somes and lysed preparations in oxygen, nitrogen and nitrous oxide up to 30 min did not alter membrane permeability and enzyme activity in comparison with exposure to air.

a) Intact lysosomes (table 1). Irradiation in air induces a significant release of acid phosphatase in the medium which achieves the highest value at 12,600 rads dose ($p < 0.001$) and keeps constant up to 25,200 rads ($p < 0.001$). Lysosomes irradiated in oxygen and nitrogen show the same membrane permeability as the corresponding not-irradiated controls in the dose range from 0 to 12,600 rads. Irradiation in nitrous oxide in the same range induces a significant decrease of acid phosphatase activity detectable in supernatants from intact lysosomes, reaching its minimum value at 6300 rads dose ($p < 0.01$), keeping constant up to 12,600 rads ($p < 0.001$) (figure 1).

b) Free enzyme activity (table 2). Behaviour of enzyme activity after irradiation in air, oxygen and nitrogen is identical, showing 10% decrease at 12,600 rads dose in comparison with controls. The effects of irradiation in nitrous oxide are much more relevant: detectable enzyme activity decreases to 79% at 3150 rads dose ($p < 0.05$), falling to 50% at 12,600 rads as compared to not-irradiated controls ($p < 0.001$) (figure 2).

Discussion. Our data on the effects of radiation on free acid phosphatase in lysed lysosomal preparations show an identical pattern of enzyme activity in air, oxygen and nitrogen, i.e. a slight decrease to about 90% in comparison with controls. Enzyme inactivation is stronger after irradiation in nitrous oxide, reaching 50% at 12,600 rads.

So far as intact lysosomes are concerned, an increased acid phosphatase release after irradiation in air significant in comparison with controls was noted. Following irradiation in nitrogen and oxygen, no difference was observed in detectable enzyme activity between irradiated and not-irradiated samples. Acid phosphatase activity sharply decreased after irradiation in nitrous oxide.

Linking together the 2 series of results, we may conclude that increased enzyme release after irradiation in air is really the expression of an increased lysosomal permeability; in contrast, radiation appears to stabilize lysosomal membrane when working in oxygen and nitrogen. Sharp decrease of detectable enzyme activity in the supernatant from intact lysosomes irradiated in nitrous oxide may be ascribed to immediate inactivation of newly released acid phosphatase: in fact free enzyme activity in lysed lysosomal preparations irradiated in the same gas shows a significant decrease probably induced by inactivating radicals. However, one cannot exclude the hypothesis that enzyme inactivation may occur inside the lysosome. Our results on the effects of radiation on mouse liver lysosomes, agree with studies on rat liver, as regards irradiation in air, nitrogen and oxygen; these data on irradiation in nitrous oxide are of some interest and might be a first approach for the characterization of free radicals involved in lysosomal damage by X-rays.

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2 Y.E. Rahman and A. Lindebaum, Radiat. Res. *21*, 575 (1964).
3 I.D. Desai, P.L. Sawant and A.L. Tappel, Biochim. biophys. Acta *86*, 277 (1964).
4 J.W. Harris, Radiat. Res. *28*, 766 (1966).
5 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
6 M. Andersch and A.J. Szczypinski, Am. J. clin. *17*, 571 (1947).

Narciclasine, an inhibitor of protein synthesis. Action on *Allium cepa* L. root meristems

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Summary. Shortening of nucleologenesis time in a synchronous cell population, labelled as binucleate, by a caffeine pulse and fall in frequency of prophases are related to narciclasine inhibition of protein synthesis in *Allium cepa* L. meristems.

Narciclasine, an alkaloid present in *Narcissus* bulbs, is characterized by its antitumoral action on sarcoma 180 cells in an ascites form; it also inhibits root growth in wheat grains[3]. Narciclasine inhibits peptidic bond formation by preventing the union between the 3'-OH end of the donor substrate and the peptidil-transferase centre of the 60S subunit in eucariotic ribosomes[4,5].

We have isolated the alkaloid, and proved its inhibitory effect on protein synthesis in *Allium cepa* L. root meristems. We postulate that the analysis of mitosis and nucleologenesis under a drug may constitute a rapid and effective screening method for new potential inhibitors of protein synthesis.

Material and methods. We used the Piozzi et al.[6] method to isolate narciclasine from *Narcissus pseudonarcissus*, King Alfred variety bulbs. Root meristems from *Allium cepa* L., growing in tap water under continuous aeration at a temperature of 25±0.5 °C, were used. The roots were treated with 0.1, 0.5, 1 and 10 µg/ml (3×10^{-7}, 1.5×10^{-6}, 3×10^{-6} and 3×10^{-5} M) of narciclasine for 6 h. The alkaloid was previously dissolved in absolute ethanol because of its insolubility in water, the final ethanol concentrations being 3% at 10 µg/ml and 0.03% at 0.1 µg/ml of narciclasine.

To evaluate protein synthesis, some cut roots were incubated for 30 min in 5 µCi/ml ^{3}H leucine (sp. act. 18 Ci/mmole), and some others for 30 min in 10 µCi/ml ^{3}H lysine (sp. act. 10 Ci/mmole). After washing the roots

Effect of narciclasine on protein synthesis. Incorporation after 30-min pulses with ^{3}H leucine and ^{3}H lysine in control and treated meristems. Each value represents the mean in cpm of 10 meristems ± SE

	^{3}H Leucine	^{3}H Lysine
Control	621.17± 8.5	556.39±4.46
0.1 µg/ml	590.17± 9.7	370.45±5.39
0.5 µg/ml	316.94±12.1	264.95±3.29
1 µg/ml*	146.75± 5.0	354.50±3.02
10 µg/ml*	314.71±10.1	345.10±9.24

*These roots always developed heavy bacterial contamination. The values includes protein synthesis in the contaminating microorganisms.

several times, the 2nd mm from the root tips were cut and fixed in 3:1 ethanol acetic acid. Then the root segments were incubated at 37 °C for 1 h to evaporate any fixer residue, and were introduced into a scintillation solution (20% butanol, 5% PPO and 0.2% POPOP in toluene) containing 30% soluene-350 as a tissue solubilizer. After keeping the root segments in the solution for 24 h at 37 °C, the incorporated radioactivity was measured in a liquid scintillator counter.

The assay for nucleogenesis included 1 h with 0.1% caffeine simultaneously with 1 µg/ml of narciclasine, followed by narciclasine on its own. Onion roots were fixed in 3:1 ethanol acetic acid stained in acetic orcein and squashed. Nucleolus study involved formol hydroquinone root fixation, 1% $AgNO_3$ impregnation and squashing[7].

Results and discussion. The results in figure 1 show that, from the beginning of the treatment, narciclasine inhibits root growth at concentrations of 1 µg/ml and higher.

Protein synthesis inhibition produced by narciclasine in other cell systems[3-5] is confirmed in onion bulbs. The table shows that, at 0.5 µg/ml, there is a 50% reduction in the incorporation of ^3H aminoacids. The alkaloid inhibits the protein synthesis in onion at a concentration (3×10^{-6} M), about 66% lower than the minimum inhibitory concentration (10^{-4} M), found on HeLa and Krebs II ascites cells by Jiménez et al.[5]. Incorporation data in meristem cells at 1 and 10 µg/ml of narciclasine fail to show the inhibition of protein synthesis, since an important bacterial contamination consistently appeared in these roots making it difficult to discern which portion of the incorporation corresponded to the meristem.

Action on cycle kinetics. Analysis of mitotic index during treatment shows that narciclasine has an antimitotic action on all the concentrations studied (figure 2). We studied the developing of phase indexes during the first h of narciclasine treatment. The results during treatment with 1 µg/ml narciclasine are represented in figure 3. An inhibition in prophase entrance was evidenced by the prophasic index fall, probably due to an altered sensitivity during early G_2, where progression in the cell cycle relies on the protein synthesis[8]. At the higher concentration, the mitotic index fails to fall in the first h, probably due to the reversal of prophase which potent protein synthesis inhibitors produce[9].

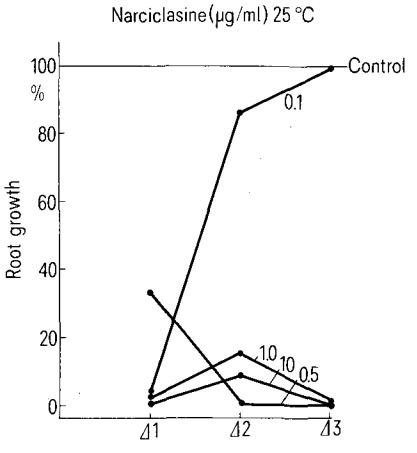

Fig. 1. Root growth in *Allium cepa* L. bulbs after different narciclasine concentrations, expressed as percent of root growth in untreated control roots. Δ_1, Δ_2 and Δ_3 correspond to 24, 48 and 72 h of treatment. Each point represents the mean value for 3 different meristems.

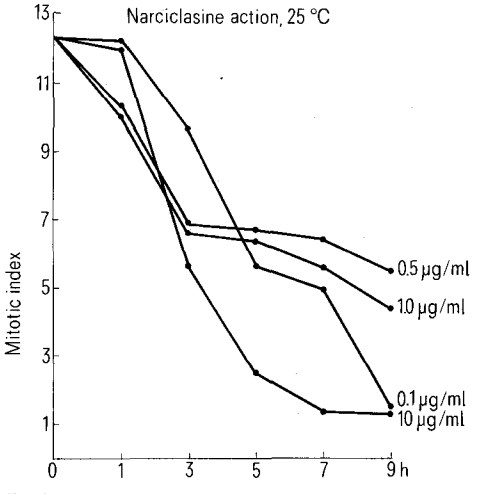

Fig. 2. Mitotic index in root meristems growing under steady state conditions when introduced into different narciclasine concentrations. Each point represents the recorded value for at least 1000 cells per meristem. 3 meristems were studied at each fixing time.

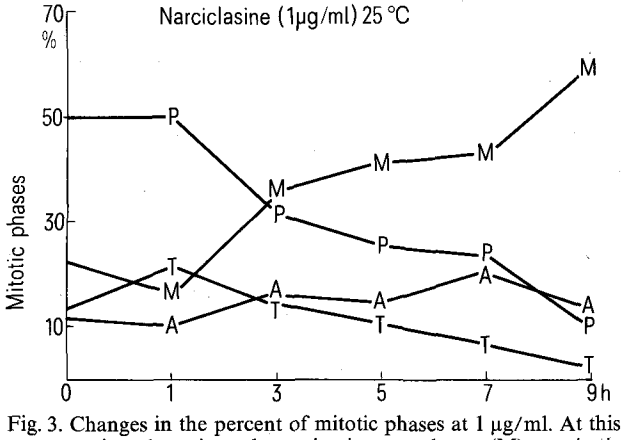

Fig. 3. Changes in the percent of mitotic phases at 1 µg/ml. At this concentration there is a sharp rise in metaphases (M), c-mitotic action. The percent of prophases (P) falls, as well as that of telophases (T). Anafases (A).

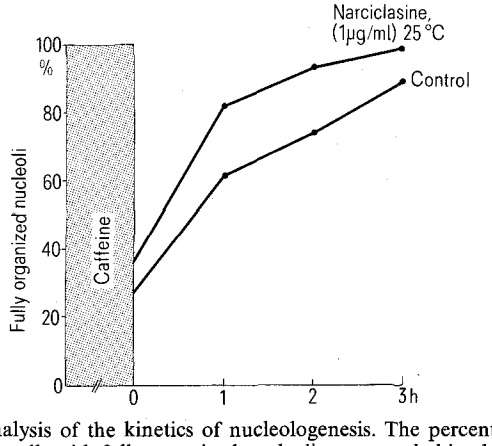

Fig. 4. Analysis of the kinetics of nucleologenesis. The percent of binucleate cells with fully organized nucleoli was recorded in about 200 binucleate cells per meristem, each point representing the mean value recorded for 3 different meristems. 1 µg/ml narciclasine produces an acceleration of the nucleologenesis rate as compared to control binucleate cells.

A c-mitotic action is also detected by the rise in metaphase number. Since this c-mitotic action, earlier described by Ceriotti[3] on animal material, is absent in other protein synthesis inhibitors such as in anisomycin or cycloheximide[10], 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 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 anisomycin[11]. 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.

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3 G. Ceriotti, Nature *213*, 595 (1967).
4 L. Carrasco, M. Fresno and D. Vázquez, FEBS Lett. *52*, 236 (1975).
5 A. Jiménez, L. Sánchez and D. Vázquez, FEBS Lett. *55*, 63 (1975).
6 F. Piozzi, C. Fuganti, R. Mondelli and G. Ceriotti, Tetrahedron *24*, 1119 (1968).
7 M.E. Fernández-Gómez, J.C. Stockert, J.F. López-Sáez and G. Giménez-Martín, Stain Techn. *44*, 48 (1969).
8 G. García-Herdugo, M.E. Fernández-Gómez, J. Hidalgo and J.F. López-Sáez, Exptl Cell Res. *89*, 336 (1974).
9 A. González-Fernández, G. Giménez-Martín, M.E. Fernández-Gómez and C. de la Torre, Exptl Cell Res. *88*, 163 (1974).
10 M.E. Fernández-Gómez, C. de la Torre and G. Giménez-Martín, Cytobiologie *5*, 117 (1972).
11 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 limited[2]. 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 previously[3-5]. 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 phospholipids 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.