A c-mitotic action is also detected by the rise in metaphase number. Since this c-mitotic action, earlier described by Ceriotti³ on animal material, is absent in other protein synthesis inhibitors such as in anisomycin or cycloheximide¹⁰, it may be that this effect is a secondary one not related to the primary inhibition which works on protein synthesis.

Action on nucleologenesis. We analysed the frequency of binucleate cells with an organized nucleolus at different times after the caffeine treatment, both in control meristems, and in meristems treated with 1 µg/ml narciclasine. This study was especially valid since nucleologenesis takes place during or immediately after the caffeine action, and

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- G. Ceriotti, Nature 213, 595 (1967).

the binucleate cells are made up of a synchronous population. The analysis shows that narciclasine increases the frequency of cells that have finished nucleologenesis, in comparison to control meristems. The accelerated kinetics of nucleologenesis induced by narciclasine, is very similar to the action of other protein synthesis inhibitors upon nucleologenesis, for example cycloheximide 10, and anisomycin¹¹. We postulate the importance of the nucleologenesis test for detecting metabolic responses in eucariotic cells, because the nucleologenesis test shows clearly and simultaneously whether there is any inhibition of protein synthesis produced by nucleologenesis acceleration, or whether the drug is inhibiting RNA synthesis and then blocking the nucleologenesis.

- L. Carrasco, M. Fresno and D. Vázquez, FEBS Lett. 52, 236
- A. Jiménez, L. Sánchez and D. Vázquez, FEBS Lett. 55, 63 (1975).
- F. Piozzi, C. Fuganti, R. Mondelli and G. Ceriotti, Tetrahedron 24, 1119 (1968).
- M.E. Fernández-Gómez, J.C. Stockert, J.F. López-Sáez and G. Giménez-Martín, Stain Techn. 44, 48 (1969)
- G. García-Herdugo, M.E. Fernández-Gómez, J. Hidalgo and J.F. López-Sáez, Exptl Cell Res. 89, 336 (1974).
- A. González-Fernández, G. Giménez-Martín, M.E. Fernández-Gómez and C. de la Torre, Exptl Cell Res. 88, 163 (1974).
- M.E. Fernández-Gómez, C. de la Torre and G. Giménez-Martín, Cytobiologie 5, 117 (1972). G. Giménez-Martín, C. de la Torre, M.E. Fernández-Gómez
- and A. González-Fernández, J. Cell Biol. 60, 502 (1974).

Phospholipid composition of Dipylidium caninum

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Summary. The phospholipid composition of Dipylidium caninum has been studied. Chloroform-methanol-soluble fraction amounted to 2.4% and phospholipids to 0.5% of the wet weight of the parasite. Phosphatidyl choline and phosphatidyl ethanolamine represented the bulk of the phospholipids, whereas phosphatidyl serine, phosphatidyl inositol, lysolecithin and lysophosphatidyl ethanolamine were present in minor amounts. Sulfatides were also identified in this parasite.

The dog tapeworm Dipylidium caninum is a parasite belonging to the class of cestodes. It inhabites the intestine and is responsible mainly for the intestinal problems like abdominal discomfort, indigestion, hunger pain, diarrhoea and constipation. At present, our knowledge of the nature of biochemical components of parasites in general is very limited². As part of a detailed chemical and immunological study of the parasite, the lipids of the adult *Dipylidium* caninum have been investigated. This note pertains to the phospholipid composition of this parasite.

Materials and methods. Dipylidium caninum tapeworms were removed from the intestinal tract of dogs immediately following the sacrifice and were placed in physiological saline. After repeated washing, they were blotted on filter paper and weighed. Extraction, purification and isolation of phospholipids and their separation, identification and characterization were as described previously³⁻⁵. Cerebrosides and sulfatides were separated on silica gel G plates in chloroform-methanol-ammonia (80:20:0.4, vol/vol). Plates

were developed for several h to achieve better resolution of these lipids. Sugar-containing lipids were identified with alpha naphthol sulfuric acid spray.

Results and discussion. The total chloroform-methanol soluble fraction of D. caninum amounted to 2.4% and phospho-

lipids to 0.5% of the wet wt of the parasite. When these lipids were subjected to TLC, 8 components were identified with iodine vapors (figure). Of these components, 6 were found to be phosphorus-positive when sprayed with molybdate spray. These phospholipids were identified as lysophosphatidyl choline, phosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine and phosphatidyl ethanolamine. The presence of these phospholipids was further confirmed by cochromatography with authentic standards. The phospholipid composition of D. caninum is presented in the table. It makes clear that phosphatidyl ethanolamine and phosphatidyl

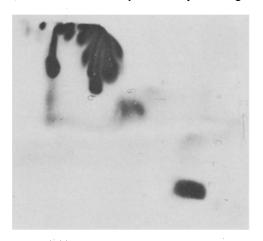
Phospholipids of Dipylidium caninum

Phospholipids	% of total phospholipids*
Lysolecithin	3.5 ± 0.5
Phosphatidyl choline	54.5 ± 2.5
Lysophosphatidyl ethanolamine	4.4 ± 0.8
Phosphatidyl inositol	7.1 ± 1.2
Phosphatidyl serine	10.4 ± 1.4
Phosphatidyl ethanolamine	20.1 ± 1.6

^{*}Each value is the mean ± SD of 3 different determinations.

choline represent the major phospholipids of this parasite. On chromatography of these lipids in chloroform-methanol-ammonia (80:20:0.4, vol/vol) 1 sugar positive component was identified with alpha naphthol sulfuric acid. This component was identified as sulfatides with authentic markers. The presence of this lipid was further confirmed by its chromatography with the respective glycolipid.

The chemical constitution of the phospholipids has received detailed attention only in recent years. In general,



Two-dimensional thin layer chromatogram of lipids of Dipylidium caninum. Chloroform-methanol-water (65:25:4, vol/vol) was used in the first direction and chloroform-methanol-acetic acid-water (50:35:8:1, vol/vol) in the 2nd direction. Components are visualized with iodine vapors: 1, lysolecithin; 3, phosphatidyl choline; 4, lysophosphatidyl ethanolamine; 5, phosphatidyl inositol; phosphatidyl serine; 7, sulfatide; 8, phosphatidyl ethanolamine; 9, neutral lipids.

it appears that the major phospholipid group known from vertebrates occur also in parasites. The phospholipids detected in D. caninum, namely lysolecithin, phosphatidyl choline, lysophosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine and phosphatidyl ethanolamine were the same as those reported in Hymenlepis diminuta⁶ and Taenia hydatigena⁷. They differed, however, from those of T. saginata⁸ and T. taneniae formis⁹ in that the former contained no lecithin, and the latter no phosphatidyl inositol. Ethanolamine and choline containing lipids comprise about 74% of total phospholipids similar to Ascaris lumricoides¹⁰ and Ancylostoma caninum¹¹. The identification of sulfatides in D. caninum is similar to its presence in nematodes and trematodes³. The function of these lipids in the metabolism of D. caninum is unknown and warrants further work.

- Author for reprint request.
- T. Von Brand, in: Biochemistry of Parasites, Academic Press, New York 1966.
- G.K. Khuller and P.J. Brennan, Biochem. J. 127, 369 (1972).
- G.K. Khuller and D. Subrahmanyam, Experientia 24, 851 (1968)
- S.K. Jain and D. Subrahmanyam, Indian J. Biochem. Biophys. 13, 155 (1976).
- C.D. Ginger and D. Fairbairn, J. Parasit. 52, 1097 (1966).
- A.I. Kassis and G.T. Frayha, Comp. Biochem. Physiol. 46B,
- S. Cmelik and Z. Bartl, Physiol. Chem. 305, 170 (1956). M.J. Thompson, E. Mosettig and T. Von Brand, Exptl Parasit. 9, 127 (1960).
- D. Subrahmanyam and S. Venkatesan, Comp. Biochem. Physiol. 25, 733 (1968).
- G.K. Khuller, S.K. Jain and V.K. Vinayak, Experientia 33, 1585 (1977).

Chaetogaster limnaei K von Baer 1872 on Lymnaea tomentosa: Ingestion of Fasciola hepatica cercariae

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Summary. The fresh water mollusc, Lymnaea tomentosa, the intermediate host of the liver fluke is heavily infested under natural conditions with ectocommensal annelid, Chaetogaster limnaei. These annelids which destroy the larval stages of Fasciola hepatica have been observed in the laboratory. The association of C. limnaei with the snail intermediate host is of value in exploiting a control measure against economically important liver fluke disease.

Lymnaea tomentosa is the intermediate host of the liver fluke, Fasciola hepatica, in Australia. Its ecology has been studied in detail² and many biological and chemical measures aimed at controlling populations of this snail have been suggested³. The former include artificially creating competition with other snails, encouraging the predatory habits of sciomycid flies and rearing ducks and other water fowl in snail localities. A competition between the larvae of echinostomes and *F. hepatica* in snails also seems to lessen the prevalence of *F. hepatica* cercariae (Boray; personal communication). Although such methods have been found to be quite successful in controlling lymnaeid snails in other parts of the world, none were found successful for the control of L. tomentosa in Australia.

An alternative biological control measure for fascioliasis might derive from Chaetogaster limnaei, an oligochaete annelid, which is found attached to the bodies of snails. This species feeds on protozoa, rotifers, nematodes, crustaceans, water mites and chironomid larvae⁴. There are also reports that they feed on miracidia^{2,5,6} and cercariae^{7,8}. Becklund⁸ and Khalil⁶ considered that these annelids play a

part in limiting the distribution of trematodes. This view seems to be supported by the fact that a) Boray² found few or no sporocysts of F. hepatica in snails infested with this annelid, and b) Khalil⁶ noted an inverse relationship between the prevalence of F. hepatica infections in snails and the number of C. limnaei present. C. limnaei may also kill snails since Bendezu9 found many snails dying due to excessive protozoan accumulation in the intrashell space following the death of the annelid. This report provides additional observations of the feeding behaviour of C. limnaei on cercariae of F. hepatica.

Experimental observations. Adult L. tomentosa collected from Brooks' Creek, which flows through sheep grazing areas near Canberra, Australia, were examined for the presence of larval stages of F. hepatica. It was observed that all snails carried heavy infestations of C. limnaei (ranging from 70 to 90 per snail) on their external surfaces and shells, but they were apparently not infected with trematodes. 88 snails were each exposed in the laboratory to 20 miracidia of F. hepatica. During the next 2 months many snails died and most of them were devoid of any larval