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REVUE ROUMAINE DE BIOLOGIE

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SOMMAIRE

SUR LA VALIDITÉ DES ESPÈCES *SARCOPHAGA DUX* THOMSON ET *SARCOPHAGA EXUBERANS* PANDELLÉ, AVEC LA DESCRIPTION D'UNE NOUVELLE ESPÈCE AFRICAINE DU GENRE *LIOSARCOPHAGA* ENDERLEIN *(DIPTERA, SARCOPHAGIDAE)*

ANDY Z. LEHRER

The validity, the taxonomical position and the geographical distribution of the species *Sarcophaga dux* Thomson and *Sarcophaga exuberans* Pandellé are discussed. The holotype of *Liosarcophàga dux* (Thomson) and a new African species, *Liosarcophaga babiyari* sp. n., are described. A key for the identification of this species is given.

Dans un intervalle d'un siècle et demi, les espèces *Sarcophaga misera* Walker, 1849 et *Sarcophaga dux* Thomson, 1869 ont provoqué beaucoup de confusions et controverses taxonomiques. Elles ont été synonymisées alternativement et ont été confondues ou identifiées volontairement avec d'autres espèces affines comme: *S. exuberans* Pandellé, 1896, *S. harpax* Pandellé, 1896, *S. tuberosa* Pandellé 1896 et *S. orchidea* Böttcher, 1913.

Parmi les grands connaisseurs de la famille Sarcophagidae, Rohdendorf (1937) a reconnu une *Parasarcophaga (Liosarcophaga) exuberans* (Pand.) avec le tergite anal du mâle d'un rouge-brunâtre jusqu'au rouge-orange et une *P. (L.) misera* (Walk.) avec le tergite anal noir. Également, la première a le distiphallus pourvu d'apophyses latérales longuement bifides, étant répandue dans l'Europe du Sud, l'Afrique septentrionale, le Crimée et le Nord du Caucase; la dernière a les apophyses latérales simples ou avec une petite dent subterminale, en se trouvant en Chine et en Australie.

Senior-White, Aubertin et Smart (1940 : 267) n'acceptent pas la validité de *S. misera* Walker, parce que «the type of misera is a female» et, vraiment, «thus its identity with the male selected as neotype by Johnson and Tiegs can never be certain». C'est pourquoi ils admettent seulement *S. dux* Thomson et introduisent *S. tuberosa* Pand. et *S. misera* Auct. (nec. Walker) dans sa synonymie. Ainsi, la dispersion de cette espèce a été élargie à l'Europe, à l'Asie occidentale et aux régions orientale et australienne.

Cependant, ni de leurs dessins et ni des mentions de Nandi (1989 : 397) on ne peut identifier la vraie espèce existante dans la région orientale.

Séguy (1941) rétablit la «priorité» apparente de *S. misera* Walker et introduit dans sa synonymie non seulement *S. dux* Thomson, mais aussi les bonnes espèces européennes *S. exuberans* Pandellé, *S. harpax* Pandellé et *S. tuberosa* Pandellé.

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Le mélange de ces espèces à caractères distiphalliques très distincts l'a conduit à la conclusion, évidemment erronée, que l'«appareil copulateur du *S. misera* est très variable» (supra, p. 122).

Zumpt (1951) admet tout d'abord *S. dux* Thomson et considère *S. exuberans* Pandellé comme un de ses synonymes. Mais, après l'étude de Rohdendorf sur le système des Sarcophagines éthiopiénnes (1963 : 9), dans laquelle il doute que cette espèce paléarctique se trouve aussi en Afrique ("? Africa"), Zumpt (1964) reconsidère son opinion. Il accepte l'existence de *S. exuberans* Pandellé dans «apparently the whole of the Ethiopian region» (Zumpt,1972 : 160) et présente sa génitalie, d'après un «specimen from Johannesburg, Transvaal» (supra, 1964 : 72, fig. 12 et 1972 : 160, fig. 91) comme base de comparaison pour sa nouvelle espèce malgache *S. exuberansoides* Zumpt.

Fan Zi-de (1965) a introduit *Parasarcophaga (Liosarcophaga) misera misera* (Walker) dans la faune de Chine avec le synonyme «*Sarcophaga dux* sensu Hall & Bohart, 1948 (nec. Thomson, 1868)», parce que - d'après sa note infrapaginale (supra, p. 266) – («*Sarcophaga dux* Thomson, 1868 = *Sarcophaga harpax* Pand., 1896)» (!).

Les spécialistes Kano, Field et Shinonaga (1967 : 48) retiennent *Parasarcophaga misera* (Walker) (= *S. dux* Thomson), sans rappeler *S. exuberans* Pandellé, inexistante au Japon.

Dans le catalogue des Sarcophagides afrotropicales, Dear (1980) admet une «*Thyrsocnema*» (!) *exuberans* (Pand.), mais il exagère en introduisant *S. craggi* Parker, *S. exuberansoides* Zumpt et *S. dux* Auct. dans la synonymie de celle-ci.

Le catalogue des Sarcophagides paléarctiques de Verves (1986) enregistre trois espèces affines même dans deux sous-genres différents (!): *Parasarcophaga (Liosarcophaga) dux* (Thomson) de Chine, Corée du Sud, Japon et des régions orientale et australienne; *Parasarcophaga (Liosarcophaga) exuberans* (Pandellé) des régions paléarctique, afrotropicale et orientale; *Parasarcophaga (Parasarcophaga) misera* (Walker) de l'Asie et des régions orientale et australienne, mais ayant comme synonyme la bonne espèce *S. orchidea* Böttcher (!).

Cette opinion erronée a été développée par Povolný (1987) sous la forme d'une vraie conception anti-taxonomique et totalement non-justifiée au point de vue scientifique. Bien qu'il reconnaisse que «*Parasarcophaga (s. str.) misera* (Walker, 1849) was described after a female [. . .] from Australia» (supra, 1987 : 149), il l'a identifié (?!), probablement d'après son «armature génitale» (!), avec le mâle de la bonne espèce *Sarcophaga orchidea* Böttcher, en dépit du fait que ses prédécesseurs l'ont reconnue en *S. dux* Thomson, *S. harpax* Pand., *S. tuberosa* Pand., *S. subtuberosa* Parker, etc. Mais, ce qu'il semble tout à fait surprenant, c'est que Povolný reprend le leitmotive de Séguy, en affirmant que «*Parasarcophaga (Liosarcophaga) dux* (Thoms.) is purely morphologically a rather variable and obviously polytypical species» (supra, 1987 : 159). C'est la raison pour laquelle, on ne peut pas comprendre pourquoi il a introduit dans la synonymie de *P. (L.) dux* seulement les espèces *S. exuberans* Pand. (paléarctique et éthiopienne), *S. subtuberosa* Parker, *S. ceylonensis* Parker (mise en synonymie

avant lui par Senior-White, Aubertin & Smart, 1940 : 266) et *S. craggi* Parker, en acceptant seulement *S. sarracenoides* Aldrich comme «subsp. bona» de celle-ci; et n'a pas introduit aussi, conséquemment, les autres espèces affines, d'une variabilité morphologique semblable, comme par exemple: *S. harpax* Pand., *S. tuberosa* Pand., *S. marshali* Parker, *S. madeirensis* Schiner, *S. brevicornis* Ho, *Parasarcophaga portschinsckyi* Rohd., *P. jacobsoni* Rohd., *P. pleskei* Rohd. ou même *S. scopariformis* S. -W., etc.

De toutes les données présentées plus haut on constate que seulement Senior-White, Aubertin et Smart (1940) ont justifié leur opinion et ont exposé un point de vue correct en ce qui concerne l'inadmissibilité de l'espèce incertaine *S. misera* Walker et la validité de *S. dux* Thomson. Ils ont fourni aussi l'information exacte que le type de *S. dux* Thomson se trouve dans le Muséum d'Histoire Naturelle de Stockholm et une information inexacte (1940 : 267) que la génitalie de celui-ci a été examinée par Aldrich.

Dans son travail, Aldrich (1931 : 27) reconnaît très clairement que «I did not spread the genitalia of the Thomson type», utilisant une méthode inconcevable et inacceptable dans nos jours pour l'établissement de son identité spécifique. Il dit plus loin: «but have other males from Honolulu in the National Museum (de ÉUA - n. n.) wich have been spread». Sur la base d'une telle «étude comparative», il tire la conclusion que le type du Muséum de Stockholm «as I expected, this is the same as *Sarcophaga harpax* Boettcher».

D'ici résulte indubitablement que tous les raisonnements taxonomiques sur la validité et les synonymes des espèces mentionnées sont édifiés sur de simples suppositions et que la seule voie d'arrêter l'afflux des erreurs est l'examen scientifique du type de *Sarcophaga dux* Thomson qui, heureusement, se conserve encore dans les collections de Stockholm. Celui-ci n'a été connu par aucun des spécialistes contemporains et personne jusqu'à nous n'a vu l'originalité morphologique de son armature génitale mâle.

En même temps, ayant à notre disposition un riche matériel africain, nous nous sommes convaincu que dans la région éthiopienne il n'y a ni l'espèce hawaïenne *Liosarcophaga dux* (Thomson) et ni l'espèce paléarctique *Liosarcophaga exuberans* (Pandellé). Les espèces interprétées ainsi par Zumpt (1951, 1964, 1972) ne sont qu'un taxon affine différent, propre à la faune d'Afrique, dénommé par nous *Liosarcophaga babiyari* sp. n. et décrite plus bas.

Remerciements

Prof. Heikki Hippa (Naturhistoriska Riksmuseet, Stockholm) a eu la bienveillance de nous prêter le type de *Sarcophaga dux* Thomson et nous a permis de faire les préparations microscopiques de son armature génitale.

MM. Dr. B. R. Stuckenberg et Dr. D. A. Barraclough (Natal Museum, Pietermaritzburg) ont eu l'immense compréhension et noblesse de nous offrir une unique collection de Sarcophagines africaines, qui nous a donné la possibilité d'établir sûrement l'identité de *Liosarcophaga babiyari* sp. n.

Dans les collections de l'Institut pour la Systématiques et la Biologie des Populations (Muséum Zoologique) de l'Université d'Amsterdam j'ai trouvé un mâle appartenant à *Liosarcophaga exuberans* (Pandellé, 1896) de Grèce, qui m'a été prêté par Mme Dr. Sandrine Ulenberg et M. Ben

Brugge pour études détaillées.

Le Service de Référence de la Bibliothèque Canadienne de l'Agriculture (Ottawa) a complété notre bibliographie avec quelques articles absolument nécessaires pour cette étude.

Nous prions ces distinguées personnalités, collègues et institutions d'accepter le témoignage de notre sincère gratitude.

DIAGNOSES

1. Description du holotype de *Sarcophaga dux* Thomson

MÂLE

Tête. Noire et couverte d'un tomentum argenté à teinte brunâtre; le vibrissarium avec ses branches péristomale et suboculaire est brunâtre. Front, vu du dessus et au lieu le plus étroit, mesure presque 1/2 de la largeur d'un œil. La bande frontale noire brunâtre presque deux fois plus large qu'une parafrontalie. Les antennes noires, à nuance brunâtre sur les articles basaux et tachées d'orange sur le bout distal du deuxième article, sur la base et sur la moitié proximale interne du troisième article; le dernier est presque deux fois plus long que le précédent. Arista brune noirâtre; épaisse sur 1/4 basal et avec de longs poils sur les deux parties. Palpes noirs à teinte brunâtre; la trompe noire. Le péristome mesure 1/3 du grande diamètre oculaire.

Chétotaxie de la tête. Les macrochètes verticaux internes sont tombés, mais après leurs bases, ils sont longs, forts et rétroclines; les macrochètes verticaux externes manquent; les ocellaires proclines plus courts et plus fins que les préverticaux rétroclines; les macrochètes frontaux au nombre de 10 paires; on observe 1-2 macrochètes parafaciaux très fins et courts; les petites vibrisses montent un peu sur les bordures faciales; il y a 2 postocellaires et 1 postvertical de chaque côté de l'occiput; les microchètes occipitaux disposés sur un rang. Le péristome est couvert de poils noirs; la partie postérieure de la tête a de poils blancs.

Thorax. Noir à tomentum argenté; il a 3 bandes médio-dorsales longitudinales noires larges et 2 bandes latérales étroites et courtes. Les propleures glabres; prosternum poilu. Les stigmates antérieurs sont d'un jaune-brun noirâtre; les stigmates postérieurs d'un jaune-orange. Les pattes sont noires, avec les tibias d'un brun foncé.

Chétotaxie du thorax. ac = 0 + 1 (le postsutural fin), dc = 4 + 4(5), ia = 0 + 2, prs = 1, sa = 3, h = 3, ph = 2, n = 4, pa = 2, sc = 4 + l, pp = 1 (plus quelques poils), pst = 1, st = 1:1:1.

Ailes. Transparentes. Épaulette noire; basicosta et costagium jaunes. La nervure r_1 glabre; la nervure r_{4+5} est ciliée jusqu'à la proximité de r-m. Cubitulus courbé dans un angle un peu aigu et prolongé d'un pli. L'épine costale petite. Les écailles blanches jaunâtres; les balanciers d'un brun un peu plus foncé.

Chétotaxie des tibias. Les tibias antérieurs ont 3 ad proximaux et 1 pv; les tibias médians manquent; les tibias postérieurs ont 2 ad, 1 av, 2 pd et une longue pilosité ventrale, plus longue sur les parties postéro-ventrales que sur

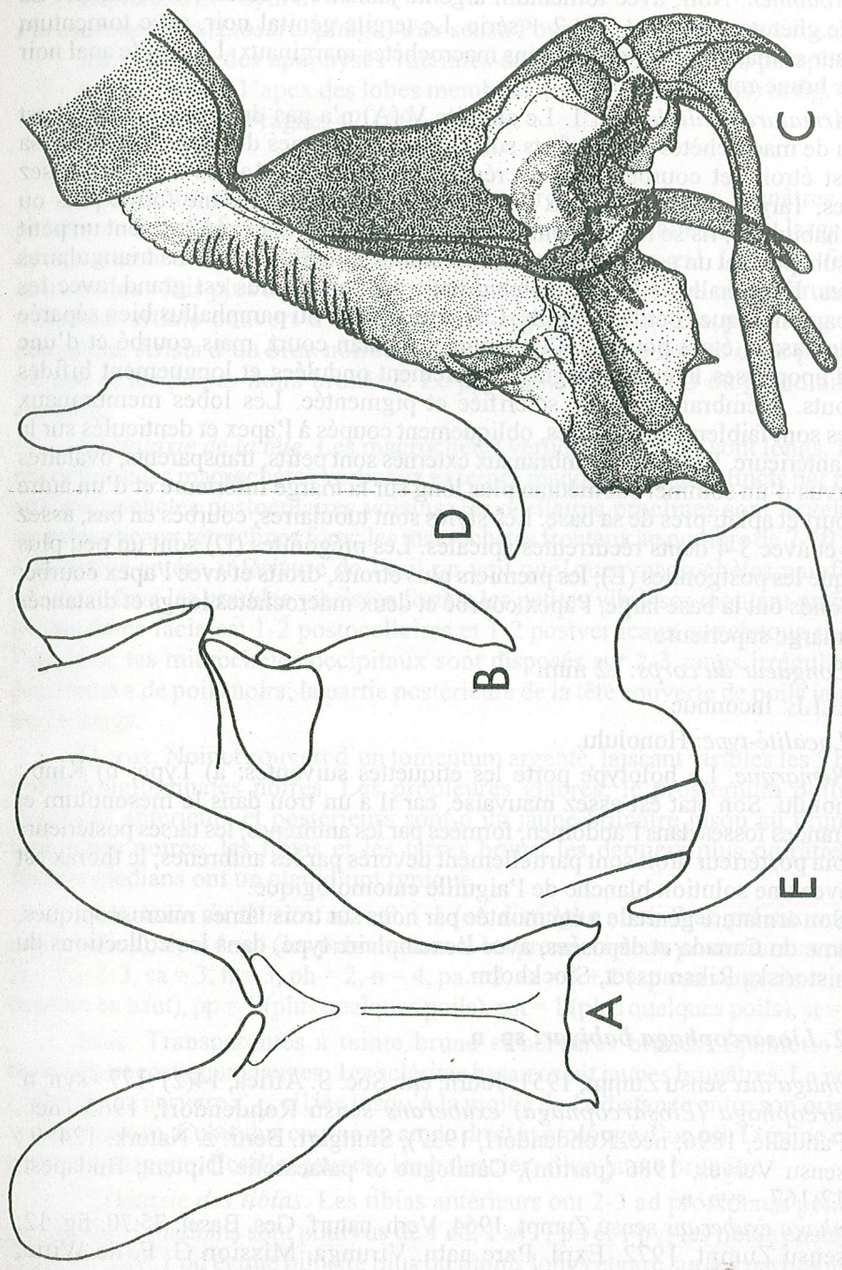

Fig. 1. – Armature génitale de *Liosarcophaga dux* (Thomson) (holotype). A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.

celles antéro-ventrales.

Abdomen. Noir, avec tomentum argenté jaunâtre et dessins en damiers. La formule chétotaxique: 0 + 0 + 2 + série. Le tergite génital noir, avec tomentum dense sur sa marge postérieure et sans macrochètes marginaux. Le tergite anal noir à teinte brune-rougeâtre.

Armature génitale: fig. 1. Le sternite V (A) n'a pas de brosses, mais il est pourvu de macrochètes drus et forts sur les marges internes des lames latérales; sa base est étroite et courbée dans la région médiane; les lames latérales assez longues, larges et arrondies aux bouts. Les cerques (B) ont une forme plus ou moins habituelle; ils se rétrécissent qraduellement vers l'apex, où forment un petit creux subapical et un sommet court; les paralobes sont plus ou moins triangulaires et larges. Distiphallus (C) relativement petit. Le paraphallus est grand, avec les lobes paraphalliques petits et étroits. La partie apicale du paraphallus bien séparée de celle basale, étant pourvue d'un sommet médian court, mais courbé et d'une paire d'apophyses latérales longues, légèrement ondulées et longuement bifides aux bouts. Membrana longue, sclérifiée et pigmentée. Les lobes membranaux internes sont faiblement sclérifiés, obliquement coupés à l'apex et denticulés sur la marge antérieure. Les lobes membranaux externes sont petits, transparents, ovalaires et pourvus d'un sommet submédian plus long sur la marge inférieure et d'un autre plus court et aplati près de sa base. Les styles sont tubulaires, courbés en bas, assez étroits et avec 3-4 dents récurrentes apicales. Les prégonites (D) sont un peu plus longs que les postgonites (E); les premiers plus étroits, droits et avec l'apex courbé; les seconds ont la base large, l'apex courbé et deux macrochètes longs et distancés sur la marge supérieure.

Longueur du corps: 12 mm.

FEMELLE. Inconnue.

Localité-type: Honolulu.

Remarque. Le holotype porte les étiquettes suivantes: a) Type; b) Kinb.; c) Honolulu. Son état est assez mauvaise, car il a un trou dans le mesonotum et deux grandes fosses dans l'abdomen, formées par les anthrènes; les tarses postérieurs et le tibia postérieur droit sont partiellement devorés par les anthrènes; le thorax est collé avec une solution blanche de l'aiguille entomologique.

Son armature génitale a été montée par nous sur trois lames microscopiques, au baume du Canada et déposées, avec l'exemplaire type, dans les collections du Naturhistoriska Riksmuseet, Stockholm.

2. *Liosarcophaga babiyari* sp. n.

Sarcophaga dux sensu Zumpt, 1951, Journ. ent. Soc. S. Africa, 14(2) :177 - syn. n.
Parasarcophaga (Liosarcophaga) exuberans sensu Rohdendorf, 1963 (nec. Pandellé, 1896; nec. Rohdendorf, 1937), Stuttgart. Beitr. z. Naturk, 124: 9 ; sensu Verves, 1986 (partim), Catalogue of palaearctic Diptera, Budapest, 12:167 - syn. n.
Sarcophaga exuberans sensu Zumpt, 1964, Verh. naturf. Ges. Basel, 75:70, fig. 12; sensu Zumpt, 1972, Expl. Parc natn. Virunga, Mission G. F. de Witte, 101:159 - syn. n.

Thyrsocnema exuberans sensu Dear, 1980 (partim), Catalogue of afrotropical Diptera, 815 - syn. n.
Parasarcophaga (Liosarcophaga) dux sensu Povolný, 1987 (partim), fig. 3 (avec les sommets des apophyses latérales de la partie apicale du paraphallus et probablement l'apex des lobes membranaux internes rompus) et fig. 4, Acta ent. Mus. Nat. Pragae, 42:159 - syn. n.

MÂLE

Tête. Noire et couverte d'un tomentum argenté à nuances brunâtres sur les parafrontalies, profrons et la moitié supérieure des parafacialies. Front large; vu du dessus et au lieu le plus étroit, mesure 1/2 de la largeur d'un œil. La bande frontale noire et deux fois plus large qu'une parafrontalie. Les antennes noires brunâtre; le troisième article couvert d'un tomentum argenté et 2,5 fois plus long que le deuxième. Arista d'un brun noirâtre et longuement poilue sur les deux parties. La trompe et les palpes noirs brunâtre. Le péristome mesure 1/3 du grand diamètre oculaire.

Chétotaxie de la tête. Les macrochètes verticaux internes sont longs, forts et rétroclines; les macrochètes verticaux externes manquent ou indistincts par rapport aux macrochètes postoculaires voisins; les ocellaires proclines sont développés; les préverticaux rétroclines forts; les macrochètes frontaux au nombre de 7-10 paires; à la marge antéro-inférieure de l'œil on voit quelques macrochètes parafaciaux courts et fins; les grandes vibrisses fortes; les petites vibrisses montent un peu sur les bordures faciales; 1-2 postocellaires et 1-2 postverticaux sur chaque partie de l'occiput; les microchètes occipitaux sont disposés sur 2-3 rangs irréguliers. Le péristome a de poils noirs; la partie postérieure de la tête couverte de poils jaunâtres assez longs.

Thorax. Noir et couvert d'un tomentum argenté, laissant visibles les 5 bandes dorso-longitudinales noires. Les propleures glabres; le prosternum poilu. Les stigmates antérieurs et postérieurs sont d'un jaune brunâtre jusqu'au brun clair. Les pattes noires; les tibias et les tarses bruns, les derniers plus noirâtres. Les fémurs médians ont un ctenidium typique.

Chétotaxie du thorax. ac = 0 + 1 (on distingue aussi 2 ac présuturaux très petits et fins), dc = 4 + 4 (les présuturaux et les premiers deux postsuturaux courts), ia = 1 + 2-3, sa = 3, h = 3, ph = 2, n = 4, pa = 2, sc = 3 + 1 (ap convergents et un peu courbés en haut), pp = 1 (plus quelques poils), pst = 1 (plus quelques poils), st = 1:1:1.

Ailes. Transparentes à teinte brune et nervures brunes. Épaulette noire; basicosta et costagium jaunes; les sclérites basaux sont jaunes brunâtres. La nervure r_1 glabre; la nervure r_{4+5}, ciliée jusqu'à la moitié de la distance entre son origine et la nervure r-m. Cubitulus courbé en angle droit et prolongé d'un pli. L'épine costale petite ou manque. Écailles jaunes; les balanciers d'un jaune brunâtre.

Chétotaxie des tibias. Les tibias antérieurs ont 2-3 ad proximaux petits et 1 pv; les tibias médians sont pourvus de 1 ad, 1 av, 1 pd et 1 pv; les tibias postérieurs ont 2 ad, 1 av, 1 pd et une pilosité plus ou moins longs et rare sur les parties antéro- et postéro-ventrales.

Abdomen. Noir et couvert d'un tomentum argenté relativement dense, qui atténue un peu les dessins en damiers et présente la tendance de former de taches longitudinales noires sur les tergites I à V. La formule chétotaxique: 0 + 0 + 2 + série. Le tergite génital n'est pas d'un noir clair, mais d'un brun-noirâtre et parfois orange; il est pourvu d'un tomentum cendré et n'a pas de macrochètes marginaux. Le segment anal et les paralobes sont oranges.

Armature génitale: fig. 2. Le sternite V (A) a une forme assez habituelle pour les espèces du genre *Liosarcophaga* Enderlein, étant dépourvu de brosses; ses base et lames latérales sont étroites. Les cerques (B) ont la marge dorsale plus ou moins ondulée, avec le tiers apical plus étroit et terminé avec un sommet court et légèrement courbé; les paralobes sont larges et d'une forme triangulaires. Distiphallus (C) assez court. La partie basale du paraphallus est courte, triangulaire et avec les lobes paraphalliques très réduits. La partie apicale du paraphallus est très courte, ayant un crochet médian mince, aigu et courbé, et une paire d'apophyses latérales longues, légèrement ondulées et inégalement bifides aux bouts. Membrana longue, pliée et plus ou moins sclérifiée. Les lobes membranaux internes sont longs, fortement sclérifiés et pigmentés, et terminés avec un sommet aigu très long et une dent superterminale courte. Les lobes membranaux externes sont bien développés, transparentes, allongés, arrondis à la partie antérieure et pourvus d'une dent antéro-submarginale. Les styles sont courts et pourvus de quelques dents distales marginales. Les prégonites (D) sont plus longs que les postgonites (E); les premiers ont un aspect lamellaire médio-longitudinal au bout distal, où il y a une cavité profonde et un apex long et courbé; les seconds ont la base un peu élargie, une forme légèrement courbée et deux macrochètes courts sur la marge supérieure.

Longueur du corps: 9-13 mm.

FEMELLE. Inconnue

Matériel-type. HOLOTYPE ♂ - Afrique du Sud, Cape Province: Hogsback, 13-l6.XII.1985 (leg. J. & B. Londt).

PARATYPES: Afrique du Sud, Cape Province: 2 ♂♂, Strandfontein, 10-12.X.1977 (leg. R. M. Miller); 2 ♂♂, Strandfontein, 5.IX.1989 (leg. J. Londt, B. Stuckenberg & P. Croeser); 1 ♂, 15 km SE Kirkwood, 4.XI.1978 (leg. R. M. Miller & J. Londt); 1 ♂, 7 km N de Steytlerville, 30.X.1978 (leg. J. Londt & R. M. Miller); 1 ♂, 10 km N de Hogsback, 14.XII .1985 (leg. J. & B. Londt); 1 ♂, Clifton Farm, 22 km NW de Grahamstown, 3-5.I.1986 (leg. J. & B. Londt & D. Gess); 1 ♂, Richtersveld, 30 km SE de Alexander Bay, 50 m alt., 28°48'30"S: 16°38'00"E, 31.VIII.l989 (leg. J. Londt & B. Stuckenberg).

Autres exemplaires: Afrique du Sud, Natal: 1 ♂, 20 km W de Tugela Ferry, 26-27.II.1977 (leg. R. M. Miller); 1 ♂, 3 km SE de van Reenen, Nindy Corner, 1600 m alt., 25. IV.1984 (leg. R. M. Miller & P. Stabbins); 1 ♂, Makaheli For., ca 5 km NE de Mangusi, 30.XI-2. XII.1982 (leg. Barraclough, Londt & Stuckenberg); 1 ♂, Hartebeespoart dom., 9.IV.1970 (leg. S. de Kock). Malawi: 1 ♂, SE de Viphia Chikangawa, 27.II-l.III.1987 (leg. J. & A. Londt).

Tous les types et les exemplaires mentionnés, ainsi que les préparations de l'armature génitale du holotype sont déposés dans les collections du Natal Museum (Pietermaritzburg).

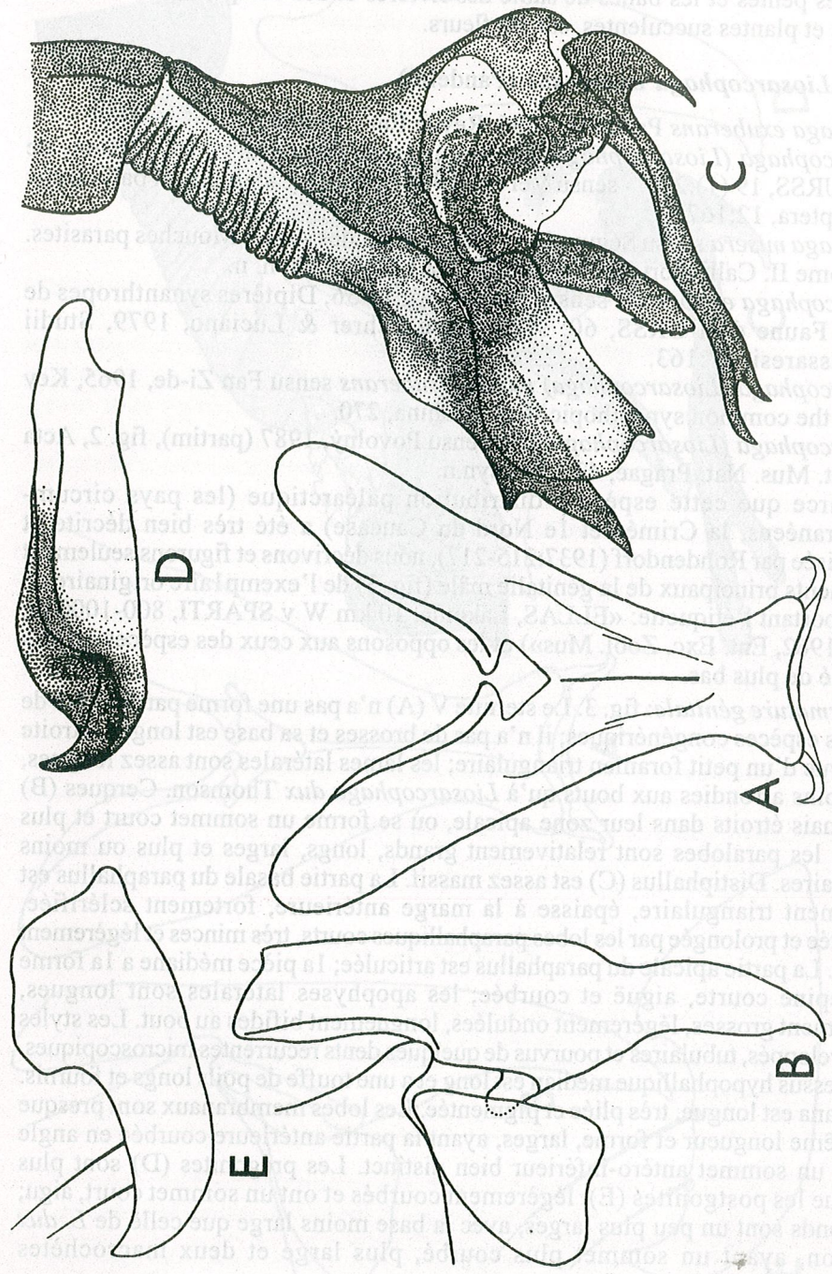

Fig. 2. – Armature génitale de *Liosarcophaga babiyari* sp. n. A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.

Biotopes. Fréquente dans les forêts et aux lisières des forêts, sur les terrains arides, les pentes et les bancs de sable des rivières et des camps, dans les vaux à buissons et plantes succulentes, sur les fleurs.

3. *Liosarcophaga exuberans* (Pandellé)

Sarcophaga exuberans Pandellé, 1896, Revue Ent., 15:186.
Parasarcophaga (Liosarcophaga) exuberans sensu Rohdendorf, 1937, Faune de l'URSS, 19 (1):215. - sensu Verves, 1986 (partim), Catalogue of palaearctic Diptera, 12:167.
Sarcophaga misera sensu Séguy, 1941 (partim), Études sur les Mouches parasites. Tome II. Calliphoridae. Encycl. Ent. (A) 21:120 - syn. n.
Parasarcophaga exuberans sensu Stackelberg, 1956, Diptères synanthropes de la Faune de l'URSS, 60: 90. - sensu Lehrer & Luciano, 1979, Studii Sassaresi, 27:163.
Parasarcophaga (Liosarcophaga) misera exuberans sensu Fan Zi-de, 1965, Key to the common synanthopic flies in China, 270.
Parasarcophaga (Liosarcophaga) dux sensu Povolný, 1987 (partim), fig. 2, Acta ent. Mus. Nat. Pragae, 42:159 - syn.n.

Parce que cette espèce à distribution paléarctique (les pays circum-méditerranéens, la Crimée et le Nord du Caucase) a été très bien décrite et caractérisée par Rohdendorf (1937:215-217), nous décrivons et figurons seulement les éléments principaux de la génitalie mâle (fig. 3) de l'exemplaire originaire de Grèce (portant l'étiquette: «ELLAS, Lakonia, 10 km W v SPARTI, 800-1000 m, 28. IX. 1962, Ent. Exc. Zoöl. Mus») et les opposons aux ceux des espèces affines par la clé de plus bas.

Armature génitale: fig. 3. Le sternite V (A) n'a pas une forme particulière de celle des espèces congénériques; il n'a pas de brosses et sa base est longue, étroite et pourvue d'un petit foramen triangulaire; les lames latérales sont assez longues, mais moins arrondies aux bouts qu'à *Liosarcophaga dux* Thomson. Cerques (B) longs, mais étroits dans leur zone apicale, où se forme un sommet court et plus atténué; les paralobes sont relativement grands, longs, larges et plus ou moins triangulaires. Distiphallus (C) est assez massif. La partie basale du paraphallus est longuement triangulaire, épaisse à la marge antérieure, fortement sclérifiée, pigmentée et prolongée par les lobes paraphalliques courts, très minces et légèrement courbés. La partie apicale du paraphallus est articulée; la pièce médiane a la forme d'une épine courte, aiguë et courbée; les apophyses latérales sont longues, relativement grosses, légèrement ondulées, longuement bifides au bout. Les styles sont développés, tubulaires et pourvus de quelques dents récurrentes microscopiques. Le processus hypophallique médian est long et a une touffe de poils longs et fournis. Membrana est longue, très pliée et pigmentée. Les lobes membranaux sont presque de la même longueur et forme, larges, ayant la partie antérieure courbée en angle droit et un sommet antéro-inférieur bien distinct. Les prégonites (D) sont plus longs que les postgonites (E), légèrement courbés et ont un sommet court, aigu; les seconds sont un peu plus larges, avec la base moins large que celle de *L. dux* Thomson, ayant un sommet plus courbé, plus large et deux macrochètes superterminaux forts et longs.

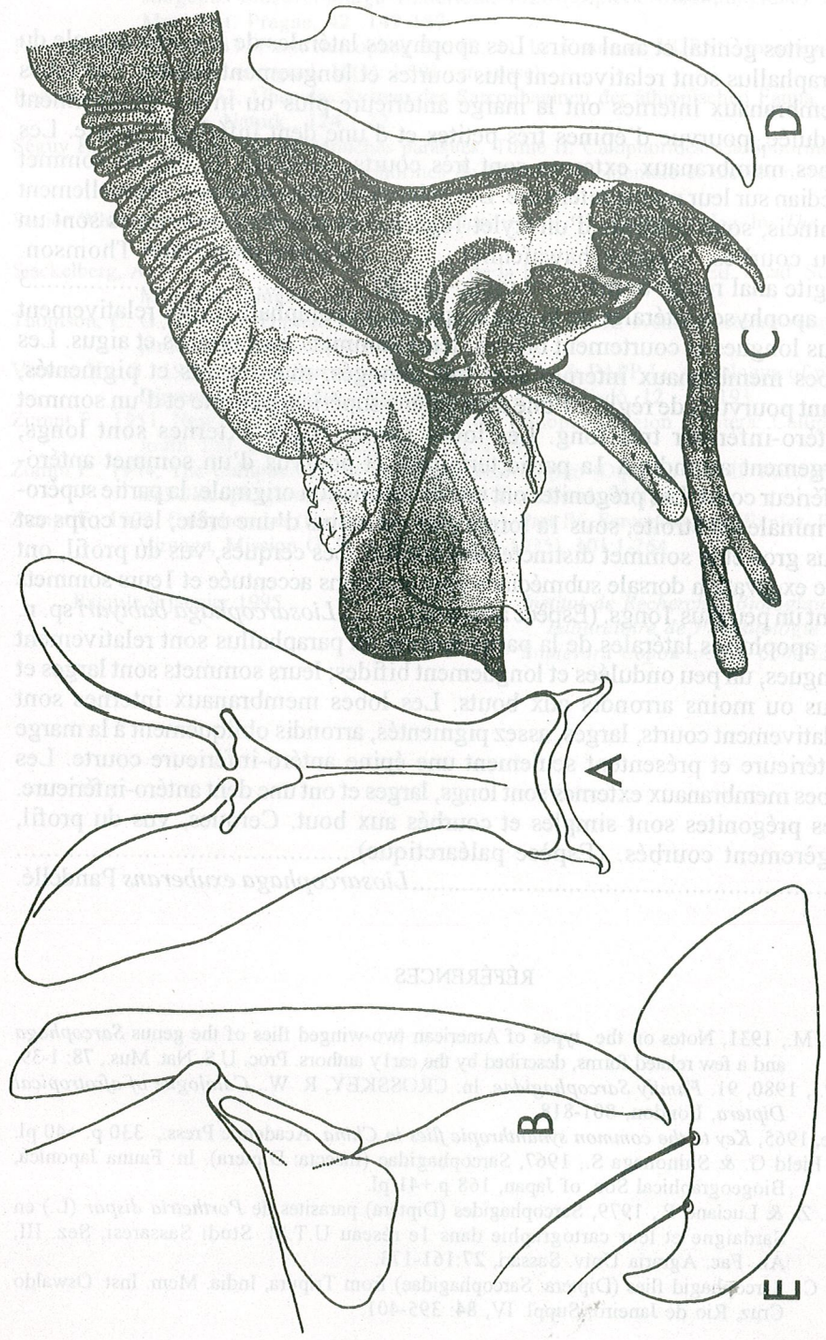

Fig. 3. – Armature génitale de *Liosarcophaga exuberans* (Pandellé). A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.

CLÉ POUR L'IDENTIFICATION DES ESPÈCES

1(2) Tergites génital et anal noirs. Les apophyses latérales de la partie apicale du paraphallus sont relativement plus courtes et longuement bifides. Les lobes membranaux internes ont la marge antérieure plus ou moins obliquement ondulée, pourvue d'épines très petites et d'une dent inférieure courte. Les lobes membranaux externes sont très courts, ovoïdaux et ont un sommet médian sur leur marge inférieure. Les prégonites sont simples et graduellement amincis, sous la forme d'un stylet légèrement courbé. Les cerques sont un peu courbés. (Espèce hawaïenne).....*Liosarcophaga dux* Thomson.

2(1) Tergite anal rouge-brunâtre ou rouge-orange.....3

3(4) Les apophyses latérales de la partie apicale du paraphallus sont relativement plus longues et courtement bifides; leurs sommets sont minces et aigus. Les lobes membranaux internes sont très allongés, mais étroits et pigmentés, étant pourvus - de règle - d'une dent antéro-supérieure courte et d'un sommet antéro-inférieur très long. Les lobes membranaux externes sont longs, largement arrondis à la partie terminale et pourvus d'un sommet antéro-inférieur court. Les prégonites ont une conformation originale: la partie supéro-terminale est étroite, sous la forme plus ou moins d'une crête; leur corps est plus gros et le sommet distinctement courbé. Les cerques, vus du profil, ont une excavation dorsale submédiane plus ou moins accentuée et leurs sommets sont un peu plus longs. (Espèce africaine).....*Liosarcophaga babiyari* sp. n.

4(3) Les apophyses latérales de la partie apicale du paraphallus sont relativement longues, un peu ondulées et longuement bifides; leurs sommets sont larges et plus ou moins arrondis aux bouts. Les lobes membranaux internes sont relativement courts, larges, assez pigmentés, arrondis obliquement à la marge antérieure et présentent seulement une épine antéro-inférieure courte. Les lobes membranaux externes sont longs, larges et ont une dent antéro-inférieure. Les prégonites sont simples et courbés aux bout. Cerques, vus du profil, légèrement courbés. (Espèce paléarctique).....*Liosarcophaga exuberans* Pandellé.

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INFLUENCE OF SOME LOW-MOLECULAR WEIGHT IMMUNOMODULATING AGENTS UPON THE SERIC COMPLEMENT

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The immunomodulating action of some new agents upon the seric complement (SC) and circulating immune complexes (CIC) has been studied on Chinchilla rabbits, for six weeks. The effect of the acetylated polyolic derivatives Pagosten-1 (Pag-1) (7.5 mg/kg body/day), Pag-2 (2.5 mg/kg body/day) and Pag-3 (2.5 mg/kg body/day) upon SC depend on the agent nature, dose and duration of treatment. In the case of Pag-1, immunostimulating effects upon SC have been observed along the whole duration of treatment, similarly with the Rodilemid agent, taken as a reference, and with Pag-3, after two weeks, while, in the case of Pag-2 – immunodepressing effects have been recorded. The HMBA agent (0.06 mg/kg body/day) has stimulating effects upon SC, while the A.52.18 antibiotic (0.15 mg/kg body/day) – immunodepressing ones, similarly with the immunodepressing agent Antifolan, taken as reference. The effects of agents upon CIC are much weaker, almost negligible, comparatively with those observed on SC.

The immune response may be induced both by various microorganisms or high-molecular weight organic products, external to the organism (antigenes) (3), (8), (12) and by some low-molecular weight substances (6), (8), (13), (14). Such immunomodulating substances may induce either immunostimulating or immunodepressing effects, thus influencing the synthesis of antibodies and their release in blood circulation (the humorally-mediated immune response) and/or the dynamics of immunocompetent cells (the cellularly-mediated immune response).

In the humorally-mediated immune response, there are involved, besides specific factors (antibodies), others – such as the seric complement (SC) system and the circulating immune complexes (CIC) (8). SC represents one non-specific factor of the humorally-mediated immune response, representing, nevertheless, a compulsory link, as completing the action of specific antibodies upon antigenes, and intervening, as well, in processes of phagocytosis (2), (8), (11). As a result of the reaction between an antigene and an antibody, antigene-antibody circulating immune complexes do appear in the organism. Such complexes have the capacity of fixing and activating the SC, and are also involved in the neutrophils' phagocitary activity, thus influencing the humorally and cellularly-mediated immune response of the organism (8), (15).

In a series of previous studies (1), (9) we analyzed the immunomodulating effects of some low-molecular weight agents, their action upon the cellularly-mediated immune response being evidenced. The observation was made that a series of new products, i.e. the acetylated polyolic derivatives (Pagosten-1, Pagosten-2, Pagosten-3),

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influence the dynamics of total leukocytes as well as the leukocyte types, as a function of the agent's nature and dose, type of leukocytes and duration of treatment, most of the effects observed being immunostimulating ones, although some immunodepressing effects are also noticed.

The present paper studies the effects of Pagosten-1 (pentaacetylglucose), Pagosten-2 (hexaacetylmanitol), Pagosten-3 (hexaacetylsorbitol), studied previously, as well as of other two new products with possible immunomodulating action – i.e., the A.52.18 antibiotic and the HMBA (hexamethylen-bis-acetamide) product of synthesis, respectively, upon the humorally-mediated immune response, in parallel with following their influence on the SC and on the CIC, comparatively with the effects induced by reference agents such as Rodilemid (an immunostimulator) and Antifolan (an immunodepressor) (4), (5).

MATERIALS AND METHODS

Chinchilla rabbits, organized in 8 groups containing 5 animals each, have been subjected to different treatments, as follows: group I-non-treated, taken as a reference; group II- treated with Pagosten-1 (Pag-1) 7.5 mg/kg body/day; group III- treated with Pag-2 2.5 mg/kg body/day; group IV- with Pag-3 2.5 mg/kg body/day; group V- with HMBA 0.06 mg/kg body/day; group VI- with A.52.18 0.15 mg/kg body/day; group VII- with Rodilemid 10 mg/kg body/day;and group VIII - with Antifolan 0.083 mg/kg body/day. The doses have been calculated starting from the human therapeutic doses; duration of treatments was of 6 weeks, the products being administered as intramuscular injections, every two days, in a 0.4 mL volume of physiological salt. The reference group has been injected only with physiological salt.

Analyses have been performed prior to initiating the treatments (T_0), for the establishment of the initial, reference values, then at two-week intervals (T_2, T_4, T_6). The total seric complement ($C'H_{50}$) has been determined with the method of L. Hartman and H. Brécy (7), (15), as improved by R. Audran (2), while the immune antigene-antibody circulating complexes – by the modified Haskova method (15).

RESULTS

The SC has recorded – with the animal groups taken into study – normal initial average values (at T_0), ranging between 16.99 and 50.58 U $C'H_{50}$/mL, the general average value being of 46.54 U/mL (Pag-1 = 21.31 U/mL, Pag-2 = 43.10 U/mL, Pag-3 = 46.22 U/mL, A.52.18 = 29.24 U/mL, HMBA = 16.99 U/mL, Rodilemid = 25.24 U/mL, Antifolan = 50.58 U/mL).

No significant variations of SC have been observed with the reference batch, on the whole duration of treatment.

The group treated with Pag-1 evidenced a continuous increase of the SC values, comparatively with those at T_0 (100 %), representing 103.14% at T_2, 105.83% at T_4 and 127.31% at T_6. However, the treatment with Pag-2 induced a

slight decrease of the SC values, up to 99.26% at T_2, 77.49% at T_4 and 94.62% at T_6, comparatively with the initial values (fig. 1). The treatment with Pag-3 has initially led to a SC increase up to 112.88% at T_2, followed by a decrease in the other phases of the experiment, up to 75.11% at T_4 and 83.34 % at T_6 (fig. 1).

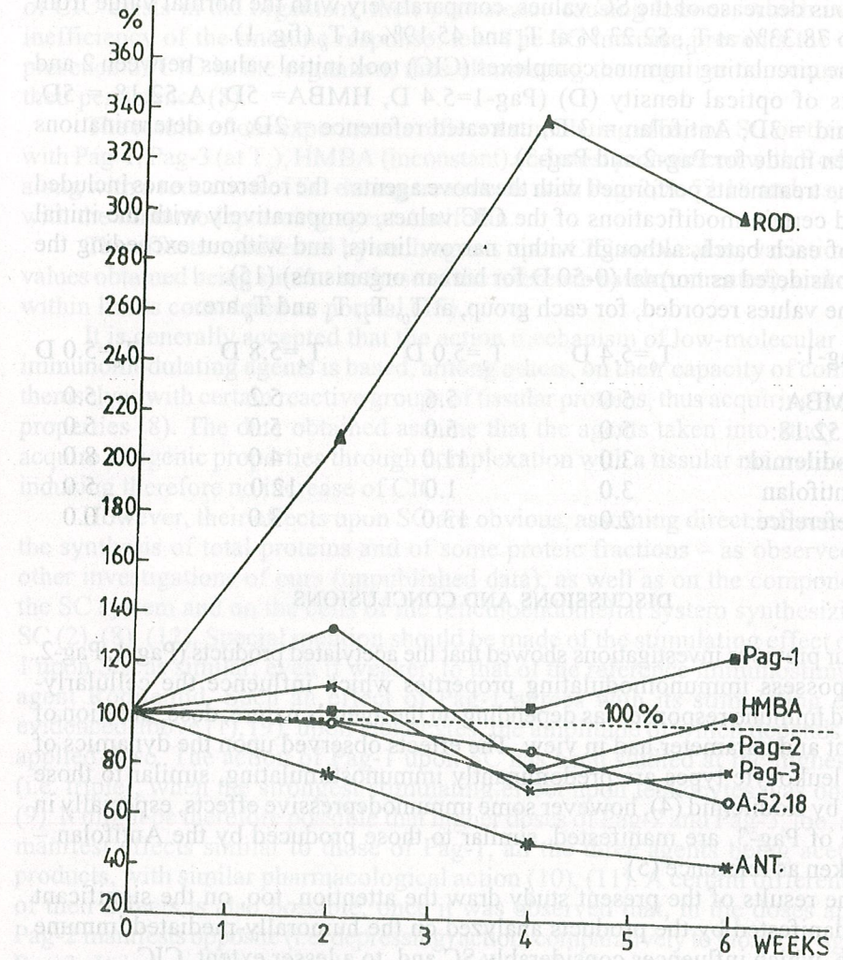

Fig. 1. – Influence of Pag-1, Pag-2, Pag-3, HMBA, A.52.18, Rodilemid (ROD) and Antifolan (ANT) on the dynamics of the seric complement.

With the HMBA- treated batch, a fluctuating increase of the SC values against T_0 has been observed, up to 135.59 % at T_2, followed by a decrease up to 81.27 % at T_4, and a final increase, up to 104.33% at T_6 (fig. 1). The A.52.18 antibiotics induced a slow, yet continuous decrease of the SC values, comparatively with the initial value (at T_0), up to 99.40 % at T_2, 88.85 % at T_4 and 70.13 % at T_6.

The immunostimulating agent taken as reference – Rodilemid – determined a significant increase of the SC values on the whole duration of the treatment, up to 231.54% at T_2, 340.33% at T_4 and 301.19% at T_6 (fig. 1). The treatment with A.52.18, an immunodepressing reference agent, induced a considerable and continuous decrease of the SC values, comparatively with the normal value from T_0, up to 78.33% at T_2, 52.23 % at T_4 and 45.19% at T_6 (fig. 1).

The circulating immune complexes (CIC) took initial values between 2 and 5.4 units of optical density (D) (Pag-1=5.4 D, HMBA= 5D, A.52.18 = 5D, Rodilemid = 3D, Antifolan = 3 D, untreated reference = 2D; no determinations have been made for Pag-2 and Pag-3).

The treatments performed with the above agents – the reference ones included – caused certain modifications of the CIC values, comparatively with the initial values of each batch, although within narrow limits, and without exceeding the limits considered as normal (0-50 D for human organisms) (15).

The values recorded, for each group, at T_0, T_2, T_4 and T_6 are:

Pag-1:	T_0=5.4 D	T_2=5.0 D	T_4=5.8 D	T_6=5.0 D
HMBA:	5.0	5.6	5.2	5.0
A.52.18:	5.0	5.0	5.0	5.0
Rodilemid:	3.0	11.0	4.0	8.0
Antifolan	3.0	1.0	12.0	5.0
Reference:	2.0	13.0	3.0	0.0

DISCUSSIONS AND CONCLUSIONS

Our previous investigations showed that the acetylated products (Pag-1, Pag-2, Pag-3) possess immunomodulating properties which influence the cellularly-mediated immune response – as depending on the agent's nature, dose, duration of treatment and parameter had in view. The effects observed upon the dynamics of various leukocyte types are predominantly immunostimulating, similar to those induced by Rodilemid (4), however some immunodepressive effects, especially in the case of Pag-3, are manifested, similar to those produced by the Antifolan – agent taken as reference (5).

The results of the present study draw the attention, too, on the significant action manifested by the products analyzed on the humorally-mediated immune response, which influences considerably SC and, to a lesser extent, CIC.

The SC system represents a non-specific, yet compulsory factor of the humorally-mediated immune response, completing the action of specific antibodies upon antigenes, and influencing phagocytosis, as well (8). Increase of the SC level indicates stimulation of the reticuloendothelial system's cells that synthesize SC (2), (8), (12). Nevertheless, decrease of SC does not affect the formation of antibodies, no dependency existing between the SC synthesis and antibodies (2), (8).

Antigenes induce the formation of immune circulating antigene-antibody complexes (deposited either in the walls of the small vessels or in various organs).

The immune complexes initiate the immune response and have the capacity of fixing and activating the complement, also influencing – indirectly – the neutrophils' phagocytary capacity. Therefore, CIC intervene both in the humorally- and in the cellularly-mediated immune response, as SC does (8). Normally, a limited amount of CIC exists in the organism, their persistence causing lesions, which indicates inefficiency of the immune response, too. The SC increase prevents the chronic presence of CIC in the organism, thus eliminating the negative consequences of their persistence (8).

The results of our experiments reflect a stimulating effect of SC on treatments with Pag-1, Pag-3 (at T_2), HMBA (inconstant) and most pronounced with Rodilemid, along with a reduction of SC during treatments with Pag-2, A.52.18 and, especially with the immunodepressing agent Antifolan.

The effects manifested by such agents upon CIC are weaker, variation of the values obtained being similar to those of the reference batch (untreated), and ranging within limits considered as normal (15).

It is generally accepted that the action mechanism of low-molecular weight immunomodulating agents is based, among others, on their capacity of combining themselves with certain reactive groups of tissular proteins, thus acquiring antigenic properties (8). The data obtained assume that the agents taken into study do not acquire antigenic properties through complexation with a tissular macromolecule, inducing therefore no increase of CIC.

However, their effects upon SC are obvious, assuming direct influences on the synthesis of total proteins and of some proteic fractions – as observed from other investigations of ours (unpublished data), as well as on the components of the SC system and on the cells of the reticuloendothelial system synthesizing the SC (2), (8), (12). Special mention should be made of the stimulating effect of Pag-1 upon SC, as similar, even if weaker, to that of the reference immunostimulating agent Rodilemid. Such an effect of Pag-1 agrees with its stimulating effects, evidenced above (1), (9), upon leukocytes, the amplitude of which depends on the applied dose. The action of Pag-1 upon SC has been studied at the highest dose (i.e. triple), when the strongest stimulating effect upon leukocytes was observed (9). It might be therefore possible that higher doses of Pag-2 and Pag-3, too, should manifest effects similar to those of Pag-1, all the three agents being acetylated products, with similar pharmacological action (10), (11). A certain differentiation of their effects is also possible, once it was observed that, in the doses applied, Pag-2 manifests opposite (i.e. depressing) action, comparatively to those of Pag-1 and Pag-3. It is beyond any doubt that the effects depend on the agents' chemical nature, -in our case, on the nature of the structure carrying the acetyl radicals (glucose – in the case of Pag-1, manitol – for Pag-2 and sorbitol – for Pag-3).

The direction and magnitude of effects depend, too, on the duration of treatment, as observed with Pag-3 which, in the first two weeks, has a stimulating effect upon SC, followed by a depressing action.

For the obtainment of a certain immunomodulating effect, it is therefore important to consider the agents' chemical nature, the doses employed and the treatment duration. Therefore, besides the modifications observed with Pag-3,

as depending on the duration of treatment, HMBA was also noticed as modifying its action with the dose, as follows: at low doses, it shows immunodepressing effects (unpublished data), at higher doses the effects being reversed-becoming immunostimulating, as seen from the above experiments. Modification of the effect, as induced by the dose, was also observed with the action of the A.52.18 agent upon the cellularly-mediated immune response (unpublished data). Similar modifications observed in the effects of agent, as depending on the applied dose, have been discussed by other authors (8).

The above observations demonstrated the immunomodulating action of the products considered for the analysis, supporting the idea of a possible influence upon the immune response by means of such agents.

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ANTITUMORAL EFFECTIVENESS DEPENDENCE OF THE PA_2 III AND PA_3 POLYPHENOLIC PREPARATIONS OF THEIR THERAPEUTICAL DOSES

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The antitumoral treatment of the rats, bearing either of Guérin T-8 lymphotropic epithelioma or of Walker 256 carcinosarcoma, with various doses of the PA_2 III and PA_3 vegetable polyphenolic preparations has induced a cancerostatic effect differentiated as intensity. Antineoplastic effectiveness dependence of these natural products – separated and purified from *Asclepias syriaca* plant leaves – of the used therapeutical dose argues the existence of a dose - response relationship. This positive answer, at the first question of the preclinical quantitative pharmacological evaluation of the PA_2 III and PA_3 antitumoral potential, imposes the appreciation of their therapeutic efficiency in relation to that one of some standard cancerostatics, in the conditions of laboratory experiments.

In previous investigations performed on adequate experimental models, we pointed out the *in vitro* cytotoxic action – on Hela cancerous cell cultures – and the *in vivo* antitumoral effect – on some experimental tumoral systems – of the PA_2 III and PA_3 vegetable polyphenolic preparations, specifically separated and purified from *Asclepias syriaca* plant leaves [9], [10], [11].

Qualitative evaluation of the PA_2 III and PA_3 specific pharmacological effect – assured by revealing both its cancerostatic pharmacodynamic action and its reproducible character [10], [11] – has imposed the quantitative pharmacological evaluation of experimental antineoplastic potential of these natural polyphenolic products.

In the present paper there are included and discussed the experimental results obtained in the *in vivo* testing of the therapeutic effect of different doses of PA_2 III and PA_3 on Guérin T - 8 lymphotropic epithelioma and Walker 256 carcinosarcoma development. At the same time an appreciation of their antitumoral effectiveness is done on the basis of the dose-response relationship established in these experimental conditions.

MATERIAL AND METHODS

White Wistar female rats of 125–150 g, bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma, were used as experimental animals, both tumor lines being of solid type.

The cancerostatic treatment, started 24 hours after the tumoral transplant, was applied daily by the intraperitoneal (i.p.) injection of the PA_2 III and PA_3 polyphenolic preparations in different doses (mg/kg.body weight), which are presented in the tables. This therapy has lasted for 16 and 19 days, respectively, in the case of Guérin T-8 tumor and Walker 256 tumor, respectively. An equivalent volume of physiological serum was administered to the control animals.

The estimation of the antitumor effect was based on comparative follow-up of the mean tumor weight (M.T.W) in the treated and control animals after sacrifice.

The evaluation of antineoplastic action was made by the percentage determination of mean tumor regression (% M.T.R.) and by the calculation of the T/C value (where T = M.T.W. for the treated groups and C = M.T.W. for the control group), as well as of the statistic significance using the Student's *t* test [5], [7], [13].

The appreciation of PA_2 III and PA_3 antitumoral effectiveness dependence of the used therapeutical dose was realized by the comparative analysis of our values of the evaluation indices with those imposed by the selection criteria of active cancerostatic agents. These criteria were established by the preclinical screening programs of the Institute for Microbiology and Experimental Therapy from Germany [5] and of the Cancer Chemotherapy National Institute from the U.S.A. [7], for this stage of the pharmacological quantitative evaluation which follows the existence of a dose-response relationship.

RESULTS

The interference of daily antitumoral therapy – performed by the administration of PA_2 III various doses – with the development process of Guérin T-8 lymphotropic epithelioma can be followed from table 1. In comparison with the control group, it is observed that this differentiated treatment has induced:

Table 1

Cancerostatic effect of different doses of PA_2 III polyphenolic preparate (mg/kg.b.w./daily) on Guérin T-8 lymphotropic epithelioma. Figures in brackets indicate the number of experimental animals

Group/Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	15.8 ± 1.6 (15)	-	-	-
1. 0 mg/kg.b.w.	11.3 ± 1.4 (10)	28.5	0.71	$p < 0.05$
2. 5 mg/kg.b.w.	9.8 ± 1.5 (10)	38.0	0.62	$p < 0.02$
5. 0 mg/kg.b.w.	7.2 ± 1.5 (10)	54.4	0.45	$p < 0.001$
7. 5 mg/kg.b.w.	5.6 ± 1.6 (10)	64.5	0.35	$p < 0.001$
10. 0 mg/kg.b.w.	12.9 ± 1.8 (10)	18.3	0.82	N.S.

– a moderate decrease of M.T.W. in the case of the group treated with 1.0 mg/kg.b.w. which allows the estimation of a M.T.R. of 28.5% and of a T/C value of 0.71;

– a significant antitumoral activity ($p<0.02$), in the animals submitted to treatment with 2.5mg/kg.b.w., illustrated by the M.T.R. (38%) and T/C (0.62) values;

– an important cancerostatic effect in the case of the group treated with 5.0 mg/kg.b.w., which is argued by the M.T.R. (54.4%), T/C (0.45) and statistical significance ($p<0.001$) values;

– a maximum inhibitory effect on tumoral development in the conditions of the therapy with 7.5 mg/kg.b.w., this being pointed out by M.T.R. of 64.5%, T/C ratio of 0.35 and statistical significance of 0.001;

– a nonsignificant antitumoral action in the case of the animals treated with 10 mg/kg.b.w., the values of the evaluation indices being 18.3% (M.T.R.) and 0.82 (T/C ratio).

Similar results were registered on rats bearing Walker 256 carcinosarcoma submitted to the daily therapy with different doses of PA_2 III (table 2). The progressive increase of the treatment dose was correlated with a confined potentiation of the antitumoral efficiency comparatively with the untreated control group.

Table 2

Antitumor activity of various doses (mg/kg.b.w./daily) of PA_2 III product on Walker 256 carcinosarcoma. Figures in brackets indicate the number of experimental animals

Group/Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	12.3 ± 1.5 (15)	-	-	-
1. 0 mg/kg.b.w.	10.6 ± 1.2 (10)	13.1	0.87	N.S.
2. 5 mg/kg.b.w.	8.5 ± 1.1 (10)	30.3	0.65	$p < 0.05$
5. 0 mg/kg.b.w.	6.1 ± 1.0 (10)	50.0	0.50	$p < 0.01$
7. 5 mg/kg.b.w.	4.9 ± 1.4 (10)	59.8	0.40	$p < 0.002$
10. 0 mg/kg.b.w.	10.2 ± 1.3 (10)	16.4	0.84	N.S.

Thus, the nonsignificant cancerostatic effect was observed at minimum dose of 1.0 mg/kg.b.w. and at maximum dose of 10 mg/kg.b.w. (M.T.R. of 13.1% and 16.4%, respectively; T/C value of 0.87 and 0.84, respectively). When the dose was augmented at 2.5 mg/kg.b.w. the decrease of M.T.W. has conditioned the estimation of a M.T.R. of 30.3% and of a T/C ratio of 0.65. These values of the evaluation indices have argued a moderate inhibitory action of tumoral development. Therapeutic dose increase at 5 and 7.5 mg/kg.b.w., respectively, was correlated with a significant enhancement of the antineoplastic potential, the M.T.R. values being of 50.0% and 59.8%, respectively, and T/C ratios being of 0.50 and 0.40, respectively.

Dependence of the antitumoral effectiveness of PA_3 therapeutical doses can be also ascertained, in comparison with the control rats, from the results included in tables 3 and 4.

Table 3

Cancerostatic effect of different doses of PA_3 polyphenolic preparate (mg/kg.b.w./daily) on Guérin T-8 lymphotropic epithelioma. Figures in brackets indicate the number of experimental animals

Group/Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	12.4 ± 1.8 (15)	-	-	-
30. 0 mg/kg.b.w	7.1 ± 1.2 (10)	42.7	0.57	$p< 0.05$
45. 0 mg/kg.b.w	5.8 ± 1.4 (10)	53.2	0.47	$p< 0.01$
60. 0 mg/kg.b.w.	4.5 ± 1.2 (10)	63.7	0.36	$p< 0.002$

Table 4

Antitumor activity of various doses (mg/kg.b.w./daily) of PA_3 product on Walker 256 carcinosarcoma. Figures in brackets indicate the number of experimental animals

Group/Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	15.3 ± 1.0 (15)	-	-	-
30. 0 mg/kg.b.w	10.4 ± 1.6 (10)	31.6	0.68	$P < 0.02$
45. 0 mg/kg.b.w	8.3 ± 1.5 (10)	45.4	0.55	$P < 0.001$
60. 0 mg/kg.b.w.	6.6 ± 1.4 (10)	56.6	0.44	$P < 0.002$

The progressive augmentation of PA_3 dose was followed of a gradual optimization of the cancerostatic therapy on Guérin T-8 tumor development. This affirmation is done according to the values of the evaluation indices of the pharmacological effect. Thus, the M.T.R. percentage increases from 42.7% (30 mg/kg.b.w.) at 53.2% (45 mg/kg.b.w.) and 63.7% (60mg/kg.b.w.), respectively. At the same time, the T/C ratios decrease from 0.52 (30 mg/kg.b.w) at 0.47 (45mg/kg.b.w.) and 0.36, respectively (60mg/kg.b.w.).

The antitumoral treatment of the rats bearing Walker 256 carcinosarcoma with various doses of PA_3 polyphenolic preparations was correlated with M.T.R. values of 31.6% (30mg/kg.b.w), 45.4% (45mg/kg.b.w.) and 56.6% (60mg/kg.b.w.), respectively and with T/C ratios of 0.64, 0.55 and 0.44, respectively. These values of the evaluation indices prove a successive amplification of the cancerostatic therapeutic efficiency as an expression of the gradual increase of the treatment doses.

DISCUSSION

The discovery of new agents with preferential cytostatic action on the malignant cells and less on the normal cells of the host represents an actual and very important field of oncobiological research. This is in agreement with the necessity of the augmentation of antineoplastic chemotherapy effectiveness [1], [3], [6], [8], [12], [14].

Preclinical characterization of a substance as active cancerostatic agent is the consequence of the qualitative and quantitative evaluation of its specific pharmacological action on some experimental tumoral systems [2], [4], [5], [7], [15], [16]. For this purpose, the methodology, established by the national and international chemotherapeutic programs of preclinical screening on diverse and adequate experimental models, requires:

– evidences on its antitumoral action and on reproducibility of this pharmacodynamic effect, as objectives of the qualitative pharmacological evaluation;

– the appreciation of the antineoplastic pharmacotherapeutical effectiveness of the new agent by: the demonstration of the existence of a dose-response relationship; the comparative analysis of its specific antitumoral effect with that one of some standard cancerostatics of clinical use; highlighting of significant inhibitory action on tumors with different degrees of development – as criteria of the quantitative pharmacological evaluation.

Our preliminary results [10], [11] – giving a positive answer at the questions of the qualitative evaluation of the PA_2 III and PA_3 antitumoral action – have imposed additional investigations in order to assure the quantitative evaluation of cancerostatic activity of these vegetable polyphenolic preparations.

Among other things, the research has followed the relation between PA_2 III and PA_3 antitumoral efficiency and their therapeutic doses, which were used in the experimental treatment of the Guérin T-8 lymphotropic epithelioma and Walker 256 carcinosarcoma. This experimental aspect, representing the objective of the present study, has revealed different intensities of the cancerostatic effect as an expression of the treatment with various doses, inferior and superior to that which has conditioned the manifestation of the PA_2 III and PA_3 antitumoral action.

Thus, the progressive increase of PA_2 III therapeutic dose from 1.0 at 2.5, 5.0 and 7.5 mg/kg.b.w., respectively, was correlated with a corresponding intensification of the antitumoral action, estimated on the basis of the consecutive M.T.R. percentage values: of 33.8%, 43.1% and 18.5%, respectively, in the case of Guérin T-8 tumor, as well as of 131.1%, 65% and 19.6%, respectively, in the case of Walker 256 tumor. This effect is also illustrated by the dynamics of the registered T/C ratios which pointed out their concomitant decrease. However, the dose-response relationship has a limited character, because daily administration of 10mg/kg.b.w. did not induce a significant antimalignant activity. The explanation of this phenomenon could be given by the simultaneous intensification of PA_2 III toxicity, due to the increase of the treatment dose.

Gradual enlargement of the daily dose of antitumoral therapy with PA_3 polyphenolic preparation from 30 at 45 and 60 mg/kg.b.w., respectively, has conditioned a corresponding potentiation of the cancerostatic effect with 24.6% and 9.7%, respectively, on lymphotropic epithelioma, as well as with 43.6% and 24.7% respectively, on carcinosarcoma. This enhancement, estimated in relation to the M.T.R. percentages, is also suggested by the corresponding T/C values.

Appreciation of the results, obtained in the first stage of the quantitative evaluation of the antitumoral effect – conceived for the establishment of the existence of a dose-response relationship, as criterion for the estimation of the terapeutical effectiveness of the studied agent – requires their analysis according to the stipulations of the reference screening programs, imposed for this preclinical investigation stage. In concordance with the German and American programs, the dose-response relationship is confirmed if:

– the M.T.R. registered a progressive increase in relation to the rise of the therapeutic dose;

– at least one of the T/C ratios, obtained after the dose differentiated treatment, is included between the limits of the admitted range (0.42 - 0.54).

In the light of the above criteria, our evaluation indices values of the antitumoral action of PA_2 III and PA_3 polyphenolic preparations highlights the existence of a relationship between the therapeutical dose and the intensity of the specific pharmacodynamic effect.

The possibility of preclinical optimization of antineoplastic effectiveness of these natural products of vegetal origin by therapeutic dose manipulation allows us to delimit the preclinical range of the therapeutic doses. This is more restricted in the case of PA_2 III preparation (2.5 – 7.5 mg/kg.b.w.) and more extended in the case of PA_3 preparation (30 – 60 mg/kg.b.w), with possibilities to be augmented because it seems less toxic.

The existence of dose-response relationship imposes a thorough–going study of the preclinical quantitative evaluation of the pharmacotherapeutical antitumoral efficiency in a next stage by comparative analysis of PA_2 III and PA_3 antitumoral potential with that one of some standard cancerostatics of clinical use, in the conditions of laboratory experiments.

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PRECLINICAL ANTITUMORAL THERAPEUTIC EFFECTIVENESS OF THE PA_2 III AND PA_3 POLYPHENOLIC PREPARATIONS

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The treatment of the rats bearing either of Guérin T-8 lymphotropic epithelioma or of Walker 256 carcinosarcoma with PA_2 III and PA_3 natural polyphenolic preparations – separated and purified from *Asclepias syriaca* plant leaves – as well as with some standard cytostatic agents of clinical use (girostan, antipholan, levopholan and cyclophosphamid) has conditioned the expression of their different cancerostatic effect. The analysis of our values of the pharmacotherapeutic effect evaluation indices reveals the significant effectiveness of these vegetable polyphenolic preparations as compared to that one of the reference agents. It is higher, equal or near to those of the standard cytostatics. The complete quantitative pharmacological evaluation of the antitumoral action of PA_2 III and PA_3 natural products imposes the supplementary investigation of their cancerostatic efficiency on tumors with different degrees of development.

Although there is a continuous progress in cancer diagnosis and treatment, the antineoplastic chemotherapy is still characterized by a decreased effectiveness. This imposes intensive and extensive investigations for the identification of new cancerostatic pharmacological agents as well as for the discovery of new ways of action on the malignant process [3], [14], [15].

In previous researches there were highlighted the experimental antitumoral therapeutic activity of the PA_2 III and PA_3 vegetable polyphenolic preparations – specifically extracted and purified from *Asclepias syriaca* plant leaves – its reproducibility and the existence of a dose-response relationship [8], [9], [10].

In the present paper are exposed the experimental results obtained in the comparative testing of the *in vivo* anticancerous action of the PA_2 III and PA_3 polyphenolic preparations as well as of some standard agents of clinical use (girostan, antipholan, levopholan and cyclophosphamid) on rats bearing of different experimental systems. The investigation has the purpose to extend the assessment of the preclinical antitumoral therapeutic efficiency of the studied vegetable products by this comparative criterion of the quantitative pharmacological evaluation.

MATERIALS AND METHODS

White Wistar female rats of 125 – 150 g, bearing either of Guérin T-8 lymphotropic epithelioma or of Walker 256 carcinosarcoma – experimental tumoral lines of solid type – were used as experimental animals.

The antitumoral treatment has started 24 hours after the tumoral transplant and has lasted for 16 and 19 days, respectively, in the case of Guérin T-8 and of Walker 256, respectively. It was applied by intraperitoneal (i.p.) daily injection both of PA_2 III and PA_3 vegetable polyphenolic preparations and of standard cancerostatics in different doses (mg/kg.body weight), which are presented in the tables with the experimental results. The doses of the standard agents were established in relation to those used in the human clinical therapy. An equivalent volume of physiological serum was administered to the control animals.

The estimation of the antitumoral activity was based on the comparative follow-up of the mean tumor weight (M.T.W.), at the sacrifice of the treated and control animals.

The evaluation of antineoplastic action was performed by the percentage determination of mean tumor regression (% M.T.R.) and by the calculation of the T/C ratio (where T = M.T.W for the treated group and C = M.T.W. for the control group) and of the statistic significance using the Student's *t* test [5], [6], [11].

The appreciation of the cancerostatic effects was realized by comparative analysis of our values of the evaluation indices with those imposed by the selection criteria of antitumoral substances. These were established by the preclinical screening programs of the Institute of Microbiology and Experimental Therapy from Germany [5] and of the National Institute for Chemotherapy of Cancer from U.S.A. [6] used by us as references.

RESULTS

The evaluation indices values of the antitumoral action induced by PA_2 III and PA_3 polyphenolic preparations, girostan, antipholan, levopholan and cyclophosphamid, respectively, on the development of the solid Guérin T-8 tumor are included in table 1.

Table 1

Comparative investigation of the antitumoral therapeutic effect of the polyphenolic preparations and of some standard cancerostatics administered in different doses (mg/kg.b.w.) to the rats bearing Guérin T-8 lymphotropic epithelioma. Figures in brackets indicate the number of experimental animals

Group/Treatment	M.T.W. (g)	% R.T.M.	T/C value	Statistical significance
Control	13.5 ±1.7 (15)	-	-	-
PA_2 III (5.0)	6.1±1.8 (10)	54.8	0.45	$p < 0.01$
PA_3 (45.0)	5.6±1.9 (10)	58.5	0.41	$p < 0.01$
Girostan (1.0)	10.0±1.3 (10)	26.0	0.74	N.S.
Antipholan (0.15)	7.1±1.6 (10)	47.4	0.53	$p < 0.02$
Levopholan (0.30)	6.7±1.9 (10)	50.4	0.49	$p < 0.02$
Cyclophosphamid (1.6)	5.8±1.7 (10)	57.1	0.42	$p < 0.01$

It can be seen – in comparison with the control group – that the antitumoral treatment with the PA_2 III and PA_3 vegetable products has inhibited significantly ($p<0.01$) the development of lymphotropic epithelioma. This effect is expressed by M.T.W. decrease, M.T.R. values (54.8 and 58.5%, respectively) and by T/C ratios (0.45 and 0.41, respectively). On the contrary, as compared with the control, the therapy with girostan has materialized by a nonsignificant diminished M.T.W., which allowed the estimation both of M.T.R. percentage (26.0%) and of T/C value (0.74).

It is also observed that significant antitumoral effects were induced by daily i.p. treatments with antipholan, levopholan and cyclophosphamid, comparatively with the normal evolution of Guérin T-8 tumor. Thus, the values of the evaluation indices corresponding of their specific actions are:

– a M.T.R. of 47.4%, 50.4% and 57.1%, respectively;
– a T/C ratio of 0.53, 0.49 and 0.42, respectively.

The comparative testing of the cancerostatic effects of the polyphenolic preparations and of the standard cytostatics was also realized on Walker 256 tumoral system, the experimental results being presented in table 2.

Table 2

The antitumoral therapeutic effectiveness of the polyphenolic preparations and of the standard cancerostatics injected to the rats bearing Walker 256 carcinosarcoma in different doses (mg/kg.b.w.) Figures in brackets indicate the number of experimental animals.

Group/Treatment	M.T.W. (g)	% R.T.M.	T/C value	Statistical significance
Control	15.2 ±1.0 (15)	-	-	-
PA_2 III (5.0)	7.9±1.5 (10)	48.1	0.52	$p < 0.001$
PA_3 (45.0)	7.6±1.3 (10)	50.0	0.50	$p < 0.001$
Girostan (1.0)	11.7±1.7 (10)	23.1	0.77	N.S.
Antipholan (0.15)	3.5±2.0 (10)	76.8	0.23	$p < 0.001$
Levopholan (0.30)	10.2±1.9 (10)	32.9	0.67	N.S.
Cyclophosphamid (1.6)	12.0±1.6 (10)	21.1	0.79	N.S.

Once again, the evaluation indices values of the PA_2 III and PA_3 pharmacoterapeutic action (M.T.R. of 48.1% and 50.0%, respectively; T/C ratio of 0.52 and 0.50, respectively) have revealed – in comparison with the control group – a significant ($p<0.001$) inhibitory effect of Walker 256 carcinosarcoma development. The M.T.R. and T/C values of 76.8% and 0.23, respectively, – registered on rats submitted to antipholan treatment – have highlighted the high cancerostatic potential of this standard agent. On the contrary, the experimental therapies with girostan, levopholan and cyclophosphamid, respectively, have allowed the estimation of some M.T.R. of 23.1%, 32.9% and 21.1%, respectively, as well as of some corresponding T/C ratios of 0.77, 0.67 and 0.79, respectively. The values of the evaluation indices – nonsignificant in relation to the control – have proved that these reference cytostatics are characterized by a moderate antitumoral action on the Walker 256 carcinosarcoma.

The bulk of the experimental results, obtained by us in this preclinical screening stage, assures the appreciation of the cancerostatic effectiveness of PA_2 III and PA_3 vegetable polyphenolic preparations by its comparative analysis with that one of the standard cytostatic.

DISCUSSION

The identification of new pharmacological agents with antineoplastic activity represents a major and topical concern of the oncobiological research and of the medical practice, which pursues the enhancement of the antitumoral chemotherapy effectiveness.

The discovery of an anticancerous product and its introduction in the human chemotherapy is the consequence of some preclinical and clinical complex pharmacological investigations on adequate experimental models using different biological systems of testing [4], [7], [12], [13]. The chemotherapeutic programs of multistage preclinical screening, conceived to identify new active cancerostatic substances, foresee: the successive and interdependent investigation steps; the adequate experimental models;the evaluation indices of the specific pharmacodynamic effect; the qualitative and quantitative appreciation criteria of the induced antitumoral action [1], [2], [5], [6].

The preclinical characterization of a substance as antineoplastic agent is conditioned of qualitative and quantitative evaluation of the specific pharmacological effect.

Previous researches on adequate experimental models have highlighted the antitumoral pharmacotherapeutic action of PA_2 III and PA_3 polyphenolic preparations, have evidenced its reproducibility and have established the existence of a dose-response relationship [8], [9], [10].

The purpose of the present work was to extend and to complete the assessment of antineoplastic therapeutic efficiency of PA_2 III and PA_3 natural products by comparative analysis of their antitumoral potential with that of some standard cancerostatics.

The treatment of the rats bearing Guérin T-8 lymphotropic epithelioma with the polyphenolic agents and with the clinical use cytostatics, respectively, was correlated with the inducing of some specific antitumoral effects. These were quantitatively expressed by the M.T.R. values of 54.8% (PA_2 III), 58.5% (PA_3), 26.0% (Girostan), 47.4% (antipholan), 50.4% (levopholan), 57.1% (cyclophosphamid) and by the corresponding T/C ratios of 0.45, 0.41, 0.74, 0.53, 0.49 and 0.42, respectively.

According to the reference preclinical screening programs a substance is considered a possible antitumoral agent if it has conditioned a R.T.M. value of at least 35% [5] or if the induced M.T.W. decrease has allowed the estimation of a T-C ratio of 0.54 – 0.64 [6] on at least one solid tumoral system from three tested. From this viewpoint is reconfirmed the antimalignant action of the PA_2 III and PA_3 polyphenolic preparations.

The comparative analysis of evaluation indices values of the anticancerous activity reveals a significant experimental therapeutic effectiveness of the natural polyphenolic agents. This is similar (in the case of cyclophosphamid) or near (as compared with levopholan) to those of the reference agents and even higher in some cases (in relation to girostan and antipholan).

The experimental therapy of the rats bearing Walker 256 carcinosarcoma with the vegetable polyphenolic preparations and with the standard cancerostatics, respectively, has conditioned the manifestation of an antitumoral effect differentiated as intensity. The comparison of the M.T.R. values and of the T/C ratios – the evaluation indices of the inhibitory action on Walker 256 carcinosarcoma development – registered after the treatment with PA_2 III (48.1% and 0.52, respectively), PA_3 (50.0% and 0.50, respectively), girostan (23.1% and 0.77, respectively), antipholan (76.8% and 0.23, respectively), levopholan (32.9% and 0.67, respectively), and cyclophosphamid (21.1% and 0.79, respectively) highlights the significance of the cancerostatic potentials of the vegetable polyphenolic preparations. These are superior to those of girostan, levopholan, cyclophosphamid and inferior to that of antipholan.

In the light of the above results, it can be appreciated that the natural polyphenolic products present a significant antitumoral therapeutic efficiency – comparative with that one of the reference cytostatics – in our experimental conditions (at the used doses and on the tumoral systems used in screening).

The possibility of cancerostatic effectiveness optimization by manipulating the therapeutical doses [10] as well as the antitumoral potential significance of the PA_2 III and PA_3 preparations, established by comparison with the standard agents, have partially assured the quantitative evaluation of their antineoplastic pharmacodynamic effect.

The positive answers to these problems of the quantitative pharmacological evaluation impose its finalizing by the investigation of the therapeutic efficiency of PA_2 III and PA_3 polyphenolic preparations on tumors with different degrees of development.

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SPIRULINA BIOMASS UTILIZATION FOR THE ACUTE GASTRIC ULCER HEALING IN WISTAR RATS

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The effect of the *Spirulina platensis* dry biomass given orally, in the cicatrization of experimental acute gastric ulcers was investigated in Wistar rats. In 75-80% of experimental group animals gastric ulcer lesions were produced by: 40 hr fasting period, during which animals were twice exposed to constraint and cold stress (-13°C, 3 hours). Subsequently, controls were fed on standard diet, and experimental animals on bred and 2g/animal/day of *Spirulina platensis* dried biomass. After 1, 4, 7, 10, 15, 20 days of Spirulina treatment, 4-5 animals of each group were sacrificed. The number, localization, dimensions and appearance of gastric mucosa lesions were registered. After a 20 day period, 100% lesions were cicatrized in Spirulina treated animals, whereas control still presented 38% hemorrhagic ulcerations. The fragments of stomach wall containing lesion were studied at optic microscopic level, after properly histologic processing. The histologic examination confirmed the obtained macroscopic data. The factors in the *Spirulina platensis* dry biomass responsable for potentiation of the healing processes in gastric mucosa still remain unknown.

INTRODUCTION

Utilization of new natural resources for medical purposes has also considered the highly diversified kingdom of algae. *Spirulina platensis* alga, due to its high protein content, balanced content of essential amino acids, good digestibility given by cell membrane structure, high percentage of vitamins, sodium and potassium, called nutritionist's attention. *Spirulina platensis* alga does not contain toxic substances (6). Spirulina biomass utilization in numerous nutrition studies (6, 8, 18) also revealed other of its properties especially useful for medicine. For example, it was found out that Spirulina biomass helped the healing of gastro-duodenal ulcers (12, 18). In this respect, a clinical trial of Spirulina tablet utilization in the treatment of gastro-duodenal diseases was carried out in Municipal Hospital-Bucharest. As a consequence of the satisfactory results obtained then, the present investigation was done in order to assess the effect of Spirulina biomass in the ulcer healing. The morphologic changes of healing ulcerations were examined in Wistar rats with experimental acute gastric ulcer.

MATERIALS AND METHODS

Spirulina platensis culture was made in horizontal basin of 20 mp area, in Zarrouk medium (6). Stirring and recirculation of algal suspension was realized by

air barbotage. The Spirulina dry biomass was analysed chemically for protein content (Kjeldahl method) (5), total saccharides (Hagedorn-Jensen method) (17), assimilatory pigments (Holm method) (13).

White mice (115 individuals) and Wistar rats (98 individuals) were used to establish the experimental model for gastric ulcer induction. Four techniques were tested: phenylbutazone (4, 9, 11) and indomethacin (7, 19) i.p. administration, immobilization (1, 2, 3) and cold (1, 4, 16) stress. The used animals proved to be very resistant to attempted treatments and therefore the results were not as satisfactory as expected. Consequently the ulcer model was realised by associating the ulcerogenic agents as follows: 40 hr fasting, cold concomitant with restraint stress (-13° C, 3 hours of exposure in 2 days). This resulted in 75-80% of treated animals having severe hemorrhagic gastric lesions.

The Spirulina biomass effect in acute gastric ulcer cicatrization was investigated in 50 female, adult, Wistar rats (150-220g). All animals were subjected to the above mentioned treatment of ulcer induction. Further, controls were fed on standard diet (15g bred, 2g milk powder, 4 g wheat, 50 g barley, 30g maize, 1g sunflower seeds, 3g oat/animal/day) and experimental animals on bred (15g/animal/day) supplemented with 2g/individual/day dry Spirulina biomass. 4-5 rats of each group were sacrificed at 1, 4, 7, 10, 15, 20 days. The stomach was opened by incision on the high curvature line and gastric mucosa lesions were examined thoroughly under magnifier. Their number, localization, dimensions and appearance were registered. Fragments of stomach wall containing hemorrhagic lesions and ulcer scars were properly processed for microscopic examination.

RESULTS AND DISCUSSION

The chemical composition of utilized *Spirulina platensis* biomass is presented in table 1.

Table 1

Chemical composition of *Spirulina platensis* cultivated on Zarrouk medium

Humidity 9.78%

	Dry weight		
	%		mg/g
Proteins	69.62	Assimilatory pigments	
Total saccharides	5.74	Chlorophylls	12.8
Lipids	2.98	Carotenoids	5.039
Sodium	0.72	Phycobilins	106.6
Potassium	0.37		
Magnesium	0.008		

The effect of Spirulina in ulcer healing as compared with controls, is evidenced by table 2 data. The satisfactory evolution of individuals that received alga biomass is observed after 15 days of treatment. This tendency became more pronounced

within 20 days, when in Spirulina fed animals 100% lesions were cicatrized, while in controls 38% ulcerations were still hemorrhagic. Three of 6 individuals sacrificed after 20 days of standard diet feeding, presented a thickened mucosa, in 80% of cardia area, showing prominences characteristic to development of severe hypertrophic chronic gastritis.

Table 2

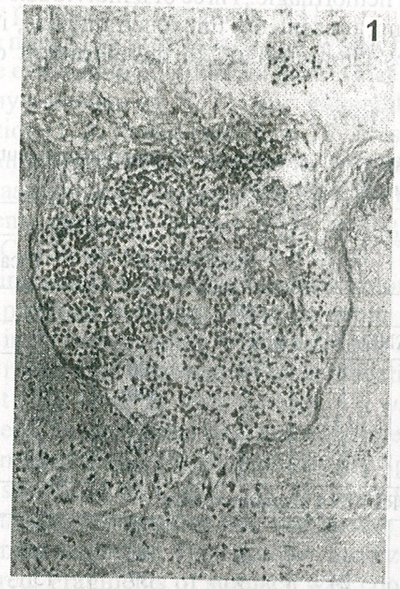
The effect of *Spirulina platensis* dry biomass on cicatrization of experimental acute gastric lesions in Wistar rats

Days after ulcer onset	Animals				Lesions			
	Sacrificed	With lesions		Total	Hemorrhagic		Cicatrized	
	no	no	%	no	no	%	no	%
STANDARD DIET								
1	4	3	75	8	8	100	0	0
4	4	2	50	5	5	100	0	0
10	5	4	80	13	3	23	10	77
15	5	2	40	6	3	50	3	50
20	6	6	100	8	3	38	5	62
BRED + SPIRULINA (2g/animal/day)								
1	4	3	75	7	7	100	0	0
4	5	4	80	10	10	100	0	0
7	5	3	60	24	9	37	15	63
10	4	2	50	17	1	18	16	82
15	4	3	75	12	1	9	11	91
20	4	1	25	4	0	0	4	100

Despite a higher lesion incidence in cardia, lesions were often found also in fundus. The ulcerations were round or oval in shape, between 0.5-5mm in diameter, the majority had 2-3mm. The ulcer craters had a reddish color due to hyperaemia and most of them were covered by a red coagulum produced by hemorrhage. The lesion boundaries were flexible without sclerous reaction. During the early period of healing hyperaemia gradually resoluted and red coagulum disappeared. In the subsequent stage of cicatrization, the injured areas became whitish, owing to newly formed tissue. Lastly, the ulcer site is marked by a depression in stomach wall, because the new cicatrization tissues are less thick than adjacent normal gastric mucosa (fig. 6).

The damage of mucosa and mucosal muscularis structure was evidenced by microscopic examination (figs. 1-3). The erosion seldom reached the submucosa layer. The affected zone was covered by a necrotic cell detritus containing numerous erythrocytes and granulocytic inflammatory infiltration (figs.1-3). At a profound level in ulceration, hyperaemia and edema were observed (figs 1, 2). The gastric epithelium recovery occurred from lesion periphery towards its center due to proliferation of regenerating cells (figs. 4, 5).

The establishing of the ulcer model raised some difficulties, despite a lot of ulcerogenic drugs and factors described in the literature (1, 2, 9, 10, 11, 14, 16, 19). The four applied models did not give satisfactory results in a short-term treatment. Even powerful stress agents like immobilization and cold induced few ulcer lesions. Only sinergic action (1, 2, 10, 16) of cold and immobilization, together



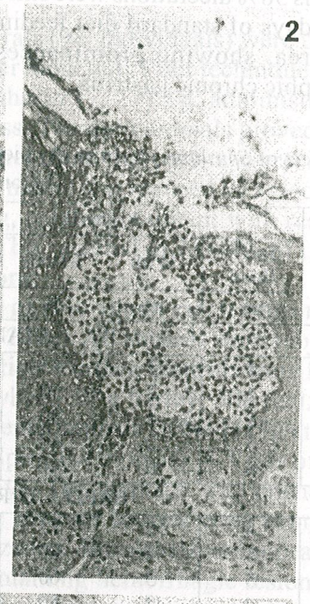
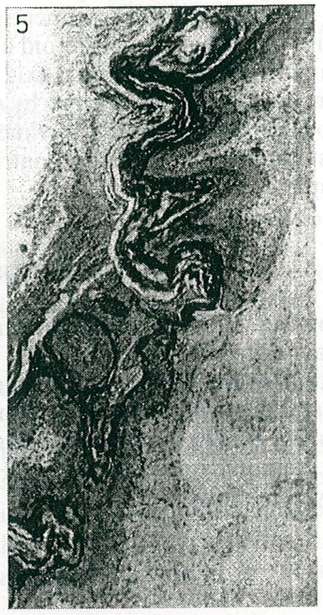


Fig. 1. – Stomach cardia hemorrhagic ulceration in control rat subjected to low temperature (–13°C)and restraint stres (3 hours), sacrificed one day after ulcer model application.
Fig. 2. – Stomach cardia hemorrhagic ulceration in control rat sacrificed after 4 days of feeding on standard diet.
Fig. 3. – Stomach cardia hemorrhagic ulceration in control rat sacrificed after 15 days of feeding on standard diet.



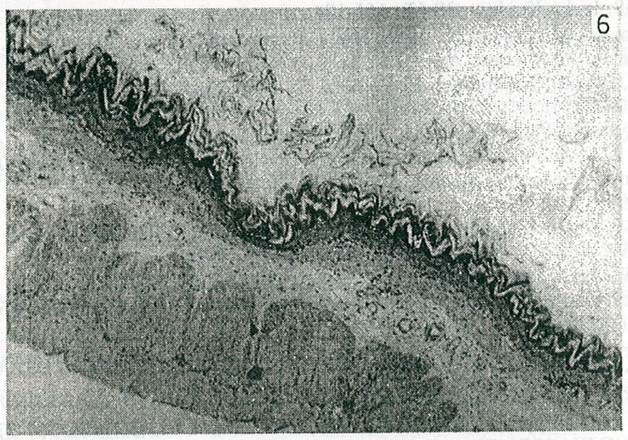


Fig.4. – Healing ulceration in stomach fundus in rat subjected to experimental ulcer and sacrificed after 7 days of feeding on bred supplemented with Spirulina biomass (2g/animal/day).
Fig. 5. – Healing ulceration in stomach cardia in rat subjected to ulcer model and fed 15 days on bred supplemented with Spirulina biomass (2g/animal/day).
Fig. 6. – Remained excavation in stomach cardia mucosa after the healing of ulcer lesion, following 20 days of feeding on bred supplemented with Spirulina biomass (2g/animal/day).

with 40 hr fasting, succeeded in producing ulcer lesions in 75-80% of animals subjected to this short period treatment. It seems that ulcerogen mechanism is correlated with increased corticosteroid and catecholamine blood levels, caused by hypothalamo-hypophysis-adrenal axis activation (15, 20).

Neither mechanism nor factors by which *Spirulina platensis* dry biomass contributed to faster the healing of experimental gastric ulcer in rats are yet unknown. Further investigations will probably contribute to use the *Spirulina platensis* alga in ulcer therapy.

CONCLUSIONS

– Following 20 days of *Spirulina platensis* dried biomass treatment (2g/animal/day, added to 15g bred), 100% experimental gastric ulcer lesions were healed, in adult, female, Wistar rats. Controls fed only on standard diet, after the same period, still presented 38% hemorrhagic ulcerations;

– *Spirulina platensis* dried biomass enhanced the cicatrization rate of experimental hemorrhagic ulcerations. However, the mechanism and factors involved are yet unknown.

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NUCLEIC ACID SPECTROPHOTOMETRIC ANALYSIS FOR THE GENETIC CHARACTERIZATION OF SOME CHINESE CARP SPECIES HABITUATED IN ROMANIA

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According to the cytogenetic analysis, three species of the Chinese carp revealed similar karyotypes, having the same number of chromosomes (2*n*=48).
The spectrophotometric analysis of nucleic acids has been used in order to develop new criteria for the genetic discrimination among the three given species.

Besides the native forms of the carp (*Cyprinus carpio L.*), the species of the phytophagous Chinese carp, such as: *Hypophthalmichthys molitrix, Aristichthys nobilis, Ctenopharingodon idella*, that have been recently introduced in the Romanian breeding stations, have got an increasing economic importance.

The habituation of these species, their integration in the new natural environment, other words their controlled breeding in hatcheries, needs a precise genetic characterization. This is a necessary prerequisite when a maximal efficient use of these species is desired.

The genetic characterization by the karyotype examination of these species revealed an identical number of chromosomes (2*n*=48), having rather similar morphologies (1, 4, 6, 10).

Therefore, this paper deals with the quantitative determination of nucleic acid, as this alternative approach might bring additional information to the genetic data obtained by the karyotype examination.

MATERIALS AND METHODS

The biological material was represented by the two summer old species of Chinese carp, that were supplied by CCPPPIP-Galați. Two muscle tissues samples were prepared for each *H. molitrix*, *A. nobilis* and *Ct. idella* species. Table 1 presents the six samples (A-F) corresponding to the three species of grass Chinese carp used in our experiment.

Table 1

The muscle tissue samples processed for the nucleic acid extraction

Species	*H. molitrix*		*A. nobilis*		*Ct. idella*	
Samples analysed	A	B	C	D	E	F
Muscle tissue content (g)	1.2	2.7	2.5	2.7	2.0	2.5

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The somatic tissue samples were prepared by the disruption of the cells with a potter and the subsequent rinse with 0.15M NaCl solution was performed. After centrifugation 5 minutes at 2500 rpm, the pellet was suspended in 0.15M NaCl solution as a proportion of 1:10 w/v (mg/ml).

The total nucleic acid extract has been obtained by the common method of ethanol precipitation (7). Samples of 10 ml extract have been used for the determination of total nucleic acid concentrations.

The nucleic acid concentrations were calculated from their absorbances at 260 nm. The readings were performed with a Zeiss Specord UV-VIS equipment. The domain used for the registration of the absorbance spectra was: 230-290 nm.

RESULTS AND DISCUSSION

Only the absorption values corresponding to the maxima of 260 nm (table 2) were taken for the calculation of nucleic acid concentration. The absorption spectra on the given wavelengths domain are represented in figures 1, 3 and 5.

Table 2

The absorption values at 260 nm and 280 nm corresponding to the nucleic acid and protein content of the samples (A - F)

Absorbance λ(nm)	*H. molitrix*		*A. nobilis*		*Ct. idella*	
	A	B	C	D	E	F
A 260	0.541	0.524	0.550	0.500	0.506	0.492
A280	0.330	0.320	0.320	0.300	0.310	0.300
A260/280	1.64	1.64	1.71	1.66	1.66	1.64

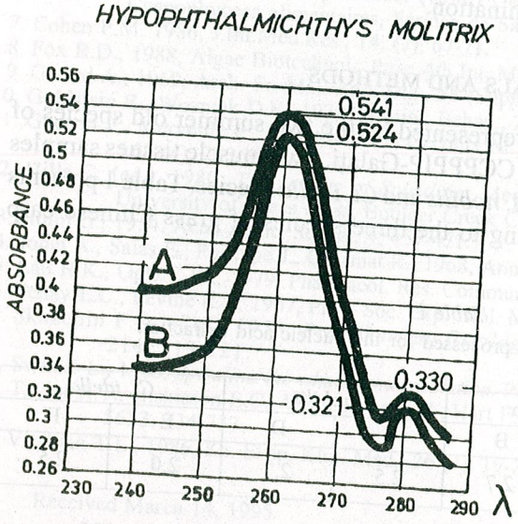

Fig. 1. – The absorption spectra for samples A and B, respectively (for the species *H. molitrix*).

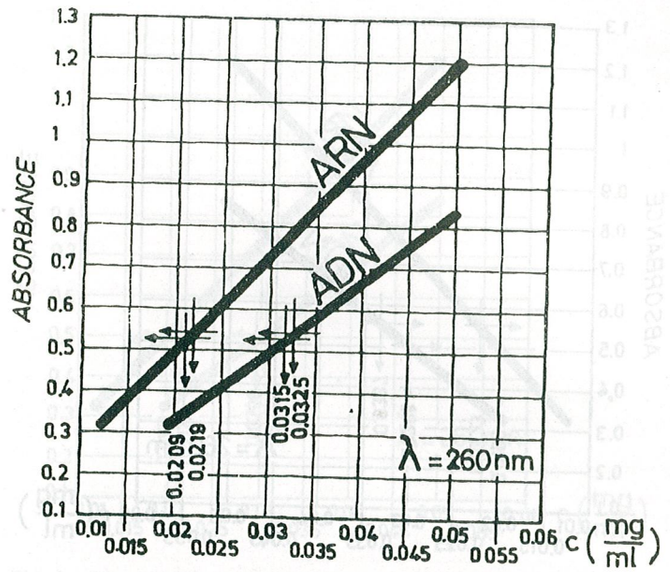

Fig. 2. – The calibration curves for DNA and RNA at 260 nm and the DNA and RNA concentrations estimated for the samples A, B.

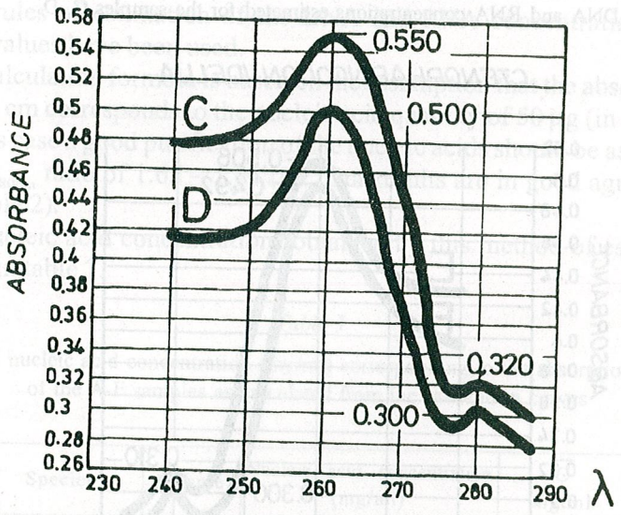

Fig. 3. – The absorption spectra for samples C and D, respectively (for the species *A. nobilis*).

The calibration curves of the DNA and RNA absorbances versus their concentrations represented in figures 2, 4 and 6 have been used for the further calculation of each nucleic acid concentration found in the muscle samples.

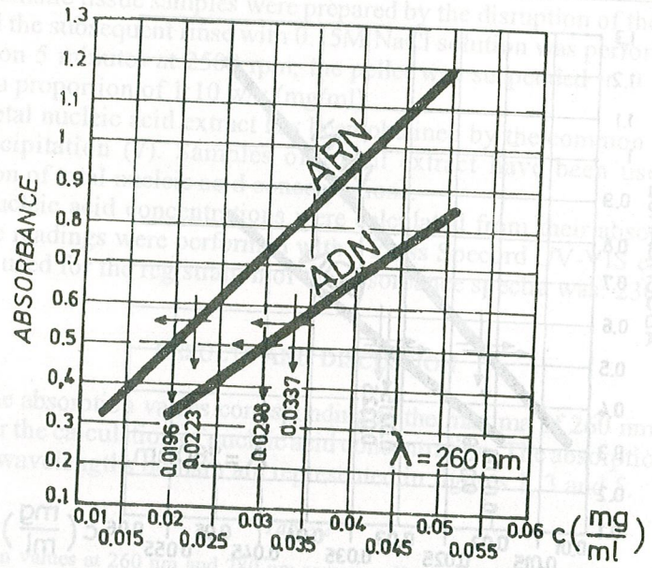

Fig. 4. – The calibration curves for DNA and RNA at 260 nm and the DNA and RNA concentrations estimated for the samples C, D.

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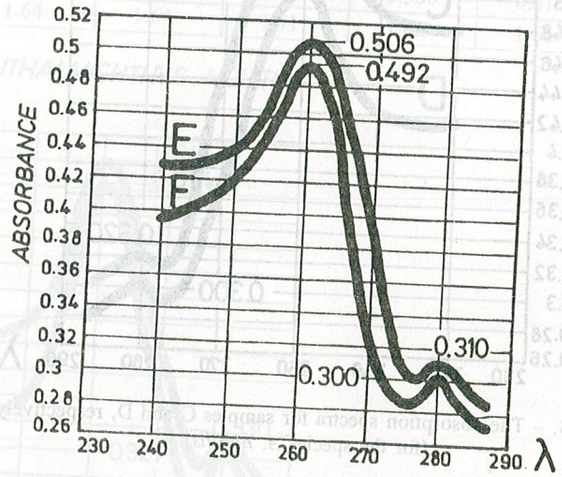

Fig. 5. – The absorption spectra for samples E and F, respectively (for the species *Ct. idella*)

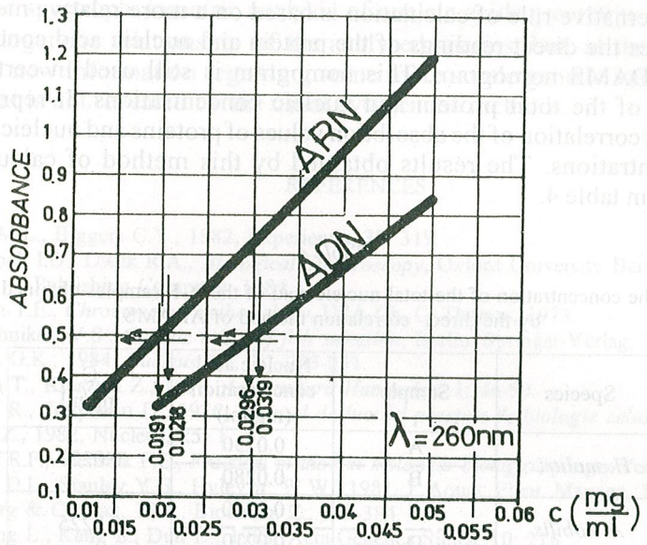

Fig. 6. – The calibration curves for DNA and RNA at 260 nm and, the DNA and RNA concentrations estimated for the samples E, F.

Two rules of estimation of the DNA and RNA concentrations from the absorption values have been used.

One calculation formula is based on the assumption that the absorption value of $A_{260\,nm} = 1$ cm corresponds to the nucleic acid quantity of 50 µg (in a 1 ml quartz cuve). In this case a good purification of the nucleic acids should be ascertained by the A_{260nm}/A_{280nm} ratio of 1.65 – 1.85 (9). Our results are in good agreement with this rule (table 2).

The nucleic acid concentrations obtained by this method of estimation are represented in table 3.

Table 3

The total nucleic acid concentration (mg/ml) corresponding to the absorption values of the A-F samples as calculated from the calibration curves

Species	Sample	Nucleic acid concentration (mg/ml)	$\overline{C}$ mg/ml
H. molitrix	A / B	0.0272 / 0.0262	0.0265
A. nobilis	C / D	0.0280 / 0.0247	0.0263
Ct. idella	E / F	0.0268 / 0.0243	0.0255

An alternative rule of calculation is based on a more relative method (11). This one uses the direct readings of the protein and nucleic acid concentrations from the ADAMS nomogram. This nomogram is still used in certain rough estimations of the total protein and nucleic concentrations. It represents the concomitant correlation of the absorbtion values of proteins and nucleic acids with their concentrations. The results obtained by this method of calculation are represented in table 4.

Table 4

The concentration of the total nucleic acid of the A-F samples obtained by the direct correlation method of ADAMS

Species	Sample	Nucleic acid concentration (mg/ml)	$\overline{C}$ mg/ml
H. molitrix	A	0.0230	0.0229
	B	0.0280	
A. nobilis	C	0.0240	0.0225
	D	0.0210	
Ct. idella	E	0.0210	0.0205
	F	0.0200	

CONCLUSIONS

The advantage of the spectrophotometric method used for the DNA and RNA content determination is that it provides a rapid and undestructive technique of measurement, as well as a good sensitivity (as low as 2.5 μg/ml).

The experiment shows that all the three species revealed similar average values of the total nucleic acid concentration in their cells (0.0265, 0.0263 and 0.0255 mg/ml tissue) respectively for *H. molitrix, A. nobilis, Ct. idella*, according to the first method of calculation based on the callibration curves comparison (table 3).

The results in table 4 obtained by the ADAMS nomogram are close to those represented in table 3; the difference appeared at the third zecimal might be accounted for the rough estimation offered by the ADAMS method.

Consequently, the three species are genetically closely related. However, the data corresponding to *Ct. idella* are indicating a slight difference.

These spectrophotometric results are in good correlation with the karyological analysis. These latter ones are less rigorous and the data obtained by the first method brought more details for the genetic discrimination among the given species.

On the basis of the karyotype examination, all the three species have the same chromosome number ($2n$=48) and similar chromosome morphologies and dimensions. However, as results also from the nucleic acid determination the chromosomes of *Ct. idella* species apparently revealed less chromatin.

The spectrophotometric analysis of nucleic acids ascertains the taxonomic and phylogenetic relationship of the analysed species, but, at the same time, it provides new information regarding some more subtle genetic variations among them, that is not revealed by the usual cytogenetic methods.

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CHARACTERIZATION OF MAIN BIOCENOTIC COMPONENTS OF "SFÂNTA ANA" NATURAL RESERVATION FROM THE BUCEGI MOUNTAINS

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"Sfânta Ana" natural reservation is an area of a 8 ha with natural vegetation installed on calcareous rocks, in the Bucegi Mountains.
The paper presents vegetation types and characteristic plant associations with the description of a new subassociation installed an semifixed rocks debris.
Diversity indexes and the distribution of trees, osmotic pressure, glucidic content and acidity of cell sap are some of other studies of primary producers.
It is also emphasized the numerical structure of the invertebrates from canopy, herbaceous layer and soil. Good natural regeneration of trees, a new plant subassociation and some rare animal species show optimum natural conditions for the development of this reservation.

"Sfânta Ana" natural reservation is a unique area from the Bucegi Mountains, through its beautiful scenery, mobile, semifixed and fixed calcareous rocks with rare plant and animal species, as well as characteristic plant associations for calcareous substrate. A general survey of the main biocenotic components of plants and animals, as well as of some ecophysiological parameters of representative plant species was undertaken in 1994, in order to emphasize the present situation of this natural reservation from the Bucegi Mountains.

MATERIAL AND METHODS

Random samples of plants, animals living in soil, on herbaceous layer and canopy, were taken seasonally, in spring, summer and autumn. In each season, a quadrate with 50/50 cm was used for plants (25 samples): for dendrological measurements, 50 sq.m. areas were selected; 10 samples for soil invertebrates (different technics, dependent on soil animal group): 10 × 50 sweepings using entomological net with 30 cm diameter, for dwelling invertebrates of herbaceous layer: 10 × 50 shakings for canopy invertebrates, using a 60 cm diameter entomological net. Osmotic pressure of the cell sap was determined using the cryoscopic method (after Steubring) and the glucidic content was determined using Abbé refractometer. The extraction of animals from samples was performed differently, depending on the soil animal group. Dehydrogenase activity was performed after Cassida method (1962).

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RESULTS AND DISCUSSIONS

Saxicole vegetation installed down on the sunny calcareous rocks is affiliated to *Sedo hispanici – Poëtum nemoralis* Pop et Hodişan 1985 *cardaminopsietosum arenosae* Sanda et Popescu 1995 (table 1).

Table 1

Sedo hispanici – Poëtum nemoralis Pop et Hodişan 1985 *cardaminopsietosum arenosae* subass. nova

Number of survey	1	2	3	4	5	6	7	8	9	10	K
Area (sq. m)	100	100	100	100	100	100	100	100	100	100	
Veg.height (cm)	40	45	30	35	45	50	40	45	50	55	
Cover area (%)	30	60	40	50	65	70	45	50	65	70	
Exposure	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	
Slope (degrees)	40	40	40	40	45	35	40	40	40	40	
Char. ass.											
Poa nemoralis	4	4-5	4	4	5	5	4	4	4	4	V
Sedum hispanicum	+	+	+	.	+1	+1	+	+	+	+	V
Diff. subass.											
Cardaminopsis arenosa	+1	+	.	.	+1	+	+	.	.	+	III
Galium album	+	.	+	.	+1	.	+	.	.	+	III
Teucrion montani											
Thymus comosus	.	+	+	.	.	.	.	+	.	+	II
Cnidium silaifolium	.	.	.	+	+	+	.	.	.	+	II
Teucrium montanum	.	+	+	+	.	+	+	+	.	+	IV
Seslerietalia											
Acinos alpinus											
ssp. hungarica	.	.	.	.	.	+1	+	+1	.	.	II
Sesleria rigida	.	.	.	.	+	.	+				I
Dianthus											
spiculifolius	.	+	.	.	.	.	.	.	+	.	I
Melica ciliata	.	+	.	+	+	+1	+1	+	+	+	IV
Polygala alpestris	.	.	.	.	+	+	.	+	+	+	III
Bupleurum falcatum	.	+	+	+	+	+	.	.	.	.	III
Anthyllis vulneraria											
ssp. *alpestris*	+	.	.	+	.	.	+	.	.	+	II
Anthemis tinctoria											
ssp. *fusii*	.	.	.	+	.	+	.	.	+	.	II
Asplenietea											
Doronicum columnae	+	.	+	+	.	+	+	+	+	.	IV
Sedum fabaria	+	+	.	+	+1	.	+	.	+	+	IV

continued table 1

0	1	2	3	4	5	6	7	8	9	10	K
Seslerio-Festucion pallentis											
Carduus candicans	+	.	+1	.	+1	.	+	+	.	+	III
Sempervivum schlehanii	.	.	.	+	+	.	.	+	.	.	II
Festucion rupicolae											
Jurinea mollis	+	+	.	+	.	.	+	.	.	+	III
Festuca rupicola	.	+	.	.	+	.	.	.	.	+	II
Thesium arvense	+	.	.	.	.	.	.	+	.	.	I
Festuco-Brometea											
Sedum sexangulare	+	+	.	+	.	+	+	+	.	+	IV
Medicago lupulina	+	.	.	.	+	.	.	+	.	.	II
Verbascum chaixii	.	+	.	.	.	+	.	.	.	.	I
Coronilla varia	+	.	.	+	.	+	.	+	+	+	III
Silene otites	+	.	.	.	.	.	.	.	.	+	I
Vincetoxicum hirundinaria	.	.	.	+	+	+	.	+	.	.	II
Arrhenatheretea											
Veronica chamaedrys	+	.	.	.	+	+	.	+	+	.	III
Arrhenatherum elatius	.	+	.	+	.	.	.	.	.	+	II
Poa pratensis	.	.	.	+	.	.	+	.	.	.	I
Rhinanthus glaber	.	.	.	.	.	+	.	.	.	+	I
Accompanying species											
Clinopodium vulgare	+	.	.	.	.	.	.	.	+	+	II
Lotus corniculatus	.	.	.	.	.	.	+	.	.	+	I
Origanum vulgare	.	+	+	.	.	+	.	.	.	+	II
Chrysanthemum leucanthemum	.	.	+	.	.	.	+	+	.	.	II
Veratrum album	.	.	.	.	+	.	+	.	.	+	II
Trifolium repens	.	+	.	.	.	+	.	.	+	.	II
Hypericum maculatum	.	+	+	.	.	.	.	+	.	.	II

Species only one survey: *Dactylis polygama* (3), *Gymnadenia conopsea* (7).
The date of surveys: 1-5, 26.05.1994; 6-10, 16.08.1994

From the alliance *Teucrion montani*, representative species are *Thymus comosus* and *Cnidium silaifolium*. The fixation tendency of rocks is marked by the presence of some plant species of *Seslerio – Festucion pallentis* and *Festucion*

rupicolae as are: *Sempervivum schlehanii* and *Festuca rupicola*. On the eastern part of tectonic rocks with 40-55° inclination of the slopes, there are characteristic species not only of association *Asperulo capitatae – Seslerietum rigidae* (Zóly. 1939) Coldea 1991, but also of the alliance *Seslerion rigidae* and *Seslerietaria* Order.

Indicatory species for these xerophytic phytocenoses, installed on calcareous substrate, there are some other species of Class *Asplenietea rupestris*, as *Poa nemoralis, Asplenium ruta–muraria* and of the alliance *Seslerio-Festucion pallentis*, as *Cnidium silaifolium* and *Sempervivum marmoreum*. On the mobile rocks there are incipient stages of herbage, the covering level of new vegetation being between 30-75%, where *Galietum albi* (erecti) Pop et Hodişan 1964 is representative with *Galium album* as dominant species.

Woody vegetation installed on the slopes with strong inclination has a limited trophic base and is characterized by the *Pulmonario rubrae-Abieti-Fagetum* Soó 1964. The arborescent stratum, dominated by the species *Fagus sylvatica* and *Abies alba*, contains in its structure species *Picea abies*, *Acer pseudoplatanus, Ulmus glabra* and some few other species of *Vaccinio-Picetea* Class as *Sorbus aucuparia, Hieracium rotundatum, Larix decidua, Vaccinium myrtillus.*

The presence of protected species *Daphne blagayana, Taxus baccata* and *Syringa vulgaris* give a prominent specificity to this natural reservation.

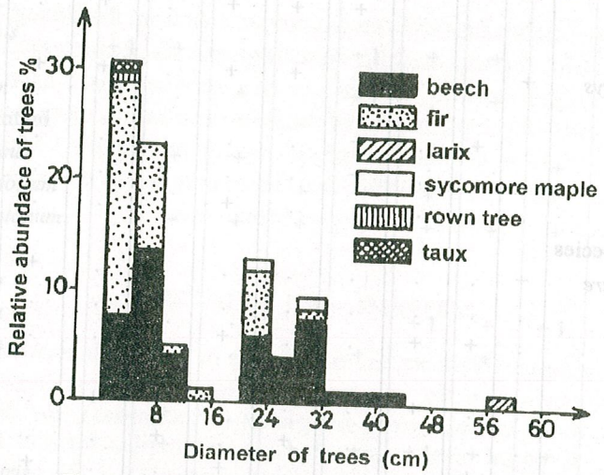

Fig. 1. – The plant species distribution in the tree layer.

Simpson/Pielou diversity indexes of fire and beech mixed forest are rather high (0.58) because not only of a great number of plant species of this stratum, but also of an equilibrated percent of these: 57.7% beech; 30.8% fire tree; 7.7% sycamore maple; 3.8% larch tree. The rowan tree and yew tree have small height and take part into the structure of the stratum. Trees are rather discontinuous with 520 individuals/ha, brush populations have accounted 1060 individuals/ha. Depending on their diameters, beech old trees are grouped to an average of 25.9 cm and fire tree to 18.6 cm (fig. 1).

Analyzing frequency and numerical density indices could be emphasized significant participation of the species of *Seslerion rigidae* and of the Order Seslerietalia (tables 2, 3).

Table 2

Frequency and numerical density indices (m^{-2}) of the main herbaceous species from *Sedo hispanicae – Poëtum nemoralis* association

Species	spring		summer		autumn	
	F	D	F	D	F	D
Poa nemoralis	90	455.6	93.3	132.2	93.3	818.7
Sedum hispanicum	20	0.8	28	26.1	6.7	1.9
Cardaminopsis arenosa	60	24.0	53.3	7.7	20.0	2.1
Thymus comosus	40	92.4	66.6	107.7	66.6	120
Cnidium silaifolium	40	9.2	13.3	2.7	46.6	3.5
Bupleurum falcatum	50	13.2	33.3	6.9	53.3	13.3
Galium album	50	40.0	20.0	8.3	40.0	6.4
Rhinanthus minor	-	-	86.6	13.3	20.0	1.1
Origanum vulgare	-	-	93.3	25.3	80.0	23.7
Arrhenatherum elatius	40	13.2	40.0	7.7	6.7	1.3
Coronilla varia	60	9.2	73.3	9.9	66.7	8.5

Table 3

Frequency and numerical density indices (m^{-2}) of the main herbaceous species from *Asperulo capitatae – Seslerietum rigidae association*

Species	spring		summer		autumn	
	F	D	F	D	F	D
Sesleria rigida	90	732	72	450	93.3	905.6
Thymus comosus	50	132	44	200.6	80	234.6
Cnidium silaifolium	30	3.2	48	8.8	53.3	3.7
Dianthus spiculifolius	40	77.2	48	49.3	73.3	245.6
Poa nemoralis	30	56.4	64	185.2	46.7	37.9
Bupleurum falcatum	20	4.8	44	15.7	93.3	17.3
Helianthemum nummularium	10	6.4	60	35.8	46.7	22.9
Asperula capitata	10	2.4	-	-	-	-
Cardaminopsis arenosa	10	1.6	-	-	-	-
Sempervivum marmoreum	-	-	4	0.6	6.7	8
Campanula carpatica	10	0.4	4	3.5	-	-
Campanula sibirica	20	3.2	32	4.48	33.3	2.9
Galium album	-	-	12	4.48	26.7	4
Acinos arnensis	10	11.2	4	1.0	13.3	0.8

Calcareous substrate, small edaphic volume, small relative humidity of soil and the strong insolation explain partially xerophitic characteristics of phytocoenoses from this reservation. They are adapted to these conditions by a high osmotic pressure, high glucidic content and high acidity of the cell sap (tables 4, 5), that is, an intense metabolic activity.

Table 4

Osmotic pressure of cell sap to primary producers from *Seslerietum rigidae* association

Species	spring	summer	autumn
Acer pseudoplatanus	19.94	18.50	19.00
Salix capraea	18.00	18.76	18.90
Ulmus glabra	20.00	19.20	20.40
Sesleria rigida	16.10	15.90	16.24
Origanum vulgare	15.70	16.00	16.15
Cnidium silaifolium	14.80	15.00	15.64
Verbascum chaixii	14.36	14.78	14.60
Poa nemoralis	13.80	13.69	14.00
Onobrychis viciifolia	13.00	13.48	13.78
Thymus comosus	-	-	13.96
Dianthus spiculifolius	-	-	13.54
Bupleurum falcatum	-	-	12.90
Salvia verticillata	-	-	14.12
Cirsium oleraceum	-	-	15.08
Arrhenatherum elatius	15.08	15.08	-
Polygala comosa	14.36	14.60	-
Cardaminopsis arenosa	13.82	-	-

Table 5

Dynamics of glucidic content and acidity of the cell sap of herbaceous species from *Asperulo capitatae – Seslerietum rigidae* association

Species	Glucides %				pH			
	spring	summer	autumn	mean val.	spring	summer	autumn	mean val.
Sesleria rigida	11.4	16.3	16.3	14.6	5.81	5.70	5.97	5.83
Cnidium silaifolium	13.8	17.1	14.1	15.0	5.65	5.38	5.49	5.51
Polygala comosa	9.3	9.6	-	9.5	5.40	5.39	-	5.40
Thymus comosus	-	-	16.7	16.7	-	-	5.75	5.75
Dianthus spiculifolius	-	-	16.5	16.5	-	-	5.39	5.39
Poa nemoralis	9.4	7.7	11.8	9.9	5.58	5.81	5.73	5.80
Cardaminopsis arenosa	6.5	-	-	6.5	5.23	-	-	5.23
Verbascum lychnitis	7.6	10.1	11.7	9.8	4.93	4.98	5.30	5.07
Arrhenatherum elatius	5.5	8.0	-	6.8	5.88	5.80	-	5.84
Origanum vulgare	-	9.6	19.3	14.5	-	5.43	5.02	5.23
Coronilla varia	-	12.2	19.6	15.9	-	5.09	5.32	5.21

Table 5 (continued)

Species	Glucides %				pH			
	spring	summer	autumn	mean val.	spring	summer	autumn	mean val.
Bupleurum falcatum	-	-	20.2	20.2	-	-	5.50	5.50
Ulmus glabra	13.6	16.8	18.3	16.2	5.79	5.49	5.63	5.64
Acer pseudoplatanus	10.6	13.9	14.2	12.9	3.95	3.81	4.53	4.43
Salix caprea	9.2	13.3	14.6	12.4	5.54	5.59	5.61	5.58
Mean values	9.69	12.23	16.10	13.16	5.40	5.31	5.42	5.42

Both beech and fire tree species are lasting mixed, because of a good natural regeneration; this phenomenon is not met for the other species – sycamore maple, larch tree and yew tree. Nonuniform distribution of trees, and especially their less united canopy determine a characteristic qualitative and quantitative composition of invertebrates at this level (table 6). Numerical abundance of major groups of

Table 6

Numerical and relative abundance of canopy invertebrates

Taxon	$\bar{x}$	%	S^2	S	$S\bar{x}$	CV
ORTHOPTERA	0.3	1.27	0.064	0.25	0.50	84.62
THYSANOPTERA	0.5	2.11	0.055	0.23	0.48	47.14
HETEROPTERA	0.5	2.11	0.055	0.23	0.48	47.14
HOMOPTERA	9.2	38.82	15.16	3.89	1.97	42.33
HYMENOPTERA	0.6	2.53	0.08	0.28	0.53	47.14
COLEOPTERA	7.7	32.49	4.33	2.08	1.44	27.03
LEPIDOPTERA	0.1	0.42	0.002	0.047	0.22	47.14
DIPTERA	3.7	15.61	1.533	1.04	1.11	33.44
ARACHNIDA	1.1	4.64	0.13	0.37	0.61	33.47

invertebrates (insects and arachnida) is small, one of explanations being the special microclimate realized by this less united canopy. In the numerical structure of insects there was identified species *Rhynchaenus fagi* (Coleoptera, Curculionidae) which, in turn, did not attain the attack level of this pest insect. Even though the herbaceous level developed on the calcareous rocky stones has a variable united level, between 30% and 75%, some structural parameters of population invertebrates inhabiting this level showed rather high values (table 7).

Mean density m^{-2} of these invertebrate populations was large with a uniform distribution of zoophage and phytophage species, which play an important role in the trophic equilibrium at this level.

Having a natural reservation status, some rare species of Thysanoptera were indentified in this area: *Cryptothrips nigripes*, with a last citation, here, in 1957 and *Poecilothrips albopictus,* the second citation in the country.

Table 7

Numerical density (m^{-2}) of invertebrates from herbaceous layer

Taxon	$\bar{x}$	%	S^2	S	$S\bar{x}$	CV
ORTHOPTERA	1.0	1.45	3.0	1.73	0.77	173.21
THYSANOPTERA	6.2	8.95	40.3	6.34	2.83	102.26
HETEROPTERA	4.2	6.07	9.7	3.11	1.39	74.15
HOMOPTERA	12.0	17.34	35.5	5.96	2.66	49.65
HYMENOPTERA	8.6	12.43	18.8	4.34	1.94	50.42
COLEOPTERA	14.8	21.39	26.2	5.12	2.29	34.58
LEPIDOPTERA	0.2	0.29	0.2	0.45	0.2	223.61
DIPTERA	18.2	26.30	147.2	12.13	5.43	66.66
ARACHNIDA	4.0	5.78	7.0	2.65	1.18	66.14

The invertebrates soil fauna was strongly influenced by the nonuniform and rather small soil volume because of its calcareous rocky stones.

Numerical and biomass density of the edaphic fauna (fig. 2) is rather small for Nematoda and Collembola, because of soil small trophicity and of its acidity.

Even though soil conditions are not proper, numerical density of earthworms was 64 individuals m^{-2}, dominant species being *Lumbricus terrestris*. This species is known having a large scale of vertical movement and, surprisingly, it is a dominant species in this calcareous stone soil.

Saprophagous enchytraeids were identified through 3 species of Fridericia genus. There numerical densities showed mean values for this type of ecosystem. Among Acarina, oribatides are dominant, the following species being the most representative: *Oppiella ornata, O. subpectinata, Quadropia quadricarinata*. All these species are microphytophagous ones.

Soil microbiota has presented average values of dehydrogenase activity, specific for humic rendzina, which indicates a decomposing activity with a medium intensity (table 8).

Table 8

Level or dehydrogenase actual and potential activity (mg. T. P. F./100 g dry soil)

Level	spring		summer		autumn	
	actual	potential	actual	potential	actual	potential
L	3.145	5.019	2.704	4.615	7.821	11.896
S_1	2.566	4.712	2.194	3.648	6.657	10.921
S_2	2.062	3.164	1.638	2.547	4.589	6.764

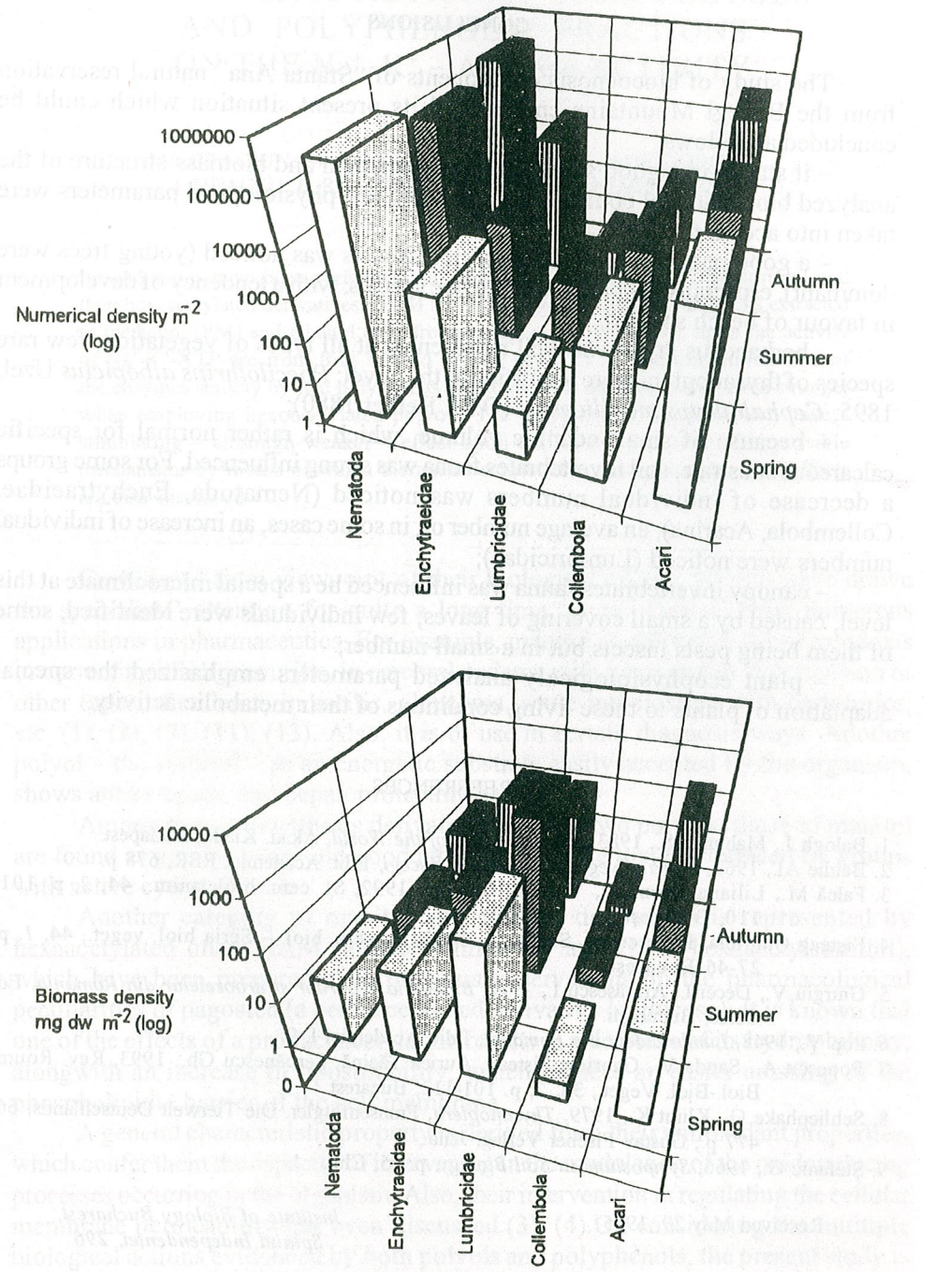

Fig. 2. – Numerical (mean number m^{-2}) and biomass (mg dw m^{-2}) density of invertebrate soil fauna

CONCLUSIONS

The study of biocoenosis components of "Sfânta Ana" natural reservation from the Bucegi Mountains emphasized its present situation which could be concluded as follows:

– it stands in a good status, both as numerical and biomass structure of the analyzed biocoenotical components and some eco-physiological parameters were taken into account:

– a good natural regeneration of tree species was noticed (young trees were dominant), especially for beech and fire tree species, with a tendency of development in favour of beech species;

– herbaceous layer was well represented at all types of vegetation, few rare species of thysanoptera were identified at this level: *Poecilothrips albopictus* Uzel, 1895, *Cephalothrips monilicornis* (O.M. Reuter 1880);

– because of small edaphic volume, which is rather normal for specific calcareous substrate, soil invertebrates fauna was strong influenced. For some groups a decrease of individual numbers was noticed (Nematoda, Enchytraeidae, Collembola, Acarina); an average number or, in some cases, an increase of individual numbers were noticed (Lumbricidae);

– canopy invertebrates fauna was influenced be a special microclimate at this level, caused by a small covering of leaves; few individuals were identified, some of them being pests insects but in a small number;

– plant ecophysiologicaly analyzed parameters emphasized the special adaptation of plants to these living conditions of their metabolic activity.

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THE SPECIFIC ACTION OF SOME POLYOLS AND POLYPHENOLIC FRACTIONS ON THE Na^+–K^+ – ATP-ase ACTIVITY

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Investigations have been performed on the effects of both manitol and sorbitol and their hexaacetylated derivatives (HAM and HAS) and polyphenolic fractions extracted in methanol (FM) and ethanol (FE) from *Asclepias syriaca* leaves, upon the activity of Na^+-K^+-ATP-ase from frog striated muscle fibers. A pronounced stimulation of the enzymes activity by two polyols has been observed, the effect being even stronger when employing hexoacetylated polyols. The polyphenolic fractions, too, have a stimulating – although weaker – effect upon the ATP-ase activity. The possible mechanisms by means of which the agents subjected to testing exercise such effects are also discussed.

Considered from viewpoint of their biological effects, polyols have drawn the specialists' attention for quite a long time, as reflected in their numerous applications in pharmaceutics. For example, manitol is utilized in the prophylaxis or correcting of oligoanuritis, in cerebral oedema with intercranian hypertension or other types of oedema, in ascitis, glaucoma, acute intoxication with barbiturics, etc. (1), (2), (9), (11), (13). Also, it is of use in certain diagnosis ways. Another polyol – the sorbitol – as an energetic substrate easily accepted by the organism, shows antiketogene and hepatoprotecting action.

Among the semisynthesis derivatives of these two polyols, those of manitol are found as good carriers and good attenuators of the toxicity induced by groups with active cytostatic role.

Another category of manitol and sorbitol derivatives is represented by hexaacetylated ones: HAM (hexaacetylmanitol) and HAS (hexaacetylsorbitol), which have been prepared after previous observations on the pharmacological peculiarities of pagosten (a pentaacetylated derivative of glucose). It is known that one of the effects of a product acetylation results in a decrease of its hydrophilicity, alongwith an increase in liposolubility, which induces an easier crossing of the phospholipidic barrier of the cell membrane.

A general characteristic property is derived from their antioxidant properties, which confer them the capacity of intervening in the modulation of the oxidoreducing processes occurring in the organism. Also, their intervention in regulating the cellular membrane permeability has been discussed (3), (4).On considering the multiple biological actions evidenced by both polyols and polyphenols, the present study is devoted to the effects which some of them have on the activity of membranary Na^+ -K^+ - ATP - ase.

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MATERIALS AND METHODS

Experiments have been performed on striated muscle fibers of frog. The frog's Sartorius muscle has been taken over through dissections, that avoided to destroy the membrane integrity. Each experimental variant made use of muscle sample taken from 5 animals, which have been incubated for 3 hours in Ringer solutions, to which the agents considered for the study have been added. The results represent the average values obtained with the 5 pairs of Sartorius muscle against 100 g fresh muscular tissue.

Manitol and sorbitol have been added to the Ringer solution in a concentration of 5 mg/100 ml. HAM and respectively HAS have been solved in propyleneglycol (PGL) prior to being added to Ringer solution, concentrations of 5 mg/100 ml and respectively 0.5 ml PGL being attained. Also, with a view to evidencing the possible specific effect of the solvent, determinations were made with preparations in Ringer with 0.5 % PGL.

The polyphenolic fractions extracted from *Asclepias syriaca* leaves, in either methanol (FM) or ethanol (FE), have been added to the Ringer solution in concentrations of 5 mg dry matter/ml Ringer.

Determination of the Na^+ -K^+ -ATP-ase activity involved dosing of the inorganic phosphate resulted from the ATP hydrolysis under the action of ATP-ase, at room temperature.

EXPERIMENTAL RESULTS

Table 1 shows that in the presence of manitol, the activity of ATP-ase is highly stimulated, i.e. 27 % higher than in normal Ringer (taken as reference). In the presence of HAM, the enzyme activity is even more strongly stimulated (47.8% higher than that of the reference sample), although PGL in which HAM has been previously dissolved, has a slight inhibitory action upon ATP-ase activity.

Table 1

Effects of polyols and polyphenols on the Na^+ -K^+ - ATP- ase activity

Experimental conditions	ATP-ase activity (mg P.i./100 g)	Difference vs. the reference (as %)
R.N	116.5 ± 4.1	0
Manitol	148.0 ± 1.8	+ 27.0
RN + PGL	112.4 ± 3.2	- 3.5
RN + PGL + HAM	172.2 ± 3.1	+ 47.1
Sorbitol	128.5 ± 3.6	+ 10.3
RN + PGL + HAS	139.7 ± 5.3	+ 20.0
RN + FM	126.9 ± 2.1	+ 8.9
RN + FE	136.0 ± 4.0	+ 16.7

In its turn, sorbitol stimulates, too, the activity of Na^+ -K^+ -ATP-ase, although to a lower extent than manitol does, while its hexaacetylated derivative (HAS) has a much higher stimulating action than sorbitol.

Polyphenols, as well, stimulate the Na^+ -K^+ -ATP-ase activity, fraction extracted in ethanol (FE) having, nevertheless, an almost twice stronger effect than the fraction extracted in methanol.

DISCUSSIONS AND INTERPRETATIONS

As generally known, the unequal distribution of the Na^+ and K^+ ions on one side and another of the cell membrane is maintained by the activity of the Na^+ -K^+ -ATP-ase, which expels the Na^+ that penetrates passively into the cells, thus reintroducing the K^+ that leaves the cells, again passively, in the direction of the concentration gradient. Under normal physiological conditions, for each molecule of hydrolyzed ATP, three Na^+ and two K^+ are taken out and, respectively introduced into the cell (7), (14), (16).The uneveness of the active movements of K^+ and Na^+ may lead directly to a clear-cut charge separation through the membrane and, consequently, to the membrane hyperpolarization (10), (15).

Mention should be already made of the fact that both manitol and sorbitol stimulate considerably the activity of Na^+ -K^+ -ATP-ase, the manitol effect being, nevertheless, almost three times higher than that of sorbitol. However, in the case of their hexaacetylated derivatives, a double stimulating effect upon the ATP-ase activity has been recorded, as already discussed. Once accepted the idea that both manitol and sorbitol intensify the active transport through stimulation of the cellular metabolism, thus increasing the transport energy source (polyols being known for their energetic value), the observation may be made that acetylation of such products still increases their energetic value.

Also, manitol and sorbitol have hypepolarizing effect on the cell membrane (unpublished results), which can be well correlated with that of stimulating the ATP-ase activiy, if considering that the Na^+/K^+ active transport in a 3/2 ratio may be electrogenic. More than that, such a correlation is also supported by the observation that the hyperpolarizing effect of manitol is stronger than that of sorbitol, while hexaacetylation of these polyols causes an increase not only of the activity of ATP-ase, but also of the hyperpolarizing effect.

The fact that polyols' hyperpolarizing effect is not as strong as that manifested upon the ATP-ase's activity permits the conclusion that, apart from the stimulating effect of the Na^+ and K^+ active transport by increasing the source of metabolic energy, the polyols considered for the study might have another effect, as well, on the level of the cell membrane, namely, that of an increased passive permeability for Na^+ and K^+. In such a case, the increase of the Na+, and K^+ passive flows through the membrane should represent another stimulating factor of the ATP-ase activity, as this enzyme has been found (5), (6), (12) promptly stimulated by the increase of the Na^+ intracell and, respectively, of K^+ extracell concentration. Consequently, the increase of the membrane passive permeability might induce lowering of the electrogenic (hyperpolarizing) effect of the Na^+ and K^+ active transport stimulation.

The polyphenolic fractions considered in the study have, too, a stimulating effect on the ATP-ase activity, the ethanolic fraction (FE) being more efficient than the methanolic one (FM). Nevertheless, in such a case, no hyperpolarization but – on the contrary – depolarization of the membrane occurs.

As ATP-ase is known as being very sensible to the increase of the Na^+ intracell concentration, and of the K^+ extracellular one, as well, our conclusion is that it is not through increasing the metabolic substrate that polyphenols intensify the activity of this enzyme, but through the increase of the membrane passive permeability and, consequently, of the Na^+ and K^+ passive flows through the membrane, in the direction of their concentration gradients.

Such an effect induces both membrane depolarization and stimulation of the ATP-ase activity.

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