Im Anschluss an die früheren Befunde über die Abnahme des ATP-Gehaltes im Alter[4] und der wahrscheinlich dadurch bedingten Reduktion der KP-Reserven[5] kann angenommen werden, dass diese Störung durch die Änderung der ATP-Resynthese bedingt ist.

Summary. The activity of aldolase and succinatdehydrogenase (SDH) in white and red skeletal muscle of young (3–7 months) and old (20–30 months) rats has been determined. In addition also the SDH of liver was measured. The activity of aldolase is higher in white than in red muscles, while SDH shows a higher activity in red than in white muscles. The activity of aldolase is not influenced by ageing in white muscles, but decreased in red muscles by 23%. In old animals the activity of SDH is 34% less in white and 52% less in red muscles. In liver the activity is 44% less. The significance of these changes for the energy metabolism of skeletal muscle is discussed.

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The Presence of Isocitrate Lyase and Malate Synthase Activity in Germinating *Ginkgo biloba* seeds[1,2]

The glyoxylate cycle has been found operative in bacteria[3], algae[4], fungi[5], conifers[6] and angiosperms[7]. This cycle provides a mechanism for the conversion of fats into sugar. In the attempt to demonstrate that this metabolic pathway is very strictly associated with high levels of lipids in the tissue and that the cycle might be considered an ancestral mechanism of utilization of fats, we investigated the lipid content of *Ginkgo biloba* seeds, at 0 time of germination. Subsequently we studied the presence of the two key enzymes of the cycle, the isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) in seedlings 8 cm long.

Moreover, the fatty acids composition of the triglyceride fractions is investigated and compared to the composition of species where the cycle has been found to be operative, in order to provide a possible correlation between the enzymes assayed and the material they are utilizing as substrate.

It is interesting to underline that the *G. biloba* tree belongs to a division of the Ginkgophita which goes back to the carboniferous and the *G. biloba* is the only living Ginkgophita[8].

Experimental. Culture of *G. biloba.* At 0 time of germination, the seeds deprived of the sarcotesta and sclerotesta were used for lipid determinations. For the enzyme assay, the seeds without sarcotesta but with sclerotesta, were cultivated in a chamber at constant temperature of 25 °C in the dark on moistened sand. The proper quantity of water was added to insure complete imbibition and normal germination. At the desidered time of germination, the material was collected and used for enzyme determinations.

Lipids extraction and determination. For the extraction of lipid material, we adopted the method of Folch et al.[9]. A small aliquot of lipid extract was withdrawn and evaporated in a oven at 60 °C for 20 h to obtain the weight of total lipids. For the separation of the lipid classes, we used the thin-layer chromatography as described by Malins and Mangold[10]. The triglyceride and phospholipid fractions were assayed after scraping from the plate by the methods of Carlson[11] and Martin et al.[12], respectively. The fatty acid composition of triglyceride fractions were obtained by gas chromatographic analysis.

Enzymes extraction and determination. The enzymatic extracts were prepared by the method of Firenzuoli et al.[6]. Isocitrate lyase and malate synthase were assayed at 25 °C with continuous optical method[13] slightly modified by us[6].

Protein determination. The total proteins of the enzyme extract were determined by the biuret method according to Beisenherz et al.[14].

Results and discussion. In Table I (a) are reported the levels of total lipids and of 2 fractions more representative and obtained by thin-layer chromatography. It is evident that the seeds at 0 time of germination are mostly rich of triglyceride.

The activities of 2 key-enzymes of glyoxylate cycle in seedlings 8 cm long are reported in Table I (b). The highest values are those of the isocitrate lyase. Table II shows the fatty acid compositions of the triglyceride fraction. Evidently, the saturated fatty acids are quantitatively less represented, unsaturated fatty acids are predominant. The most abundant unsaturated fatty acids are linoleic and oleic acids; these values of *G. biloba* compared with those of the 2 species show that always C18:1 and C18:2 are predominant fatty acids and also linoleic is the most represented one; the ratio C18: 1/C 18:2, studied in all species, is almost constant. These data may indicate the

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[3] H. L. Kornberg, Nature, Lond. *179*, 988 (1957).

[4] W. G. Haig and H. Beevers, Arch. Biochem. Biophys. *107*, 152 (1964).

[5] J. A. Olson, J. biol. Chem. *234*, 5 (1959).

[6] A. M. Firenzuoli, P. Vanni, E. Mastronuzzi, A. Zanobini and V. Baccari, Plant Physiol. *43*, 1125 (1968).

[7] Y. Yamamoto and H. Beevers, Plant Physiol. *35*, 102 (1960).

[8] H. C. Bold, *The Plant Kingdom* (Eds. W. D. McElroy and C. P. Swanson; Prentice Hall Inc., Englewood Cliffs 1964), p. 3.

[9] J. Folch, M. Lees and G. M. Sloane-Stanley, J. biol. Chem. *226*, 497 (1957).

[10] D. C. Malins and H. K. Mangold, J. biol. Chem. *226*, 576 (1960).

[11] L. A. Carlson, J. Atheroscl. Res. *3*, 334 (1963).

[12] J. B. Martin and D. M. Doty, *Methods of Biochemical Analysis* (Interscience Publishers J. Wiley and Sons, New York 1956), vol. 3, p. 1.

[13] G. H. Dixon and H. L. Kornberg, Biochem. J. *72*, 3 (1959).

[14] G. Beisenherz, H. J. Boltze, Th. Bücher, R. Czok, K. H. Garbade, E. Meyer Arendt and G. Pfleiderer, Z. Naturforsch. *8b*, 555 (1953).

Table I. Lipid content, glyoxylate cycle enzymes and proteins in seeds (a) and in seedlings (b) of *Gingko biloba*

a) Seeds		
Total lipid	Triglycerides	Phospholipids
19.1	14.6	1.7
b) Seedlings		
Isocitrate lyase	Malate Syntase	Proteins
3.7	28.2	12.2

Total lipid, triglycerides and phospholipids are expressed as mg/g fresh wt. Two enzymes as μmoles of substrate transformed per h × g fresh wt.; proteins as mg/g fresh wt.

Table II. Fatty acid composition of the triglyceride fractions of 3 species that use the glyoxylate cycle

Fatty acids	*Ginkgo biloba* seeds (%)	*Pinus pinea* seeds[6] (%)	*Abies alba* seeds[6] (%)
16:0	7.2	6.6	3.8
16:1	4.7	0.4	0.7
18:0	0.9	3.4	2.1
18:1	36.0	37.5	30.3
19:0	2.3	—	5.0
18:2	43.0	46.8	42.1
20:0	0.6	0.7	13.1
18:3	3.5	1.4	0.6
18:2 / 18:1	1.1	1.2	1.4

existence of some correlation among the operativity of cycle and the levels of oleic and linoleic acids, these can probably provide the main carbon source; from these results it is evident that the glyoxylate cycle is operative during germination of *G. biloba* seeds. Finally from a comparative point of view, the presence of the cycle in *G. biloba* ancestral plant and its absence in Mammalia[15,16] in Graminacee[17] could confirm that his cycle represents a primitive metabolic pathway for lipids utilization.

Riassunto. Si è dimostrata la presenza del ciclo del gliossilato durante la germinazione di semi di *Ginkgo biloba*. Isocitrico liasi e malato sintasi, e due enzimi chiave del ciclo, sono stati messi in evidenza in pianticelle di 8 cm. Le attività dei due enzimi sono invece assenti nel seme dove elevato è il contenuto in lipidi. Tra gli acidi grassi i più rappresentati sono l'oleico e il linoleico.

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20 September 1971.

[15] W. D. CARPENTER and E. G. JAWORSKI, Biochim. biophys. Acta *58*, 369 (1962).
[16] M. ROTHSTEIN and H. MAYOH, Comp. Biochem. Physiol. *17*, 1181 (1966).
[17] A. M. FIRENZUOLI, P. VANNI, G. RAMPONI and V. BACCARI, Plant Physiol. *43*, 260 (1968).
[18] We are deeply indebted to Dr. S. RUGGIERI and A. FALLANI of General Pathology Department of University of Florence for GLC analysis and for valuable suggestions in lipid determination.

Trace Elements in Human Hair

It has been known for many years that human hair contains a complex mixture of trace mineral elements[1], but little is known of the physiological and pathological processes that determine the concentration in hair of a particular element. We have therefore determined the concentration of copper, iron and zinc in hair from a large group of people, then analyzed our results to determine the effects, if any, of age, sex, ethnic origin, diet, pregnancy, health, and pharmaceuticals on each element.

Approximately 1.0 g scalp hair from the occipital region was taken from 222 persons living in the Lusaka area. Each person was interviewed to obtain full personal and medical details. The hair was washed in the laboratory with nonionic detergent or purified diethylether[2], dried overnight at 110 °C, then ashed at 520 °C for 8 h. The ash was dissolved in the minimum amount of concentration HCl and the copper, iron and zinc concentrations of the solution measured by atomic absorption spectrometry (Unicam SP900A). Recoveries were 95 to 102% and reproducibility ± 3% for each element. Not all elements were measured on each hair specimen, but 222 results were obtained for zinc, 192 for copper, and 133 for iron.

The Table gives a breakdown of our findings for each element, expressed as mean and standard deviation of particular groups, divided by age, sex, ethnic origins, health status, etc. *T*-tests were conducted to assess the significance of any apparent differences.

For copper in hair, females are higher than males ($P < 0.002$), and caucasians higher than negroes ($P < 0.002$). Healthy subjects have higher values than hospitalized patients ($P < 0.002$), irrespective of the type of illness. Women taking oral contraceptives had values strikingly higher than other adult women ($P < 0.001$).

The only factor apparently influencing iron in hair was the taking of anti-malarials ($P < 0.002$ when compared to the rest of the study and $P < 0.001$ when compared with a matched group of similar people not taking these drugs). This result may be a reflection of better iron stores, for Lusaka is a malaria region and anemia due to this disease is common in unprotected people.

Ethnic origin influenced zinc in hair ,with asiatics giving the highest results and negroes the lowest ($P < 0.05$ for this difference). Hair zinc content is low in pregnancy; a fact reported by another worker[3]. This is probably a reflection of the lowered plasma zinc level during pregnancy[4].

[1] W. S. SPECTOR, *Handbook of Biological Data* (W. Saunders and Co. Inc., Philadelphia 1956).
[2] W. W. HARRISON, J. P. YURACHEK and C. A. BENSON, Clin. chim. Acta *23*, 83 (1969).
[3] L. M. KLEVAY, Am. J. clin. Nutr. *23*, 284 (1970).
[4] J. HALSTEAD, B. M. HACKLEY and J. C. SMITH, Lancet *2*, 278 (1968).