## Extracellular $Ca^{2+}$ and $Mg^{2+}$ and Cellular Metabolism of Phospholipids

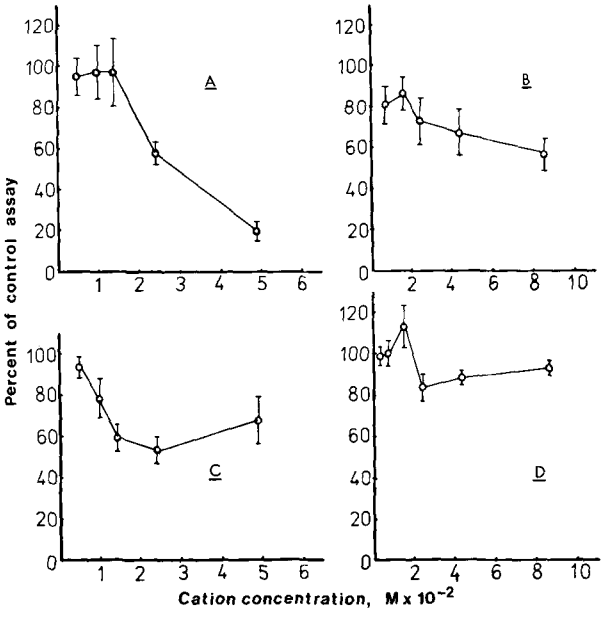
The experimental observations of several authors indicate that $Ca^{2+}$ influences the morphology, the physiology and the growth of the cell[1–5]. In the present work, the effects of $Ca^{2+}$ and $Mg^{2++}$ on the metabolism of the cell phospholipids have been studied.

6–7-day-old Novikoff hepatoma ascites growths maintained by weekly transplantation in Holzman rats, and liver cells from normal animals were used. The ascites cells obtained from 3-animal groups were washed 5 times with cold saline, and the liver cells were obtained, according to the technique described elsewhere[6]. The incorporation of $^{32}P$ was studied by incubation of the cells in a medium containing, 0.9 g glucose, 6.5 g NaCl, 0.4 g KCl, 8.7 g HEPES (N-2-hydroxyethylpiperazin-N-2 ethansulfonic acid), 10 ml 100 × Minimal Eagle Vitamins, 20 ml 50 × Minimal Eagle Amino Acids, 6 ml of 5% glutamine and 500,000 U penicillin dissolved in 1 l of distilled water, brought to pH 7.4 with 2 *N* NaOH and filtered through millipore membrane filter (0.2 μm pore size). The concentrations of $Ca^{2+}$ and $Mg^{2+}$ were adjusted by addition of the required amount of isotonic $CaCl_2$ and $MgCl_2$ solutions. In each series of experiments, identical amounts of cells suspended in saline (10 to 20 mg of cell protein in each tube) were added with a final volume of 5 ml. 250 μCi $^{32}P$-orthophosphate in 5 μg carrier phosphorus were added to each tube, and then incubated with continuous shaking at 38 °C for 6 h. After incubation 0.1 volume of 100% (w/v) trichloracetic acid solution was added to each tube. After standing overnight at 4 °C, centrifuged and washed 5 times with 5% trichloracetic acid, the phospholipids were extracted from the residual material by means of ethanol:chloroform (1:1). An aliquot of the extract was used for $^{32}P$ measurement in a liquid scintillation counter. The content of lecithin and cephalin in the extract was determined by fractionation on Alumina columns[7]. The residual material after phospholipid extraction containing the nucleic acids and phosphoproteins was digested with 1 *N* NaOH, and $^{32}P$ counted as described above.

The Figure shows the results corresponding to 7 series of experiments with ascites cells and 5 series of experiments with liver cells. They show that increasing concentrations of $Ca^{2+}$ produce a remarkable inhibition of $^{32}P$ incorporation into ascites cells phospholipids. Thus, with a 0.05 *M* $Ca^{2+}$ this uptake is only 1/5 (80% inhibition) of that corresponding to the control tube (without $Ca^{2+}$ addition) (Figure A). On the contrary, the same concentration of $Mg^{2+}$ produces only a slight inhibition (Figure B). The $^{32}P$ incorporation into liver phospholipids is not so greatly affected by a high concentration of $Ca^{2+}$, and a plateau of about 40% inhibition was observed for high concentrations (Figure C). As in the case of ascites cells, liver cells are not significantly inhibited by $Mg^{2+}$ (Figure D).

The $^{32}P$ incorporation by the rest of phosphocompounds (nucleic acids + phosphoproteins) is inhibited to a much lower extent than the uptake by phospholipids. 0.05 *M* $Ca^{2+}$ decreases this incorporation by ascites cells and liver cells in 38.5% and 11.9% respectively. $Mg^{2+}$ showed no effects on this uptake. The chromatographic analysis of the $^{32}P$-labelled phospholipids indicates that 0.05 *M* $Ca^{2+}$ increases in 20–30% the $^{32}P$ incorporation into the lecithin fraction.

The possible interference in the metabolism of P due to $Ca^{2+}$ precipitation of phosphate ions, was tested by incubation in absence of cells followed by filtration through millipore filter (0.2 μm pore size). No significant differences were observed between the control and the other tubes. In addition, in order to establish if the increasing $Ca^{2+}$ concentration affects the intracellular calcium, ascites cells incubated (under the same experimental conditions of the experiments with $Ca^{2+}$ and $Mg^{2+}$) with different $Ca^{2+}$ concentrations were washed 3 times with cold Ringer solution, digested in $HClO_4$ + $HNO_3$, and the




Effects of $Ca^{2+}$ and $Mg^{2+}$ concentration on $^{32}P$ incorporation into phospholipids isolated from Novikoff hepatoma ascites and liver cells (as % of the control assay). A) $Ca^{2+}$ in Novikoff ascites. B) $Mg^{2+}$ in Novikoff ascites. C) $Ca^{2+}$ in liver cells. D) $Mg^{2+}$ in liver cells. Mean value ± SD corresponding to 7 experiments with ascites cells and 5 experiments with liver cells.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Effects of extracellular $Ca^{2+}$ concentration on the intracellular content of calcium

| $Ca^{2+}$ concentration (*M*) | μg Ca/mg cell protein |
|---|---|
| 0.048 | 0.61 ± 0.18 |
| 0.024 | 0.22 ± 0.05 |
| 0.014 | 0.12 ± 0.02 |
| 0.009 | 0.10 ± 0.02 |
| 0.0046 | 0.09 ± 0.02 |
| 0.0023 | 0.09 ± 0.02 |

Mean value ± SD of 10 experiments.

calcium content determined by atomic absorption spectrometry. The Table shows the values corresponding to 10 experiments. The increasing cellular calcium content (Table) indicates that under these experimental conditions the variation of the extracellular $Ca^{2+}$ concentration seems to affect the flux of $Ca^{2+}$ into the cells, and that it is very likely that the change in the intracellular calcium might be involved in the observed inhibition of phospholipid synthesis.

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*Summary.* High extracellular concentration of $Ca^{2+}$ inhibits the incorporation of $^{32}P$ into the cellular phospholipids. This effect is more significant in neoplastic than in normal cells, and it is accompanied by an increase of the percentual incorporation into the lecithin fraction.

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## The Composition and Utilization of Lipids and the Operativity of the Glyoxylate Cycle During the Germination of *Lupinus* Seeds

The glyoxylate cycle provides a mechanism for the conversion of fats into sugars in endosperms, which are particularly rich in lipids[1–3]. Recently we noted[4,5] that seeds of *Lupinus* cultivars (in dicotyledons the seed contains the embryo that consists of an axis, the hypocotyl, bearing 2 cotyledons), apparently free of endosperm, are particularly rich in lipids in the cotyledons. For this reason we prepared an oil and investigated the composition of the lipid classes from 0 time to the 24th day of germination. The presence and operativity of the two key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, were also demonstrated.

*Seed germination.* Seeds of *Lupinus* cultivars (the biological materials were about 1-year-old) were germinated on dampened paper in Petri dishes with distilled water at room temperature in diffused daylight. The experimental period was from October 1 to May 30.

*Lipids extraction and assay.* The extraction of lipid material was obtained by the method of FOLCH et al.[6]. The total lipids were determined by weighing[2]. The oil was obtained from ground *Lupinus* cultivars seeds by continuous solvent extraction with *n*-hexane for 24 h in a Soxhlet apparatus. The oil from seeds which had been germinating for 10 days was obtained by the same method. The fatty acids composition of the whole oil was investigated by GLC of the methyl esters. Mass spectra were also performed to confirm these findings, with Perkin-Elmer instrument Model 270 S at 70 eV. For the separation of the lipid classes, we used TLC on silica gel, as described by MALINS and MANGOLD[7]. The triglyceride fraction was scraped from the plate, eluted with ethyl ether and analyzed for fatty acids composition by gas-liquid chromatography of the methyl esters. The analysis was carried out at 170 °C on a column 2.5 m in length, 3.4 mm i.d. packed with 15% diethylene glycol succinate on 80

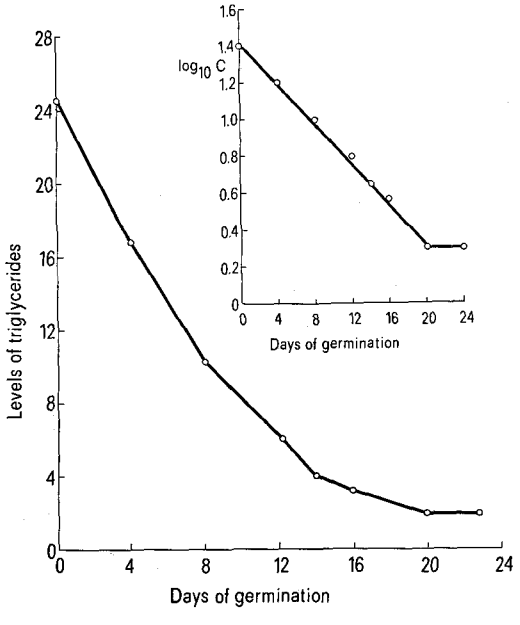


Fig. 1. Triglyceride content from 0 time to the 24th day of germination. The values are expressed as mg per cotyledon. In the inset, the log of the triglyceride content is reported against the time of germination.

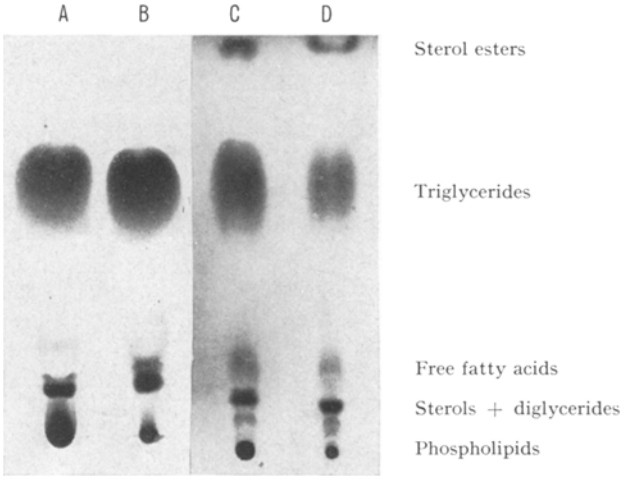


Fig. 3. TLC of the lipid classes. A) and C) $CHCl_3$MeOH extraction at 0 time and the 10th day of Germination. B) and D) Hexane extraction (oil) at 0 time and the 10th day of germination.

Table I. Total lipids content from 0 time to the 24th day of germination

| | Days of germination | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 14 | 16 | 20 | 24 |
| Lipids (mg) | 53 | 32 | 28 | 30 | 10 | 11 | 8 | 9 |

The lipid values are expressed in mg per cotyledon.