The saturation values were calculated from the double reciprocal plot of the data according to Bishop[10].

In the table the percentage of ribosomal DNA is shown for the plant species investigated. The number of genes coding for 25 S and 18 S rRNA was calculated for the haploid DNA content taking the molecular weight for 25 S rRNA as 1.25×10^6 daltons and for 18 rRNA as 0.64×10^6 daltons[7] or correspondingly the number of nucleotides as 5700. The amount of rDNA given in the table does not represent the actual total rDNA percentage, because it takes no account of the DNA transcribed in those parts of the prescursor rRNA molecule which are cut off during processing and of the non-transcribed spacer DNA. The restriction endonuclease pattern of soybean rDNA (Friedrich, Hemleben, Meagher and Key, in preparation) suggests that the amount would increase by about 37% provided that the non-transcribed spacer has the same size in Compositae.

The number of 5 S genes in the 4 species in which it was determined is about 10,000. There are roughly 3 times as many 5 S genes as there are ribosomal RNA cistrons for the large rRNAs. The 4 determinations allow no further conclusions, except that there is no proportionality between the number of 5 S genes and that of the ribosomal cistrons.

Numbers for the latter have been determined in 8 related species. For 6 of these, the exact nature of their relationship is fairly well established[5]. *Microseris laciniata* can be regarded as ancestral to the 4 annual species of *Microseris* and possibly to *Agoseris*. The main trend among these species is a reduction of genome size. In all of these except *M. douglasii*, the number of ribosomal cistrons is roughly proprotional to the total genome size and constitutes about 0.65% of the total genome. In *M. douglasii* the proportion is roughly double this amount. The simplest explanation for this is based on gene numbers. While 3 of the annual *Microseris* species have lost about half of their ribosomal genes, *M. douglasii* has retained (or regained) the ancestral number of 3000. The repetition frequency of the ribosomal genes make them part of the intermediate renaturing fraction as defined in the earlier paper[5]. Their behavior, however, is exactly the opposite of this fraction. While *M. douglasii* is the only annual that has reduced the amount of intermediate renaturing DNA relative to the slowly renaturing fraction by one-half, it is also the only one that has retained all of the ribosomal cistrons. Strikingly enough, *Agoseris grandiflora*, derived from *M. laciniata* by an independent route of evolution and still a perennial with a genome larger than that of *M. douglasii*, seems to have undergone about the same proportional changes among its genome fractions as *M. douglasii*. The genus *Pyrrhopappus* is only distantly related to the other species studied here. In terms of absolute numbers of ribosomal genes, it is similar to them. The 2 species studied appear to differ by a duplication of the ribosomal gene numbers. *P. carolinianus* with clearly more nuclear DNA than *P. multicaulis*, has the smaller number of ribosomal cistrons.

The results reinforce our earlier conclusion that genome evolution in the Microseridinae progresses by doublings and halvings of bulk fractions relative to one another. They add the observation that the ribosomal cistrons behave as an independent fraction. Particularly with respect to the bulk of the intermediate renaturing DNA, they can change their proportion, and they seem to do it by doublings and halvings. There is no obvious pattern concerning the number of ribosomal genes in all angiosperms[11]. The detailed investigation of a group of closely related species undertaken here shows that within such a group the evolution of ribosomal gene numbers follows a pattern that complements and supports our conclusions drawn from renaturation kinetics of bulk DNA fractions.

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In vitro studies on lysosomes radiosensitivity in different gaseous atmospheres

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Summary. Radiosensitivity of lysosomes was investigated in vitro in different gaseous atmospheres. Results show a higher sensitivity when X-irradiation was performed in nitrous oxide. Possible reasons for this observation are considered.

It is well known that lysosomes are very radioresistant in vivo[2]; however, biochemical tissue changes following X-irradiation make the fine analysis of experimental results difficult. Therefore many authors have investigated radiation effects in vitro on lysosomal suspensions, usually prepared from rat liver or rabbit neutrophyle granulocytes[3,4]. Aim of this research is the evaluation of the behaviour of mouse liver lysosomes after irradiation in different gaseous atmospheres (air, nitrogen, oxygen, nitrous oxide).

Materials and methods. Balb/c inbred mice, 3 months old, fasting for 24 h, were killed by bleeding. Homogenized livers were diluted with sucrose (8.5%; pH 7.2), 4 ml/g liver. Sediment from a first centrifugation at 2500 rpm for 25 min was discarded and supernatant restored to the starting volume and stored at 4 °C. Protein concentration was measured by the method of Lowry et al.[5]. Lysosomal suspension was subdivided in 2 parts: the 1st was used for the study of acid phosphatase release; the 2nd was lysed by Triton X 100 at a final concentration of 0.2% in order to

investigate the direct effects of radiation on enzyme activity. All the operations were carried out at 4 °C.

Controls were set up incubating lysosomes in different gaseous atmospheres for 30 min, so as to exclude direct effects of the gases on membrane permeability and enzyme activity.

Irradiation: both lysosomal preparations were placed in plastic tubes (1 ml per tube) and X-irradiated (220 kV, HVL 0.8 mm Cu, 840 rads/min) in ice bath in a gas tight plexiglass container filled with gas. Gases used were oxygen, nitrogen, nitrous oxide and air; oxygen, nitrogen and nitrous oxide, 99.99% pure, were purchased from S.I.O. (Italy). Radiation doses ranged from 0 to 12,600 rads; in some experiments 25,200 rads dose was reached; controls for intact lysosomes suspension were: a) 2 samples where total lysis was induced by Triton X 100 at a final concentration of 0.2%; b) 2 not-irradiated samples for every gaseous atmosphere.

Controls for lysed lysosomal preparation consisted only of not-irradiated samples. After irradiation tubes were centrifuged at 12000 rpm for 20 min and supernatant divided in 0.1 ml fractions.

Acid phosphatase activity was assayed according to Andersch et al.[6]: 0.1 ml of supernatant was incubated for 30 min at 37 °C with 1 ml of 50 mM citrate buffer pH 4.8 containing 5.5 mM natrium-p-nitrophenylphosphate. Reaction was stopped by adding 10 ml of 0.02 M NaOH. Optical densities were estimated by Saitron Monospec 10 B spectrophotometer at 405 nm and converted into enzyme activity units (mU/ml). Statistical analysis was performed by Student's t-test. We refer to acid phosphatase present in lysed lysosomal preparations as free enzyme activity. Results are expressed as a percentage ratio between enzyme activity units detectable in the irradiated samples and the corresponding not-irradiated controls.

Results. Effects of incubation in air up to 6 h at different temperatures on lysosomes permeability and free enzyme activity were preliminarily tested. Results showed both increasing enzyme release from intact lysosomes and enhanced inactivation of free acid phosphatase with rising incubation temperature: on this basis further experiments were carried out at 4 °C. Incubation of both intact lyso-

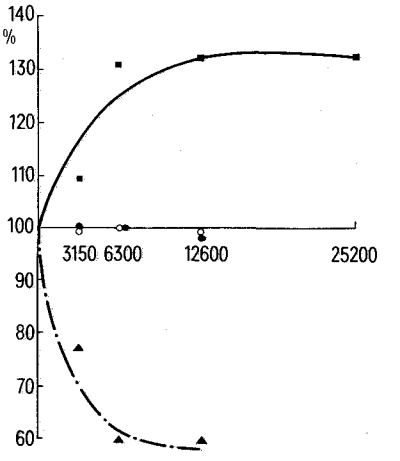

Fig. 1. Effects of X-irradiation on acid phosphatase release from intact lysosomes in air, oxygen, nitrogen and nitrous oxide. Results are expressed as in table 1.
■, Air; ○, O_2; ●, N_2; ▲, N_2O.

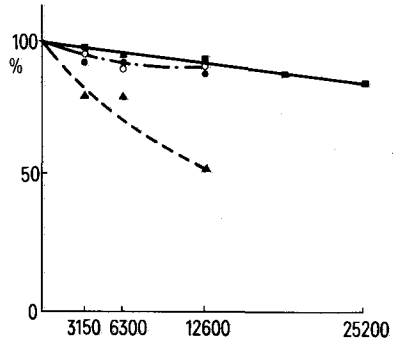

Fig. 2. Effects of X-irradiation on acid phosphatase activity detectable in lysed lysosomal suspensions (free enzyme activity) in air, oxygen, nitrogen and nitrous oxide. Results are expressed as in table 1.
■, Air; ○, O_2; ●, N_2; ▲, N_2O.

Table 1. Effects of X-irradiation on acid phosphatase release from intact lysosomes in air, oxygen, nitrogen and nitrous oxide. Results are expressed as a percentage ratio between enzyme activity units detectable in the irradiated samples and the corresponding not-irradiated controls.

rad / gas	0	3150	6300	12600	25200
AIR	100	110	132	132	132
O_2	100	100	110	100	
N_2	100	95	100	100	
N_2O	100	78	62	61	

Table 2. Effects of X-irradiation on acid phosphatase activity detectable in lysed lysosomal suspensions (free enzyme activity) in air, oxygen, nitrogen and nitrous oxide. Results are expressed as in table 1.

rad / gas	0	3150	6300	12600	25200
AIR	100	98	95	91	85
O_2	100	100	87	93	
N_2	100	100	89	93	
N_2O	100	79	79	50	

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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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.