Fatty acid composition in spinach leaves (*Spinacea oleracea*), as determined by gas-liquid chromatography

Fatty acids	16:0	16:1	16:1 *trans*	16:3	18:0	18:1	18:2	18:3
Total lipid	17.0	tr[a]	2.4	7.4	0.5	6.0	14.6	51.4
Acetone soluble lipid	6.7	tr	0.5	12.3	0.5	4.1	8.8	67.0
Acetone insoluble lipid	24.7	tr	4.8	tr	tr	9.2	25.0	36.3
Phosphatidyl glycerol	20.0	tr	31.7	–	0.6	2.6	8.0	37.1
Lyso phosphatidyl glycerol[b]	32.4	tr	0.5	–	1.2	2.4	2.1	61.4
Fatty acid liberated from phosphatidyl glycerol[b]	11.1	tr	58.9	–	–	3.1	14.5	12.4

[a] Traces of compounds less than 0.5%. [b] Use was made of phospholipase A from *Crotalus adamanteus*.

trans) and a hexatrienoic acid (16:3) were found to be present (Table). As outlined by DEBUCH these unsaturated C_{16} fatty acids are specific for the chloroplast. Fractionation by means of acetone-$MgCl_2$ precipitation rendered an interesting distribution of both fatty acid constituents. The 16:1 *trans*-fatty acid was recovered in the precipitate containing, together with other lipids, practically all of the phospholipids, whereas the 16:3 fatty acid residue appeared to be present in the soluble fraction which is rich in galactolipids (Table). After two chromatographic runs of the acetone insoluble fraction on silicic acid columns, we obtained a chromatographically and analytically pure fraction of phosphatidyl glycerol (glycerol-phosphorus ratio: 2.04). Surprisingly, gas chromatography of the fatty acid methyl esters demonstrated that the 16:1 *trans*-fatty acid represented as much as 30% of the total fatty acid residues of phosphatidyl glycerol (Table). The fatty acid methyl ester was isolated in a pure state by means of gas chromatography. The IR-spectrum and oxidative degradation in perfect agreement with the work of DEBUCH[9] showed this acid to be identical with Δ^3-*trans*-hexadecenoic acid.

The fatty acid composition of the lecithin fraction from spinach leaves was found to be very similar to that reported by SASTRY and KATES[1] for lecithin from runner-bean leaves. Although the characterization of the other phospholipid species is not yet complete, the evidence available now indicates that the 16:1 *trans*-fatty acid is concentrated mainly in phosphatidyl glycerol. This finding is relevant to the recent work of NICHOLS[6], who suggested that lecithin and phosphatidyl ethanolamine are present mainly in mitochondria and nuclei, whereas phosphatidyl glycerol is specifically concentrated in the chloroplast.

The positional distribution of the fatty acids was investigated after a complete hydrolysis of phosphatidyl glycerol with phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus*[12]. By virtue of the positional specificity of this enzyme the Δ^3-*trans*-hexadecenoic acid appeared to be located exclusively at the 2-position of phosphatidyl glycerol (Table). It is intriguing that a predominant part of linolenic acid occupies the 1-position. Hence, the distribution of monoenoic and polyenoic fatty acid constituents in phosphatidyl glycerol differs significantly from the distribution usually found in several other phospholipid types from the animal and plant kingdom.

Zusammenfassung. Der Gehalt an Δ^3-*trans*-Hexadecensäure im Phosphatidylglycerol aus grünen Spinatblättern (*Spinacea oleracea*) erweist sich als auffallend hoch. Die enzymatische Hydrolyse zeigt die C_{16}-Monoensäure mit der β-Stelle des Phosphatids verknüpft.

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Alternative Pathways of Glucose Metabolism in Developing Chick Brain[1]

Introduction. A widely used method of comparing the relative amounts of glucose metabolized by the two major known pathways, in different tissues, is based on the differential utilization of specifically labelled glucose. C-6 of the glucose molecule is converted to carbon dioxide by glycolysis and the tricarboxylic cycle. C-1 of the glucose molecule is converted to CO_2 both by this pathway and by the hexose-monophosphate shunt[2,3]. This method was used in previous investigations in this laboratory concerning the relative importance of the two pathways in various tissues[4–6]. Study was now extended to embryonic chick brain with the purpose of comparing the relative rates of the glycolytic and hexosemonophosphate pathways at various developmental stages.

Material and methods. Embryos were removed from fertile eggs incubated for 6 to 21 days at 37°C and staged according to the HAMBURGER and HAMILTON[7] stage

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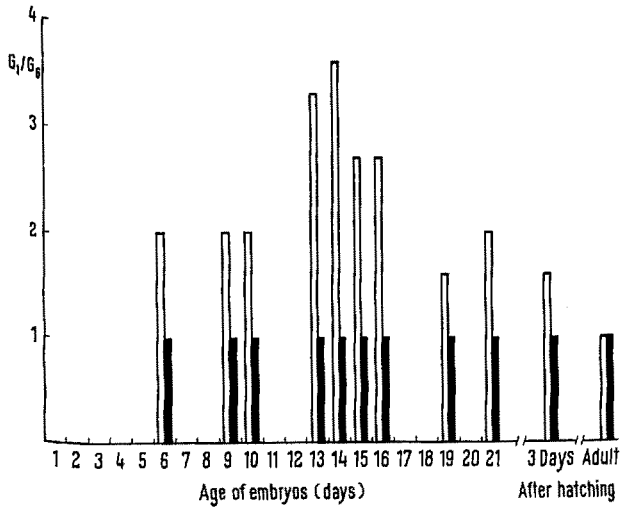
series, from which equivalent age was calculated. Embryonic, newly-hatched and adult chicken brains were removed and dissected clear from surrounding membranes. All other structures such as optic cups, were completely removed.

The brain tissue was cut into small pieces and a cell suspension (1:5 in Krebs-Ringer buffer, pH 7.2) was obtained by gentle pipetting. 0.2 ml of this suspension was placed in a Warburg flask containing 0.3 ml of Krebs-Ringer buffer + 0.1% glucose and 2.5 μC of either 1-C^{14}-glucose or 6-C^{14}-glucose (from the Radiochemical Centre, Amersham). The central well of the flask contained 0.2 ml of 1 *M* hyamine base.

The flasks were incubated in a Warburg apparatus for 2 h at 37° C, the reaction was stopped by addition of 0.2 ml of 1 *M* citric acid. The flasks were shaken for 30 min and the hyamine solution was quantitatively recovered in several methanol washings. The radioactivity was counted in a liquid scintillation counter.

Production of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C

Age	$^{14}CO_2$ production in c.p.m. (less background)		Ratio G1/G6
	Glucose-1-^{14}C	Glucose-6-^{14}C	
6 days	1260	624	2.1
	1290	526	2.9
9 days	1236	770	2.3
	1128	680	1.9
10 days	2640	1530	1.7
	2670	990	2.6
13 days	2860	890	3.2
	2685	780	3.4
14 days	3270	1290	2.8
	2790	630	4.5
19 days	1290	990	1.3
	1190	700	1.7
Newly-hatched	1930	1116	1.7
	1580	1098	1.4
Adult	2574	2640	0.9
	2700	3100	0.9
	1800	1560	1.2



Ratios of $^{14}CO_2$ yields from glucose 1-^{14}C and glucose 6-^{14}C in chick brain at various developmental stages.

Results and comment. The results of the experiments with cell-suspension embryonic, newly-hatched and adult chicken brains are summarized in the Table. From the conversion ratios of glucose carbon to carbon dioxide it appears that the activity of the hexose-monophosphate pathway is rather high during embryonic life, having a maximum between the 12th and 15th days of incubation, then gradually declining at the time of hatching, and almost completely disappearing in the adult chicken brain. The recovery of $C_{14}O_2$ from glucose-1-C^{14} was twice that obtained from glucose-6-C^{14} during the 6th to the 10th day of incubation. This increased further to reach a maximum at the 14th day (Figure).

Soon before hatching the ratio G1/G6 slowly decreased and 3 days after hatching was about 1.4. In these experiments with adult chicken brain the ratio G1/G6 was constantly found to be about 1, thus indicating that only a minor part of the glucose, if any, was metabolized by the adult brain tissue through the hexose-monophosphate shunt.

Since the recognition of the hexose-monophosphate pathway in animal tissue, several studies have shown that this pathway of glucose metabolism provides the pentoses for nucleic acid synthesis; it also contributes to the production of TPNH, an essential coenzyme for the biosynthesis of fats and of other organic compounds[8].

There is evidence that the metabolism of glucose *via* this pathway is enhanced under those conditions in which an increased production of these end products is required[9,10]. Thus embryonic development could be a physiological condition wherein the exigency of the rapidly growing tissues would require an increased activity of the pentose shunt.

In sea-urchin eggs undergoing cleavage and gastrulation, the hexose-monophosphate pathway appears to be the major route of glucose metabolism, whereas 24 h later glycolysis and the Krebs cycle become predominant[11,12].

The experimental results indicate that similar changes in the relative rates of glucose metabolism *via* the two alternative pathways, take place in the developing chick brain. From the values of the G1/G6 ratios, it appears that the pentose shunt is actively operating through all of the embryonic life. This activity attains a maximum during the second week of incubation, declines thereafter and is practically absent in the fully developed brain. It is conceivable that this pattern of carbohydrate metabolism reflects the rapid rate of biosynthetic reactions which take place in developing brain tissue.

Riassunto. È stato studiato il metabolismo di glucosio marcato in C-1 e C-6, nel cervello di pollo in varie fasi di sviluppo. I risultati ottenuti indicano che, mentre nel cervello adulto la massima parte del glucosio viene metabolizzata attraverso il ciclo di Embden-Meyeroff, nel cervello embrionale in fase di sviluppo anche la via di Warburg-Dickens è attivamente operante.

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