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

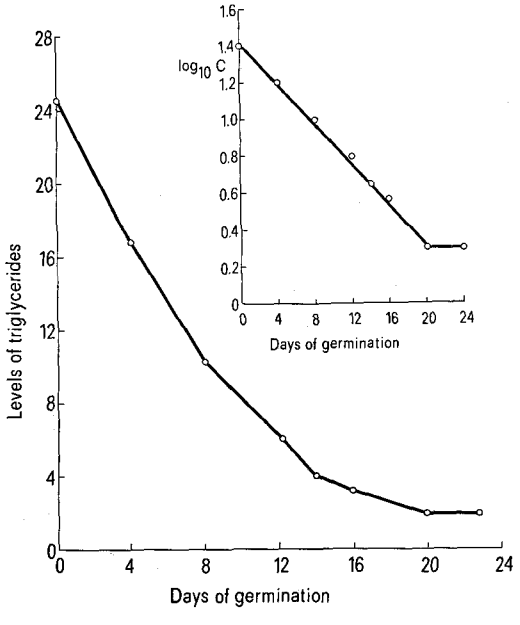

Fig. 1. Triglyceride content from 0 time to the 24th day of germination. The values are expressed as mg per cotyledon. In the inset, the log of the triglyceride content is reported against the time of germination.

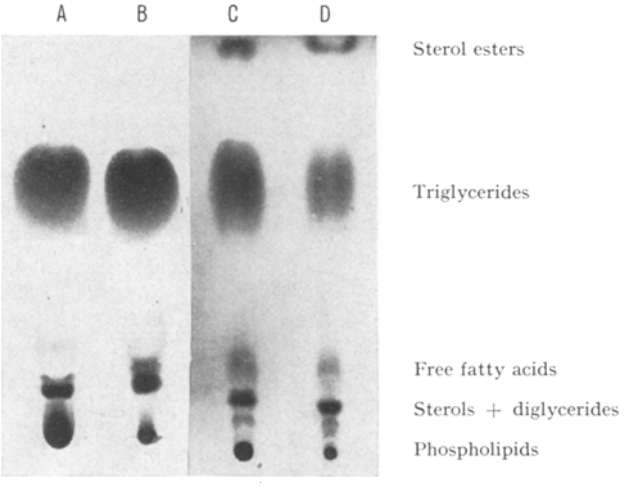

Fig. 3. TLC of the lipid classes. A) and C) $CHCl_3$MeOH extraction at 0 time and the 10th day of Germination. B) and D) Hexane extraction (oil) at 0 time and the 10th day of germination.

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.

Table II. Percentage of total fatty acids (hexane extraction) and fatty acids of the triglyceride fraction ($CHCl_3$-MeOH extraction) at 0 time and at the 10th day of germination

Acids	0 time		10th day	
	Fatty acid content (%) (hexane extraction by Soxhlet)	Fatty acid content (%) ($CHCl_3$-MeOH extraction)	Fatty acid content (%) (hexane extraction by Soxhlet)	Fatty acid content (%) ($CHCl_3$-MeOH extraction)
Palmitic	10.45	6.6	7.78	7.77
Palmitoleic	0.3	traces	traces	traces
Stearic	1.76	1.2	1.1	0.67
Oleic	48.2	54.46	48.96	49.84
Linoleic	9.9	13.70	22.09	25.61
C:20	0.92	traces	1.15	traces
C:18^{---} + C:20^{-}	16.18	17.31	14.08	14.35
C:22	7.63	4.06	3.68	1.27
C:22^{-}	4.11	2.06	1.06	0.48
C:24	0.55	traces	traces	traces

to 90 mesh Anakrom ABS. Areas under the peaks were calculated by triangulation to determine the percentage of fatty acids. The total triglyceride content was determined with respect to an internal standard of tripalmitin.

Preparation of enzyme extract and assays. The enzymatic extracts were prepared by the method of VINCENZINI et al.[8]. The isocitrate lyase and malate synthase activities were assayed at 25 °C with continuous optical method[9], slightly modified by us[10].

Reagents. Tween 80 and mercaptoethanol were purchased from Fluka A.G. Buchs. Substrates were from Boehringer, Mannheim. *Tris*, EDTA and all other products were obtained from Merck.

Results and discussion. The photograph (Figure 3) shows the TLC of the lipid extract at 0 time of germination (A and B) and on the 10th day of germination (C and D); B and D, oil composition, A and C $CHCl_3$-MeOH

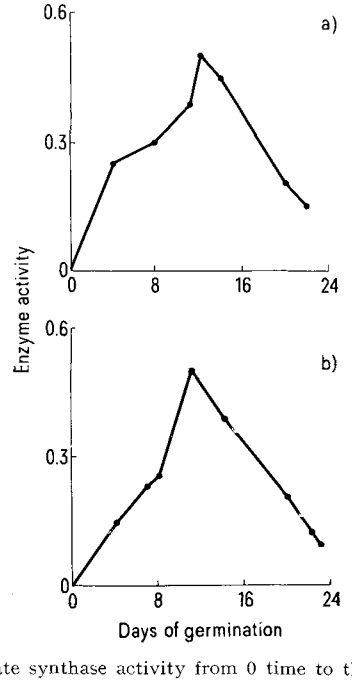

Fig. 2a) Malate synthase activity from 0 time to the 24th day of germination. The enzymatic activities are expressed as μmoles of substrate transformed per min per cotyledon. b) Isocitrate lyase activity from 0 time to the 24th day of germination. The enzymatic activities are expressed as μmoles of substrate transformed per min per cotyledon.

composition. The experiments show that the qualitative composition is the same. Beginning at the top of the photograph and reading downward, we note sterol esters, triglycerides, free fatty acids, sterols and diglycerides and phospholipids. Quantitative differences in the triglyceride content are evident between 0 time and 10th day of germination for both extraction procedures used (Figure 1). Table I shows the total lipids levels from 0 time to the 24th day of germination; there is an evident lipid catabolism. The triglyceride content of the seeds during the same investigated period is reported in Figure 1; there is an evident hyperbolic outline, as in a first order reaction. In fact, reporting the log of the triglyceride level value versus the days of germination, we obtained a straight line, as can be seen in the inset. The t/2 is 6 days and the k (velocity constant of the reaction) is 0.115 days^{-1}.

Table II shows the percentages of the total fatty acids in the oil at 0 time and after 10 days of germination. As can be seen, a parallel investigation performed on the $CHCl_3$-MeOH extract of the seeds at the same period of germination does not show significant variation in the percentages. On the other hand, some quantitative differences (particularly for $C_{18:2}$ $C_{22:0}$ and $C_{22:1}$) were found by comparing the percentages of the total fatty acid content at 0 time and at the 10th day of germination. The $C_{18:2}$ percentage doubles and the percentages of $C_{22:0}$ and $C_{22:1}$ decrease about 7 times. The oil may be suggested for possible alimentary use because of its equilibrate composition in unsaturated fatty acids and because of the low concentration of erucic acid. All the fatty acid methyl esters were identified, not only by their retention times but by their mass spectra. The identification was performed by comparison with pure

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standards. Mass spectra data were particularly useful to identify the peaks corresponding to the $C_{18:3}$ and $C_{20:1}$ unresolved in our gas chromatographic conditions, and the minor component $C_{22:1}$. In the first case the MS of the unresolved chromatographic peak showed the presence of both the parent peaks of $C_{18:3}$ and $C_{20:1}$ at *m/e* 292, and 324 respectively. The peaks at *m/e* 292 (M-32), *m/e* 236 (M-56) and 223 (M-69) for $C_{18:3}$ and at *m/e* 292 (M-32), *m/e* 250 (M-74) and *m/e* 208 (M-116) for $C_{20:1}$ were also observed. $C_{22:1}$ was identified by the parent peak occurring at *m/e* 352 and the characteristic peaks occurring at *m/e* 320 (M-32), *m/e* 278 (M-74) and *m/e* 236 (M-116). Because the mass spectra of positional isomers, with double bond at position 6:7 or higher up in the chain are practically indistinguishable from those of methyl oleate, a comparison with GLC retention time with a pure standard of $C_{22:1}$ was also performed to confirm our findings.

Figures 2a and b show the isocitrate lyase and the malate synthase levels respectively during the observation period. The key enzymes of the glyoxylate cycle increase together and reach their maximum at about the 11th day of germination. These data, compared with the results shown in Table I and in Figure 1, demonstrate that the increase in enzyme levels corresponds to the days in which the lipid catabolism is more evident.

To conclude, we demonstrated the presence and the operativity of the glyoxylate cycle in *Lupinus* seeds, and we confirmed the close correlation between this metabolic pathway and triglyceride catabolism.

Summary. The levels of total lipids and triglycerides were determined during germination of *Lupinus* seeds. The presence and operativity of the two key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, were made clearly evident and correlated to lipid metabolism. An oil, relatively free of erucic acid, was obtained from the seeds at 0 germination time.

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The Absence of an Inhibitory Effect of Metyrapone (2-methyl-1,2-di (3-pyridyl) propan-1-one) on Hepatic Microsomal Hydroxylation in Scurvy

There is evidence that the ability of the liver microsomal system to hydroxylate certain compounds is reduced in scurvy[1–3].

DEGKWITZ and STAUDINGER[3] have related the impaired hydroxylation of acetanilide in scurvy to a reduction in the cytochrome P-450 concentration. They pointed out that this would not be inconsistent with an influence of asorbic acid on the biosynthesis of haem and in support of this they point out that cytochrome b_5 too decreases in the microsomes of guinea-pigs deprived of ascorbic acid. FIELDING[4], in a similar type of experiment, also found a scurvy-induced reduction in the microsomal b_5 but found no evidence of a corresponding fall in the cytochrome P-450.

An alternative, or complementary, possibility is that ascorbic acid influences the binding of the enzyme to the substrate. Inhibitors such as Metyrapone act by interference with this binding. An experiment was therefore designed to examine the effect of Metyrapone on the microsomal acetanilide-hydroxylating system from normal and scorbutic guinea-pigs.

Methods. 12 male Dunkin-Hartley guinea-pigs were given a semisynthetic scorbutogenic diet[5]. 6 received a daily supplement of 5 mg ascorbic acid/100 g body-weight and 6 received no supplement. After 12 days the animals were killed by decapitation and exsanguination. Ascorbic acid concentrations were determined in 2 representative tissues, the spleen and adrenals[6]. Liver microsomes were extracted in 3 volumes of ice-cold 1.10% potassium chloride solution followed by homogenization in a Potter-Elvehjem type homogenizer and centrifugation at 9000 *g* at 0–4 °C for 20 min. The supernatant was centrifuged at 38,000 *g* for 60 min (MSE High Speed 18 centrifuge) and the microsomal pellet washed and finally re-suspended in ice-cold 0.2M Sörenson phosphate buffer (pH 7.4) to give a microsomal protein concentration of ca. 10 mg/ml. Liver microsomal protein was measured using the Folin-Ciocaltau reagent[7] and N-acetyl-*p*-aminophenol, the *para*-hydroxylated metabolite of acetanilide, by a modification of the method used by KRISCH and STAUDINGER[8]; no interference by the 'X-product' of KRATZ and STAUDINGER[9] was found.

The hydroxylation incubation mixture contained 2 μmoles nicotinamide adenine dinucleotide, 2 μmoles nicotinamide adenine dinucleotide phosphate, 2.5 μmoles magnesium sulphate, 4 μmoles glucose-6-phosphate, 20 μmoles nicotinamide, 0.2 ml 9000 *g* supernatant, 1–2 mg microsomal protein and the appropriate substrate, in a volume of 1.2 ml. All solutions were in 0.2 phosphate buffer, pH 7.4. Incubation was at 37 °C and the reaction was stopped by the addition of 0.1 ml 20% trichloroacetic acid.

Results and comments. The concentrations of ascorbic acid in the spleen and adrenals respectively were 1.7 ± 0.4 and 3.7 ± 0.7 (mg/100 g fresh tissue) for the scorbutic animals and 11.9 ± 1.2 and 41.7 ± 6.5 for the control animals (mean values with standard errors). The influence of different Metyrapone concentrations on the

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