, on a Sephadex G-200 column (*1*, protein concentration; *2*, enzyme activity).

The elution curve of proteins in an enzyme preparation isolated from the ileum of guinea pigs immunized with the ribosomal fraction from *S. typhi* is presented in Fig. 4. The chromatogram is totally different from that of the control experiment, peak I disappears, while peaks II and III are eluted with the same volumes of eluent, but are less active enzymatically.

After the treatment with ribosomal vaccines from *S. typhimurium* (Fig. 5) a different chromatogram is obtained: peak I is less evident, and besides peaks II and III, which are less active, a maximum of enzyme activity occurs in sample No. 20, eluted with 60 ml. In this case, immunization caused an increase of the molecular polymorphism of the ATP-ase system in the ileum and an inhibition of the molecular forms chromatographically separated.

The treatment with ribosomal vaccines from *E. coli* results in an enzyme preparation from the ileum which presents a specific behaviour (Fig. 6). Except for peak II, well outlined, with a higher specific activity than that of the control experiment and eluted with the same elution volume (30 ml), and peak III, flattened and eluted with 51—54 ml, no evident maxima are observed.

The highest inhibition percentages are demonstrated after immunization with ribosomal vaccines from *Shigella flexneri* (Fig. 7). In this case, besides peaks II and III the chromatogram presents two shoulders in samples No. 6—7 and No. 13—14 with identical enzyme activities. Immunization causes both the inhibition of total ATP-ase activity and an increase in the molecular polymorphism of this enzyme system.

In conclusion, as a result of immunization with ribosomal vaccines from *S. typhi*, *E. coli* and *Shigella flexneri*, the first peak of enzyme activity disappears. Estimation of the specific activities of peak II in the five experiments revealed inhibitory effects of immunization, which are highest in the case of ribosomal vaccine from *Shigella flexneri*, and a slight activation in the case of the ribosomes from *E. coli*. The activity of peak II is strongly inhibited in the immunization experiments, especially with the ribosomal fraction from *Shigella flexneri*.

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SOME DATA ON THE ANTI-INFLAMMATORY ACTION OF A STANDARDIZED PROPOLIS EXTRACT

BY

NICOLAE MIHAIL, RODICA GIURGEA, DRAGOMIR COPREAN, HONORIUS POPESCU and CONSTANTIN POLINICENCU

Treatment with a propolis extract given daily to rats with rheumatic fever induced by Freund's adjuvant results in attenuation and regression of the characteristic symptoms: softening of the articular cartilage and infiltrative lesions. The mechanisms and physiological connections following the treatment are discussed.

Several papers on anti-rheumatic drugs mention substances of different chemical classes that improve or resolve the inflammatory process [1], [8], [9]. A few researches show the propolis extract to have an anti-inflammatory action too, due to its content of flavonoids [5].

Starting from these premises we investigated in the present work the effects of a standardized propolis extract upon induced rheumatic fever (RF) in rats.

MATERIAL AND METHODS

White Wistar rats, males, 60 days of age (weighing about 180 g each) were chosen as experimental animals.

RF was provoked by injecting complete Freund's adjuvant (*Mycobacterium tuberculosis* var. *hominis*) freshly prepared, in the planta of the right posterior foot in a dose of 0.75 mg, in a volume of 0.3 ml in paraffin oil per animal.

The standardized propolis extract [15], [19], containing 5% bioflavonoids (expressed in crysine), was given to the animals in their drinking milk in the morning before feeding. The controls received starch dissolved in the same ration of milk and in the same concentration as the solution of propolis. The daily dose was of 15 mg/100 g body weight.

The rats were divided into the following groups: — a control group with untreated RF; — a group treated during 10 days with standardized propolis extract before inducing RF, then another 20 days after RF has been induced; —a group treated with standardized propolis extract beginning with the day of RF induction, during 30 days.

Ten rats per variant were sacrificed by decapitation at 14 and 30 days from the beginning of the treatment. Immediately after sacrification the hind limbs were amputated and fixed in 10% formalin for 48 hours. The pieces were decalcified in 5% nitric acid for 24 hours, then

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included in paraffin, 7-micron sections were made and stained with haematoxylin-eosin, Giemsa-Romanovsky in order to identify the blood elements, azan for collagen, the Mazia reaction for total proteins, the Hale reaction for demonstrating the acid mucopolysaccharides and then the LDH-reaction on pieces of frozen joints [2], [4], [13], [17].

RESULTS AND DISCUSSION

Macroscopically, 24 hours after the injection of complete Freund's adjuvant the hindleg swells and becomes red and hot, which denotes an inflammatory process. The subsequent evolution of the inflammation brings about necroses of the toes; sometimes the symmetrical foot is also affected. At the level of the knee the synovial space increases and a decalcification of the epiphyses takes place. Thirty days after RF induction a microgeodesic aspect becomes obvious radiographically in the distal part of the tibia and the tarsus. According to the data of Martel et al. [10], the lesions appear 14 days after the injection of Freund's adjuvant. In our case they appear much earlier.

Microscopically, typical symptoms of RF appear at the level of the articular cartilage, marginally, in the vicinity of the fibrocartilage after 14 and especially after 30 days (Figs. 1 and 2). The lesions are produced by inflammatory granular processes, with a chronic aspect and nodular infiltrations (Fig. 3).

Cytologically this territory is a connective tissue with many fibroblasts and round cells (leucocytes and lymphocytes). In some places enlarged capillaries may be observed, full with leucocytes or fibrin thrombi. As shown elsewhere, infiltrations appear in the synovial rheumatoid tissue and activation of the T and B lymphocytes as well as of plasma cells takes place [14]. Since our animals were sacrificed shortly after RF induction we did not find some symptoms mentioned in the literature: thickening of the synovial villosities, the development of a fibrous tissue from the capsule towards the surface of the cartilage, and a lympho-plasmocyte infiltrate.

Treatment with a standardized propolis extract during 20 days after RF induction results in regression of the articular cartilage softening and regression and fragmentation of the infiltrative lesions (Figs. 4 and 5). Therefore a prolonged treatment with a standardized propolis extract may have an intense action upon the lesions occurring in RF. When the standardized propolis extract is administered before the induction of RF there are no effects on the mentioned lesions. Comparative histochemical data show little differences between treated and untreated joints (collagen, LDH activity, total proteins), with the exception of mucopolysaccharides which evince less intense reactions at the surface of the joint cartilage of treated animals as compared with controls.

We consider that the standardized propolis extract has positive effects in the immunological reactions [7]. Genetic and endocrine factors and especially immune ones are involved in RF, the adjuvant producing an increased immune answer against a thymus-independent antigen, it being able to activate the macrophages [3], [12]. The flavonoids of

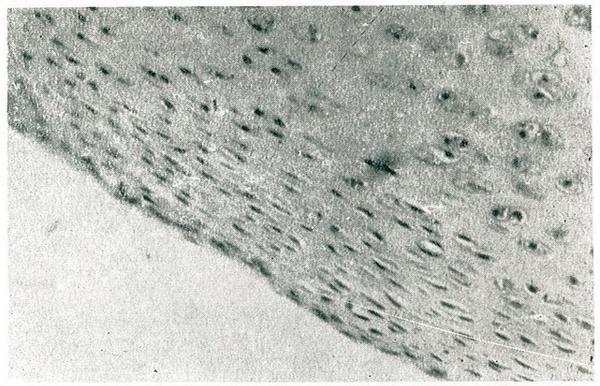

Fig. 1. — Articular cartilage of the knee in a normal rat Ob. × 20.

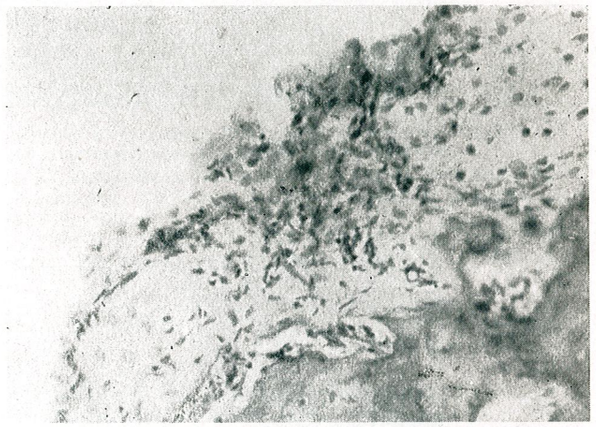

Fig. 2. — Articular cartilage with a chronic rheumatoid nodular infiltrate in a rat with rheumatic fever. Ob. × 20.

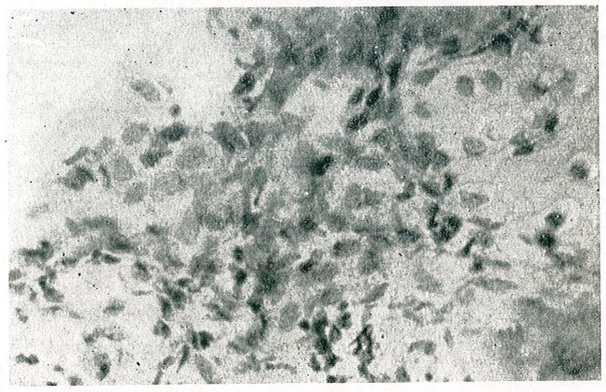

Fig. 3. — Granular infiltrate on the surface of the articular cartilage of a rat with rheumatic fever. Fibroblasts and round cells are evident. Ob. × 40.

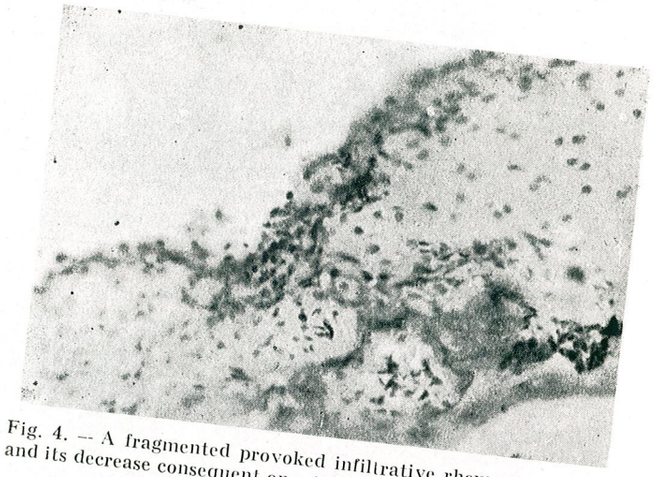

Fig. 4. — A fragmented provoked infiltrative rheumatoid lesion and its decrease consequent on a treatment with propolis extract. Ob. × 20.

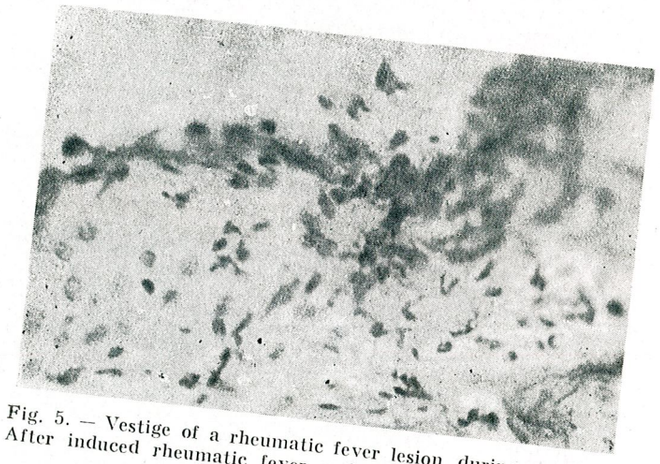

Fig. 5. — Vestige of a rheumatic fever lesion during recovery. After induced rheumatic fever and treatment with propolis. Ob. × 40.

the propolis extract work as a reversible oxido-reductive system synergistically with the ascorbic-dehydroascorbic acids [16]. Treatments with catecholamines [18] or with corticosteroids [11] are effective in RF. Thus, the attenuation of RF by flavonoids may be due to an adrenal influence. However, we were not able to demonstrate any direct action of the propolis extract upon this gland [6].

Elucidation of the mechanisms of action of propolis treatment in RF is therefore necessary.

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CYTOSTATIC AND ANTITUMOR ACTIVITY OF THE BIOSYNTHETIC ANTIBIOTIC PREPARATION A 37.4

BY

P. ROTINBERG, AL. SAUCIUC*, SMARANDA KELEMEN and ECATERINA DUCA

The *in vitro* and *in vivo* action of a new biosynthetic antibiotic preparation, A 37.4, on HeLa cell cultures and on the evolution of solid Guérin T-8 tumor was investigated. The results obtained allow to consider this isolated secondary metabolite as a potential cytostatic and antitumor agent.

One way of increasing the effectiveness of antineoplastic chemotherapy is provided by identification of new cancerostatic agents for clinical use.

Of the natural compounds synthesized by microorganisms, antibiotics arouse increasing attention due to their cytostatic and antitumor actions observed with some secondary metabolites [15], [20].

In the last 25 years a great number of antitumor antibiotics which inhibit the vital functions of both normal and malignant cells have been discovered. In the last decade some cancerostatic agents acting on human tumors have been identified among these compounds and used in antineoplastic chemotherapy [22]. This preferential action on the cancerous cell is due to its morphofunctional, biochemical, biophysical and antigenic anaplasia.

Chemotherapeutic screening programs aimed to identify new cancerostatic agents require a multistage investigation, both *in vitro* and *in vivo*, into the action of drugs with supposed antitumor effects on various biological systems characterized by different reactivities [6], [7], [9], [17], [18], [19], [21].

This paper presents the results obtained by testing the *in vitro* cytotoxic action on HeLa cells of the antibiotic preparation A 37.4 isolated at the Center for Antibiotic Research Iași, and its *in vivo* antitumor action on rats bearing Guérin T-8 solid tumors.

MATERIAL AND METHODS

Assessment of cytotoxic action on HeLa cell cultures. The cytotoxic action was assessed by comparative follow up of the total protein dynamics during the evolution of cell cultures treated with A 37.4, of cultures incubated with propylene glycol and of controls.

The test tubes were inoculated with 1×10^5 cells and after 24 hours the culture medium was replaced with a medium containing either 1.5 mg/ml of the antibiotic preparation A 37.4, or its solvent, diluted propylene glycol.

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At 24, 48 and 72 hours of culture development the medium was discarded from the test tubes and the cell layer was washed with TFS and subjected to total protein determination [10], [16].

Five culture tubes were used for each type of culture and time interval, and the statistical analysis was performed using Student's "t" test.

Evaluation of antitumor activity. White Wistar female rats weighing 150 g and bearing lymphotropic epithelioma Guérin T-8 of solid type with subcutaneous development were used.

The treatment started 24 hours after the tumor transplant and lasted for 16 days. Then, the animals were sacrificed, the tumors removed and weighed.

The antitumor treatment with the preparation A 37.4 was applied either alone in doses of 0.15 and 0.075 mg/kg body weight, administered i.p. at two-day intervals, or combined, by i.p. injection of the drug (0.075 mg/kg b.w.) at 2-day intervals concomitantly with a daily administration of chemically modified nystatin (NsMC L_2 1980) in a dose of 300 mg/kg b.w./i.p.

For a proper evaluation of the antitumor activity of the drug A 37.4, some animals received NsMC 1980 (300 mg/kg b.w./i.p. daily) or propylene glycol (diluted 1 :10) which is the vehicle for A 37.4.

The antitumor activity was assessed by determining the mean tumor regression, through the establishment of the statistical significance and of the T/C ratio (where T = mean tumor weight of the treated group and C = mean tumor weight of the control group).

RESULTS

The experimental results registered during the testing of the action of the antibiotic preparation A 37.4 on the development of HeLa cell cultures are presented in Fig. 1.

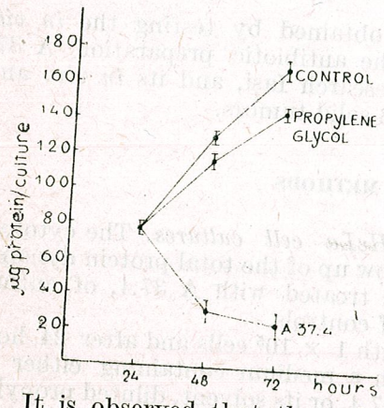

Fig. 1. — Protein content of HeLa cell cultures incubated with the antibiotic preparation A 37.4 (1.5 mg/ml).

It is observed that the values of proteins which characterize the development of treated cultures are significantly decreased ($p < 0.001$) compared to the controls. The dynamics of total proteins in treated cultures shows that the A 37.4 isolate induces an inhibition of culture development by 80 — 90 %.

A slight cytotoxic effect on HeLa cells is also observed with propylene glycol, illustrated by decreases of protein values by 10 — 15% compared to the control values.

Therefore, it is appreciated that the intensity of the inhibitory action induced by A 37.4 on cell cultures is slightly diminished by the cytotoxic effect of the solvent.

The antitumor activity of the preparation A 37.4 could not be proved when it was administered alone in the doses stated above since it induced a lethal effect after 2 — 3 injections.

The results of the experiments testing the combined treatment with A 37.4 and NsMC 1980 are presented in Table 1.

Table 1

Antitumor action of the antibiotic preparation A 37.4 (0.075 mg/kg b.w./i.p. every 2 days) administered in association with NsMC 1980 (300 mg/kg b.w./i.p. daily) on solid Guérin T-8 tumor

Group/Treatment	Mean tumor weight (g)	% Tumor regression	Statistical significance	T/C value
Control	10.0±1.1(12)	—	—	—
Diluted propylene glycol	10.0±1.2(9)	—	—	1.00
NsMC L_2 1980	9.3±1.9(9)	7.0	N.S.	0.93
A 37.4+NsMC 1980	5.3±0.6(8)	47.0	$p<0.002$	0.53

Figures in brackets indicate the number of animals.

It is observed that daily i.p. injections of propylene glycol did not affect the tumor development, since the mean tumor weight of this group was similar to that of the control group.

Administration of NsMC 1980 to rats bearing Guérin T-8 tumors induced a nonsignificant antitumoral effect, the mean tumor regression registered being of only 7%. On the other hand, in the group treated with A 37.4 associated with NsMC, the mean tumor weight showed a significant decrease ($p < 0.002$) compared to the control value. This reveals a tumor regression of 47% with a corresponding T/C value of 0.53.

DISCUSSION

The identification of new pharmacological agents with antineoplastic activity represents a major concern at present, when chemotherapy holds pride of place.

The multitude of biosynthetic, semisynthetic and synthetic substances with supposed antitumoral action led to the inclusion in the chemotherapeutic screening programs of a preliminary *in vitro* testing on HeLa and KB cancerous cell cultures of human origin which should permit the selection of some true and potent cytotoxic and/or cytostatic agents [6], [9], [17], [19], [21].

In vitro testing of the antibiotic preparation A 37.4 showed that the drug induced a significant inhibition of HeLa cell culture development. The profound alteration of the dynamics of cell total proteins by the drug allowed us to consider it as a cytostatic agent.

Although for some compounds positive correlations have been established between direct *in vitro* cytotoxic activity and the *in vivo* antitumor action [1], [8], [17], [19], [23], for the characterization of a drug as a cancerostatic agent a screening test on tumor bearing animals is required because of the internal selfdefence and control mechanisms functioning in an animal organism which can prevent the malignant transformation and evolution of some cells.

Therefore, we performed an *in vivo* test of the antitumoral action of the biosynthetic secondary metabolite A 37.4 on rats bearing Guérin T-8 tumors.

The treatment with the drug alone did not allow us to distinguish its antitumoral activity because of its high cytotoxicity and the death of animals.

However, *in vivo* testing of the antibiotic preparation A 37.4 given concomitantly with daily injections of NsMC 1980, that is a combined therapy, revealed a significant tumor regression.

The results of the present investigation have important theoretical and practical implications by the identification of a potential cytostatic and antitumoral agent, A 37.4 and also by pointing out the protective effect induced by NsMC on the organism against the high cytotoxicity of the drug.

Although the administration of the antibiotic preparation A 37.4 was not compatible with the animals' life, the combined treatment by association of NsMC 1980 with A 37.4 completely eliminated the lethal effect of the drug. The dependence of the drug antitumoral action on NsMC points out a new functional capacity of this polyene antibiotic [2]—[5], [11]—[14].

The comparison of the evaluation indexes of antitumor activity of the drug A 37.4 (mean tumor regression of 47% and T/C value of 0.53) with those imposed by the multistage screening programs of the Institute of Microbiology and Experimental Therapy in G.D.R. and of the Cancer Chemotherapy National Service Center in the U.S.A. [7], [9], [17], [19], for selection of cancerostatically active agents in a first step (mean regression of 35% and T/C ratio of 0.54), allows us to consider the isolate A 37.4 as a potential antineoplastic agent.

The results obtained by us in this preliminary investigation require its testing in the next steps of the complex screening program.

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NUCLEAR MATERIAL ELIMINATION FROM THE HEp-2 LINE CELLS DURING ADENOVIRUS 3 REPLICATION

BY

GR. MIHĂESCU, L. GAVRILĂ, I. ADERCA and D. MIȘCALENCU

Adenovirus 3 infection of thioacetamide pretreated cells of HEp-2 line reveals specific nucleolar aspects at the ultrastructural level that can be interpreted in terms of gene action (i.e. the synthesis of nucleolar fibrils on a rDNA template). At the same time, condensation and fragmentation of nuclear material take place, which are followed by chromatin blocks elimination into the cytoplasm and their enzymatic digestion. These phenomena are well correlated with thioacetamide effects as well as with viral infection.

The complex virus-host cell interactions are predominantly manifested in what is commonly called "viral order domination" which implies a deviation of cellular syntheses that normally take place in the host cell, leading to the formation of the pool of viral precursors components.

The effects of viral infection on the genetic material of host cell have been analyzed in many instances, both in animal and bacterial hosts [2], [3], [4], [9], [11].

Adenovirus infection induces a wide range of chromosomal modifications, depending on the virus serological type, the cell substrate as well as the virus dose used as inoculum.

The present paper provides ultrastructural proofs of the marked effects induced by adenovirus 3 on the genetic material of thioacetamide pretreated cells of HEp-2 line.

MATERIAL AND METHODS

Cells of the HEp-2 line from the Cell Cultures Laboratory of the Institute of Virology, grown on Eagle's medium supplemented with 10% calf serum have been used in our experiment. When the monolayer was almost complete, the cells were treated with thioacetamide in NaCl 0.85% solution for 3 days, in a quantity equivalent to 150 mg/l medium/day. After the last treatment, the cells were infected with adenovirus 3, and 24 h postinfection they were fixed in 2% glutaraldehyde buffer and postfixed in 1% osmium tetroxide. The thin sections were stained with uranyl acetate and lead citrate and examined under a Phillips 201 EM.

RESULTS AND DISCUSSION

Thioacetamide treatment leads to a quantitative diminution of the cytoplasmic ribosomes followed by nucleolus hypertrophy [1], associated

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with a ribosomal gene amplification, whose cytological proof is a large number of functionally active mininucleoli [6].

The adenovirus infection of thioacetamide pretreated cells leads to a diminished productive efficiency; viral matrices are frequently present in the nucleus of infected cells, but progenitive viral particles are encountered only in a limited number of cells [7].

Nucleolar volume increase and its fibrillar component diminution in thioacetamide pretreated cells, are easily demonstrated after adenovirus 3 infection due to rapid nucleolar segregation which also takes place in many other viral infections [8].

In our material an interesting electronmicroscopic aspect is represented by two distinct masses of the fibrillar component quantitatively diminished, united by a double linear electronclear structure (Figs 1—3). A morphologically similar structure is encountered in thioacetamide pretreated but uninfected cells (Fig. 4). The functional significance of this structure is difficult to ascertain for the moment. One can assume this structure to be rDNA template on which long rRNA molecules are synthesized, since the texture of these molecules is similar to that of the nucleolar fibrillar component. If so, these images might be taken as a morphological proof according to which the nucleolar fibrils are synthesized on a rDNA template.

Skein-like electrondense structures, which have also been observed in other viral infections [9], are encountered quite frequently in the nucleus. They suggest a chromatin condensation in which some areas, maybe the heterochromatic ones (Figs. 5, 6), are individualized, but a multiple-site chromosomal breakage, which is equivalent to chromosomal pulverization cannot be ruled out either. The process of chromosomal fragmentation is probably the result of molecular interactions during the viral component synthesis and does not reflect a primary virus-host cell interaction effect. Subsequently, dense chromatin blocks are transferred from the nucleus to the cytoplasm (Figs 7,8), where they are enzymatically degraded (Fig. 9), perhaps under the action of enzymes released from

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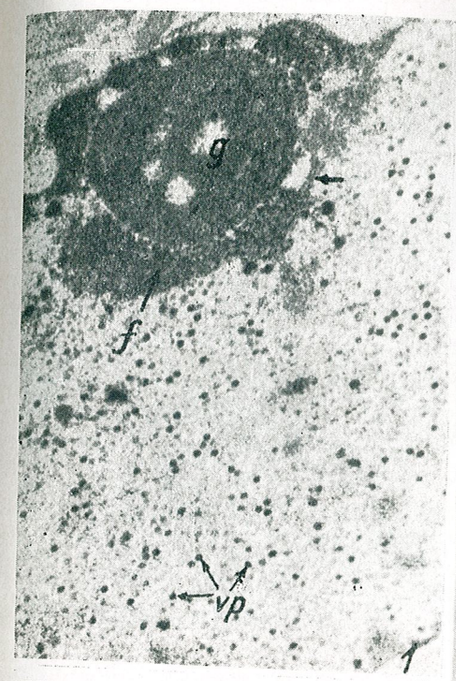
Fig. 1. — General view of the HEp-2 line cell nucleus. Nucleolus is segregated. The granular component (g) is hypertrophic. The fibrillar component (f) is subdivided into two distinct masses, united by a double structure with an electronclear core (arrow). The viral particles (vp) do not reach a crystalline arrangement. × 19,600.

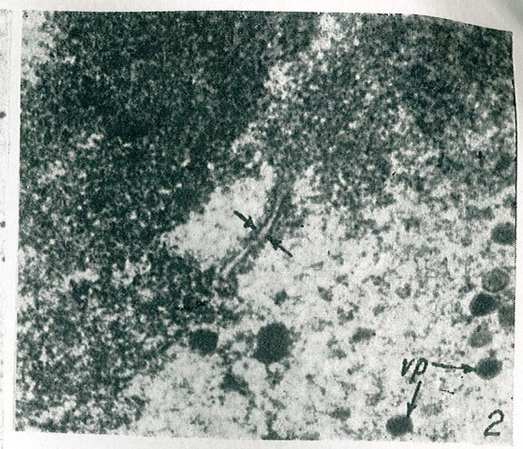
Fig. 2. — A detail of Fig. 1. Electrondense components of that double structure are closely associated with a material having the same texture as the fibrillar component of the nucleolus (arrows). × 59,200.

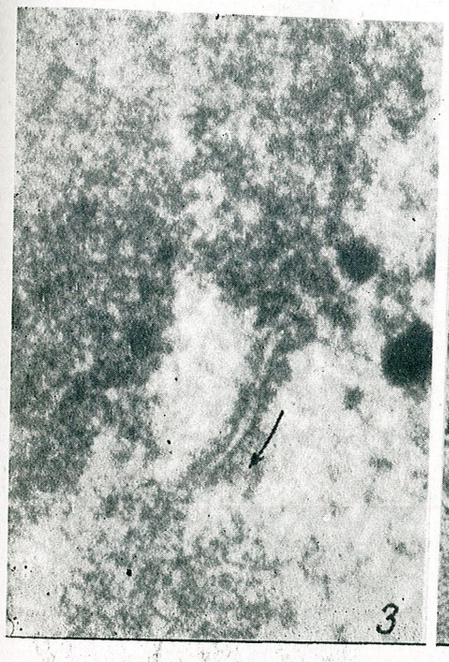
Fig. 3. — A detail of the same Fig. 1. The double structure with an electronclear core seems to be the anchorage zone of some fibrillar structures with a length gradient shown by the arrow and which are passing into nucleolar fibrils. × 160,000.

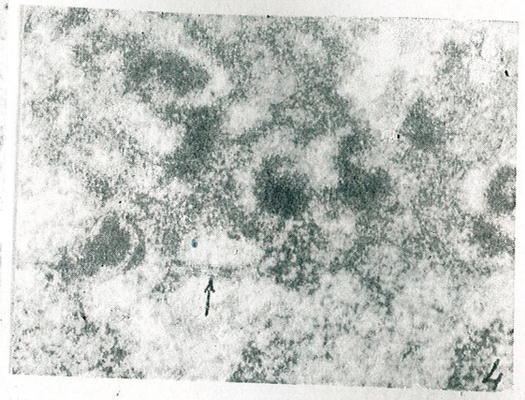
Fig. 4. — A similar structure as in Figs 2 and 3 in the nucleolus (arrow) of thioacetamide treated but uninfected cell. × 40,000.

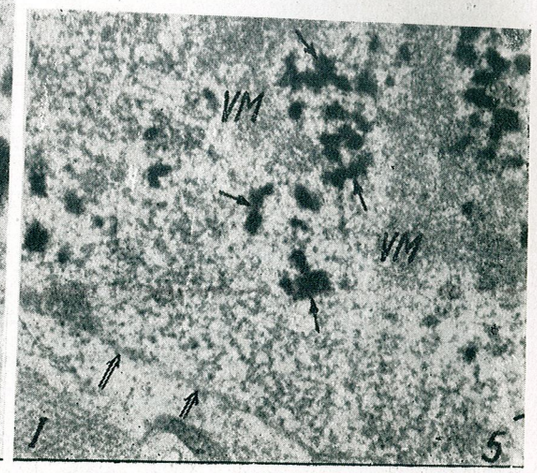
Fig. 5. — Several skein-like structures (arrows), randomly distributed among viral matrix components (VM) that appear in the nucleus. Nuclear envelope is discontinuous (double arrows) and a paracrystalline inclusion (I) with a few viral particles reached the cytoplasm. × 32,250.

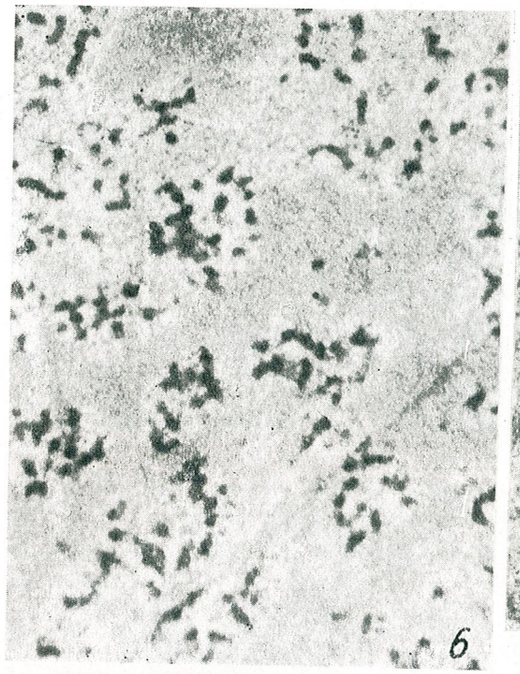


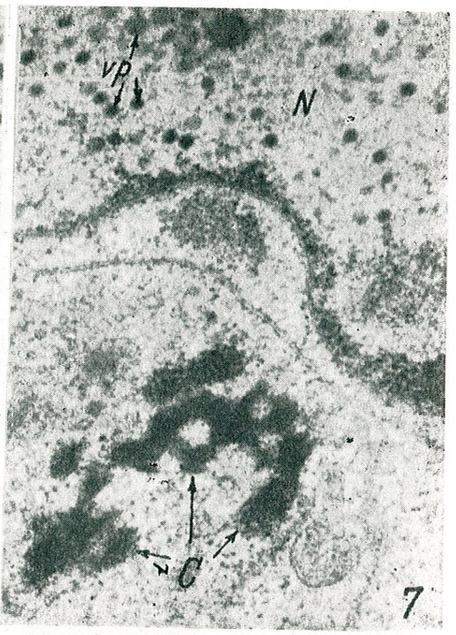


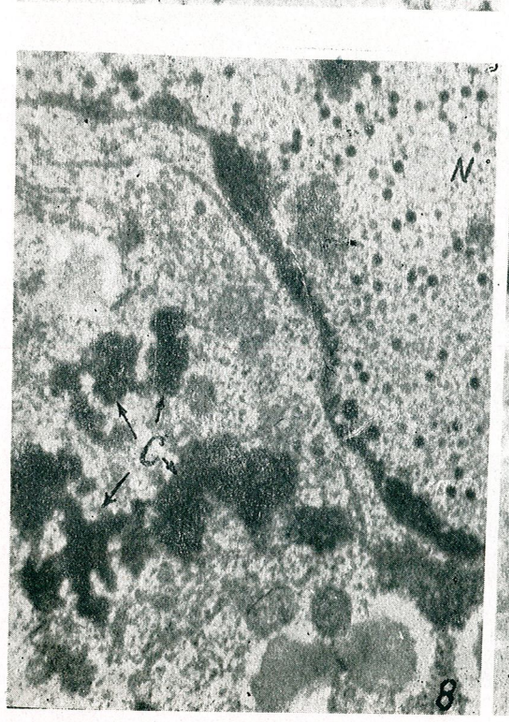












lisosomes, whose permeability is getting higher due to thioacetamide treatment. The alternating dense and clear areas, alongside of a structure which seems to be a chromosome fragment (Fig. 9) suggest an ordered sequence of chromatin areas with different levels of resistance to the enzyme action, as an expression of different degrees of chromatin spiralization and condensation.

In thioacetamide untreated cells of HEp-2 line, infected with adenovirus 3, we have not encountered these processes of chromatin condensation and cytoplasmic elimination [5]. The chromosomal modifications observed under the light microscope are especially induced by oncogenic representatives of adenoviruses [2].

Which is the role of thioacetamide in the induction of these processes? It is possible that the degradation of cytoplasmic ribonucleoproteins as a consequence of thioacetamide treatment is followed not only by an increased nucleolar activity, but also by activation of the synthesis of a wide range of proteins whose half-life is greatly reduced after treatment [1]. Subsequent infection with adenovirus 3 takes place when the cell discharges a high synthetic activity and perhaps the more intense the latter, the more marked the effects of the former. On the other hand, the increased lysosomal membrane permeability after thioacetamide treatment, amplified by viral infection [12], may represent a way through which these modifications are enhanced.

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Fig. 6. — Chromatin condensation occupying large nuclear areas. × 24,500.
Figs 7 and 8. — The large chromatin blocks (C) released into the cytoplasm. × 32,250 and 39,200 respectively.
Fig. 9. — Chromatin blocks with an ordered structure of alternating dark and clear zones are presumably enzymatically degraded in the cytoplasm. × 32,500.

AN ASSESSMENT OF THE CLASTOGENIC POTENTIAL OF TWO PESTICIDES IN HUMAN LYMPHOCYTES *IN VITRO*

BY

RUXANDRA TARNAVSCHI and CORNELIA GEORMĂNEANU

The clastogenic ability of two pesticides, chlorisopropylphenolcarbamate and isopropylphenolcarbamate was assessed in 72-h human blood cultures.
The effect of four concentrations (5, 10, 20, 40 μg/ml), administered during the last 24 h of incubation, was studied in comparison with control cultures.
In none of the treated cultures were chromosomal aberrations found to surpass control values.

A complex study of pesticides is justified by their wide utilization so that most people are exposed to their influence.

A large number of pesticides including insecticides, fungicides, herbicides, acaricides, growth regulators and inhibitors, seed sterilizers, are used either in granular form or as dusts, sprays, and aerosols.

Humans are affected either by handling these agents, because of the ecological area or by consuming toxic residues in food. Hence, a few people, such as agricultural workers, or workers in pesticide plants, are exposed daily to high concentrations of toxins, while most people accumulate small amounts of pesticides in food.

All organic pesticides are metabolized in living organisms or are chemically, thermally, photolytically, and microbiologically degraded in the environment [2]. The extent and nature of these transformations vary with time, chemical structure and physical form. The transformation of some of these agents occurs in a few minutes while that of the others requires months or even years.

Beyond the acute or chronic danger of intoxication the possible mutagenic effects can threaten the genetic health of future generations [2]. Therefore investigation of the genetic effects is of major importance.

Clastogenic, mutagenic and carcinogenic effects of pesticides on genetic material have been reported in earlier investigations [4],[6]—[9], [12]— [14] carried out by *in vivo* and *in vitro* experiments and the study of the workers handling these substances.

Because of the apparent relationship between chromosomal damage, mutagenesis and carcinogenesis, analysis of induced chromosomal aberrations has become a useful tool in evaluating the hazard involved in environmental mutagens and carcinogens. This is one of the most widely utilized methods in detecting genetic damage in man [6].

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MATERIAL AND METHOD

The two carbamates, chlorisopropylphenolcarbamate and isopropylphenolcarbamate tested by us were synthesized by the Chemical Institute Cluj-Napoca. They are herbicides with a systemic action, used in agriculture as growth inhibitors.

The experiment was carried out on human blood cultures from healthy donors who had not been submitted to any recent chemical treatment or irradiation and had not suffered of late from any viral disease.

The blood cultures were set up by the modified Arakaki and Sparkes method [1] in IC-65 medium (Cantacuzino Institute) supplemented with 20% calf serum (Cantacuzino Institute), 1-glutamine 1% and phytohaemagglutinin M (Difco).

The four pesticide concentrations (5, 10, 20, 40 μg/ml) were added to the 72-h cultures, during the last 24 h period before harvesting.

Cultures treated only with the solvent (propylene glycol) were used as controls. Colchicine was added in a concentration of 5 μg/ml 90′ before the cells were harvested.

After a hypotonic treatment with KCl 0.75% for 15′ at 37°C, the chromosome slides were prepared consecutively to four methanol-acetic acid fixations, flame dried and Giemsa stained.

A number of 100 metaphases from each concentration and for each pesticide were analysed. The same number was examined for control cultures.

RESULTS AND DISCUSSION

Results of cytogenetic analyses carried out on human blood cultures treated with four concentrations (5, 10, 20, 40 μg/ml) of the two pesticides, as well as on untreated controls are shown in Table 1.

Chromosomal aberrations scored were: breaks, fragments, spiralization defects, numerical variations and premature chromatin condensation (PCC).

Gaps were not recorded as chromosomal aberrations, but break was considered as a basic chromomere lesion.

The frequency of abnormal cells ranged from 0 to 5.45 % for treated cultures and 4.65% for controls, so that the difference is not statistically significant (χ^2 test).

Only a few cells with spiralization defects were seen: 1.94% for chlorisopropylphenolcarbamate and 0.008% for isopropylphenolcarbamate. Spiralization defects have also been described elsewhere as being induced by some pesticides [3], [15].

In our study endoreduplications were found only after isopropylphenolcarbamate treatment: 0.82% at 5 μg/ml and 0.75% at 10 μg/ml concentrations.

Among the numerical variations tetraploidy, triploidy, and small heteroploidy were observed more frequently after isopropylphenolcarbamate treatment. This could suggest a possible action of pesticides interfering with cell division processes.

The premature chromatin condensation (PCC) has only accidentally occurred after 40 μg/ml treatments.

From our experimental data it can be inferred that neither chlorisopropylphenolcarbamate, nor isopropylphenolcarbamate has significantly increased the chromosomal level in any of the concentrations used.

Table 1

Chromosomal aberrations induced by chlorisopropylphenolcarbamate and isopropylphenolcarbamate in human blood cultures

Concentration μg/ml	No. of cells examined	Aberrant cells		Gaps		Breaks		Fragments		Spiralization defects		Numerical variations		PCC	
		No.	%	No.	Per cell	No.	Per cell	No.	Per cell	No.	%	No.	%	No.	%
Chlorisopropylphenolcarbamate															
5	110	6	5.45	1	0.009	4	0.036	2	0.018	—	—	—	—	—	—
10	208	5	2.40	3	0.014	3	0.014	1	0.005	—	—	—	—	—	—
20	115	—	—	—	—	—	—	—	—	—	—	2	1.73	—	—
40	103	5	4.83	1	0.009	2	0.019	1	0.009	—	—	—	—	1	0.97
Control	43	2	4.65	1	0.023	2	0.046	—	—	—	—	—	—	—	—
$\chi^2 = 7.407$ P ~ 0.10															
Isopropylphenolcarbamate															
5	122	1	0.81	3	0.024	—	—	1	0.007	1	0.008	2	1.64	—	—
10	134	2	1.49	4	0.029	1	0.007	1	0.007	—	—	4	2.98	—	—
20	113	3	2.65	1	0.009	1	0.009	2	0.018	—	—	1	0.88	—	—
40	130	4	3.07	4	0.030	2	0.015	2	0.015	—	—	3	2.31	1	0.77
Control	43	2	4.65	1	0.023	2	0.046	—	—	—	—	—	—	—	—
$\chi^2 = 3.156$ P ~ 0.50															

The absence of the clastogenic effect has been earlier reported for other pesticides, in studies on blood specimens collected from pesticide workers [6] and on human blood cultures and rat thymocytes [10].

A dose-dependent clastogenic effect was found after pesticide treatments *in vitro*, on human blood cultures and bone rat marrow [4].

Increased chromosomal break and a constant damage of Y chromosome [13], as well as chromatid type and labile chromosome type aberrations have been reported to occur frequently both in pesticide workers and in control groups of factory employees [7].

Many structural aberrations may lead to cell death and this is sometimes considered as a result of the toxic action of pesticides [5].

A mutagenic action of pesticides has been observed by Fishbein [2] in 70% of the tested carbamates and by Seiler [12] who suggested that some of these chemicals could produce an irreversible alteration of DNA.

Many of the pesticides investigated can produce an increased frequency of the sister chromatid exchanges (SCE) without having any clastogenic action [14].

A SCE frequency increase can appear at much lower doses than those producing chromosomal aberrations. The pesticides investigated in

present study might induce a mild damage at the DNA level which not expressed as chromosomal aberrations.

Our experimental data can not be used alone as an indicator of the genetic damage induced by the chronic effect of pesticides.

For the extrapolation of the *in vitro* experimental results to chronically exposed humans, additional factors must be taken into account, as : individual data, human metabolism, genetic heterogenicity of human population, and environmental factors [4], [11].

CONCLUSIONS

The experiment conducted on human blood cultures shows no clastogenic effect of the pesticides chlorisopropylphenolcarbamate and isopropylphenolcarbamate in the four concentrations used.

Detection of pesticides free of mutagenic and carcinogenic effects is of major importance for agriculture.

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