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Phospholipid Synthesis in Aging Potato Tuber Tissue[1]

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Abstract. **The effect of activation ("aging") of potato tuber slices on their phospholipid metabolism was investigated. Aged slices were incubated with ^{14}C labeled choline, ethanolamine, methionine, serine, and acetate. In all cases, the incorporation of radioactivity into the lipid fraction increased with the length of time the slices were aged. This incorporation was shown to be true synthesis and not exchange between precursors and existing phospholipids.**

The increased incorporation of labeled choline into lipids was mainly due to an increase in its uptake by the tissue, the presence of actidione during aging prevented this increased uptake. The increase in the incorporation of labeled acetate into lipids resulted from the development of a fatty acid synthetase during aging. In the case of ethanolamine, both its uptake into the tissue and its incorporation into the lipid fraction increased.

The phospholipids formed from these precursors were identified by paper and thin-layer chromatography. The major compound formed from choline was lecithin, while phosphatidylethanolamine and a small amount of lecithin were formed from ethanolamine.

The phenomenon of wound respiration, sometimes referred to as "aging," has attracted the attention of many workers since the nineteenth century (7). In addition to a 4- to 5-fold increase in oxygen uptake, many other metabolic changes occur when a dormant storage organ is sliced and incubated aerobically. Among the responses reported are: the initiation of RNA and protein synthesis (12, 33), starch degradation (31); the onset of tricarboxylic acid cycle activity (22) and oxidative phosphorylation (21); an increase in salt uptake (20) and the development of responses to growth substances (17, 30). Recently, Willemot and Stumpf (36, 37) reported an increase of fatty acid synthetase activity during the early period of activation. Both the quantity and kind of fatty acids synthesized are different from those synthesized by the fresh tissue. The development of this activity is shown to be related to the *de novo* synthesis of RNA and proteins.

Hackett *et al.* (16) in 1960 made a comparison between the respiratory mechanisms of freshly cut and day old potato slices. Their work with tissue slices and with isolated cell fractions suggested that during aerobic incubation, there was a metabolism-dependent modification of the mitochondrial respiratory chain. Evidence for an increase in the number of mitochondria during aging of potato disks was reported by Lee and Chasson (24, 25) and Verleur *et al.* (34). Furthermore, in general an increase in the number of subcellular organelles is accompanied by a large increase in respiration. Such has been shown to be the case in sweet potato disks (2), germinating peanuts (9), castor beans (27) and cotton seeds (35).

Since phospholipids are universal components of membranes and since there is a requirement for phospholipids in many cellular processes (1), the increase in membranous materials of the parenchyma cells and the change in so many metabolic activities suggest that there is an involvement of phospholipid synthesis during aging. Therefore the synthesis of phospholipid was investigated by feeding radioactive precursors with a hope to contribute some information toward the better understanding of the aging process.

Materials and Methods

Acetate-1-^{14}C, choline-methyl-^{14}C chloride, choline-1,2-^{14}C chloride and L-serine-u-^{14}C were obtained from New England Nuclear Corporation. Ethanolamine-1,2-^{14}C was supplied by International Chemical and Nuclear Corporation and L-methionine-methyl-^{14}C was obtained from Calbiochem. Actidione (cycloheximide) was purchased from Nutritional Biochemicals Corporation and chloromycetin from Parke Davis and Company. Phospholipid standards (lecithin, phosphatidylethanolamine and a mixture of cholesterol, phosphatidylethanolamine, lecithin, and lysolecithin) were supplied by Applied Science Laboratories Incorporated.

Potato tubers (*Solanum tuberosum*, var. Russett) were bought from a local market and stored up to 2 weeks in a cold room (3–4°) in the dark. Cylindrical blocks normal to the median plane were cut inside the vascular ring with a No. 4 cork borer.

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A homemade cutter (made of stainless steel razor blades spaced with 2 washers to give a thickness of about 0.8 mm) was used to prepare slices from these blocks. The freshly made slices were rinsed with ice cold distilled water several times to wash out the materials from broken cells.

For aging, fresh slices were incubated in 10^{-4} M $CaSO_4$ solution containing chloromycetin (50 μg/ml) to prevent the growth of bacteria (23). After different periods of aging, phospholipid synthesis was determined by measuring the incorporation of labeled precursors into potato lipids. Typically 0.5 μc of the radioactive material was added to a 25 ml Erlenmeyer flask containing 10 slices in 1.0 ml $CaSO_4$/chloromycetin solution. The slices were then incubated at 25° for 2 hours in a metabolic shaker. When the effect of actidione was studied, it was added to the aging media at a concentration of 2 μg/ml but omitted during the incubation period. After the incubation, slices were rinsed with distilled water and tipped into a conical centrifuge tube containing 0.5 ml ice cold 0.1 M NaF solution (18). The slices were then boiled for 5 minutes to prevent enzymatic transesterification between phospholipids and methanol (4). The boiled slices were disrupted with a pointed glass rod and the lipids were extracted by the method of Bligh and Dyer (6) using 1 ml $CHCl_3$ and 2 ml CH_3OH. The insoluble proteins and polysaccharides were removed by centrifugation, and the supernatant was mixed with 1 ml $CHCl_3$ and 1 ml of 0.1 M unlabeled phospholipid precursor: chloine chloride, sodium acetate, ethanolamine hydrochloride, methionine or serine, depending on which of the labeled precursors had been used in the incubation. Two separate layers were thus obtained. The upper phase was discarded and the chloroform layer was washed again with 1 ml of the aqueous precursor solution. The final chloroform extract was then subjected to different analysis: scintillation counting, thin-layer chromatography of the phospholipids themselves or acid hydrolysis followed by paper chromatography of the free bases.

The uptake of radioactive precursors into the slices was measured by counting an aliquot of the medium in the flask before and after the 2 hour assay. The amount of radioactivity incorporated into the lipid fraction was measured by counting an aliquot of the lower chloroform phase. The scintillation mixture consisted of 150 g naphthalene, 15 g 2,5-diphenyloxazole (PPO), 0.3 g 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (POPOP), 720 ml absolute alcohol, 1140 ml toluene and 1140 ml *p*-dioxane.

Glass plates, 20 cm × 20 cm, were coated with Silica Gel G (Merck, Darmstadt) to a depth of 0.25 mm by mixing 25 g of the gel with 55 ml distilled water. They were activated at 100° for 30 minutes and stored in a desiccator. Potato lipids were concentrated under nitrogen, spotted on the plates and then developed in a solvent system of $CHCl_3$:CH_3OH:H_2O (65:25:4) for an hour (26). Lipid spots were revealed by exposing the plates to iodine vapor and were outlined in pencil. Radioactive spots were detected by autoradiography on X-ray film.

The base components were released from phospholipids by hydrolyzing in 4 N HCl at 100° under reflux (5). Potato lipids were dried under a stream of nitrogen followed by the addition of 1 ml of 4 N HCl. After 14 hours, the fatty acids and unhydrolyzed lipids were removed by extraction with diethyl ether; the aqueous phase was concentrated and spotted on Whatman No. 1 chromatography paper. The chromatogram was developed ascendingly either in the solvent system phenol:*n*-butanol: 80 % formic acid:H_2O (50:50:3:5, w/v/v/v), saturated with KCl (10), or in a system of *n*-butanol: ethylenechlorohydrin:NH_3:H_2O (50:10:5:16) (28) Ten hours later, the chromatogram was dried, cut into 3.8 cm strips and passed through a strip counter to detect radioactivity. Standard choline was revealed by exposing paper strips to iodine vapor (8). Standard ethanolamine, *N*-methyl and *N,N*-dimethyl-ethanolamine gave purple spots after spraying with 0.2 % ninhydrin in acetone and warming slightly.

The separation of phospholipids from neutral lipids was achieved as follows: potato lipids were dried and the residues were taken up in 2 ml diethyl ether. To each sample, 0.2 g of silicic acid (Merck, activated at 100°, overnight) was added. After 20 minutes with occasional shaking, the suspension was centrifuged and the resulting ether extract was transferred into a beaker. The silicic acid was washed 2 more times with ether to achieve complete extraction of neutral lipids. The ether extract and washes were combined and concentrated under nitrogen. Phospholipids were recovered by extracting the silicic acid 3 times with 2 ml CH_3OH. A control experiment showed only traces of cross contamination with this procedure.

Results

Incorporation of Various Precursors Into Potato Lipids. When potato tuber slices were incubated for 2 hours with ^{14}C-labeled phospholipid precursors (*i.e.*, acetate-1-^{14}C, choline-methyl-^{14}C, choline-1,2-^{14}C, ethanolamine-1,2-^{14}C, methionine-methyl-^{14}C, and L-serine-u-^{14}C), the amount of radioactivity incorporated into the lipids increased with the age of the slices (fig 1). Maximum incorporation was obtained after about 8 hours of aging, with some variation between batches of potatoes.

Aging of slices had a marked effect on the uptake of some, but not all, of the phospholipid precursors. The ability to take up choline increased rapidly as slices were aged, up to 6 hours (fig 2). The uptake of ^{14}C-choline from the medium and the incorporation of ^{14}C-choline into phospholipids both increased in sigmoidal fashion as a function of aging time; in the presence of actidione both increases were eliminated.

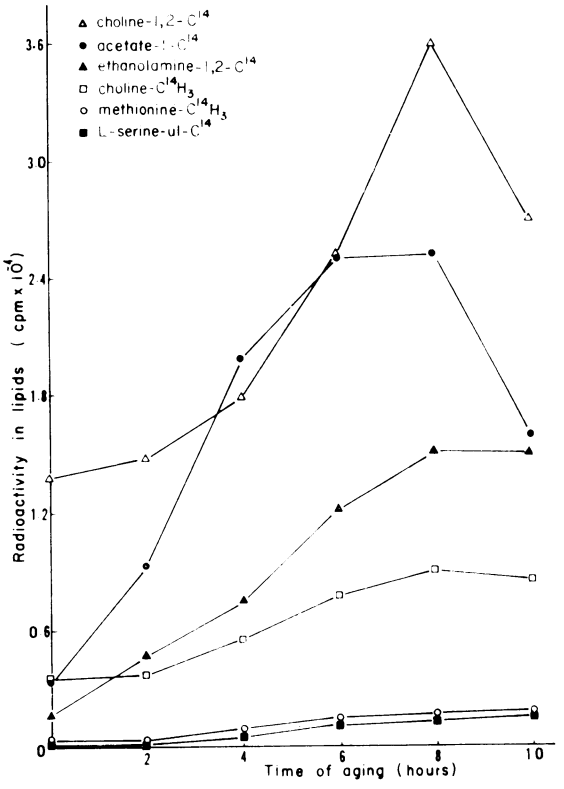

FIG. 1. Incorporation of radioactivity into lipids by potato slices incubated for 2 hours in the presence of choline-1,2-^{14}C, acetate-1,2-^{14}C, ethanolamine-1,2-^{14}C, choline-methyl-^{14}C, methionine-methyl-^{14}C and L-serine-μ-^{14}C. Before the incubation the slices were aged for 0 to 10 hours as indicated on the abscissa.

The behavior of acetate was entirely dissimilar from that of choline (fig 3). Acetate uptake from the medium was not stimulated during the aging process and actidione had no effect upon it. On the other hand, the incorporation of ^{14}C-acetate into lipids was activated and this activation was completely eliminated by actidione. These results on acetate are consistent with those reported by other workers (36, 37).

The results obtained with ethanolamine-1,2-^{14}C were intermediate between these 2 extreme cases (fig 4). There was an increase in both the uptake into the slices and its incorporation into lipids and both were inhibited by the presence of actidione. The results for methionine-methyl-^{14}C and L-serine-u-^{14}C were similar to those of ethanolamine. However for both of these precursors, the amount of radioactivity incorporated into lipids was very slight.

The ratios of lipoidal radioactivity/total radioactivity taken up from the medium have been summarized in table I. For choline this ratio is a constant regardless of the age of the slices. For all the other precursors, this ratio changes with age. In the case of acetate, the ratio undergoes a 7.5 fold increase from 0 to 10 hours.

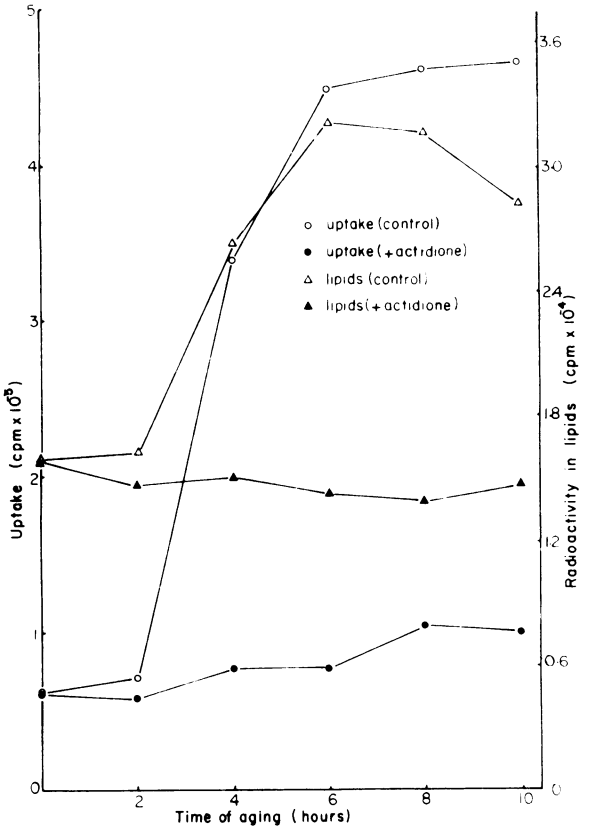

FIG. 2. Total uptake and incorporation of radioactivity into lipids by potato slices incubated for 2 hours in the presence of choline-1,2-^{14}C. Before the incubation the slices were aged for 0 to 10 hours as indicated on the abscissa. Dark symbols indicate that the slices were aged in the presence of actidione.

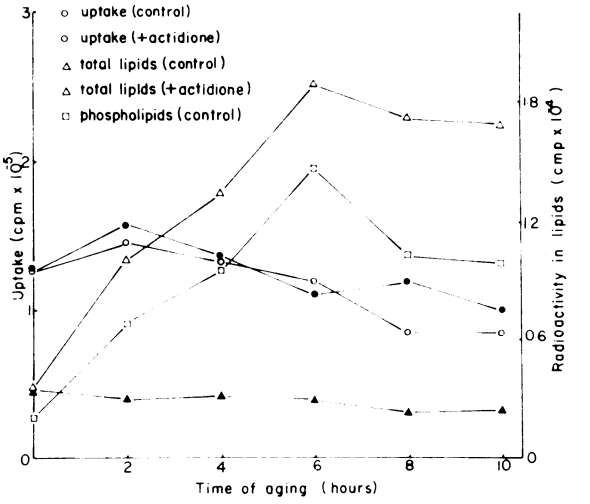

FIG. 3. Total uptake and incorporation of radioactivity into lipids by potato slices incubated for 2 hours in the presence of acetate-1-^{14}C. Before the incubation the slices were aged for 0 to 10 hours as indicated on the abscissa. Dark symbols indicate that the slices were aged in the presence of actidione.

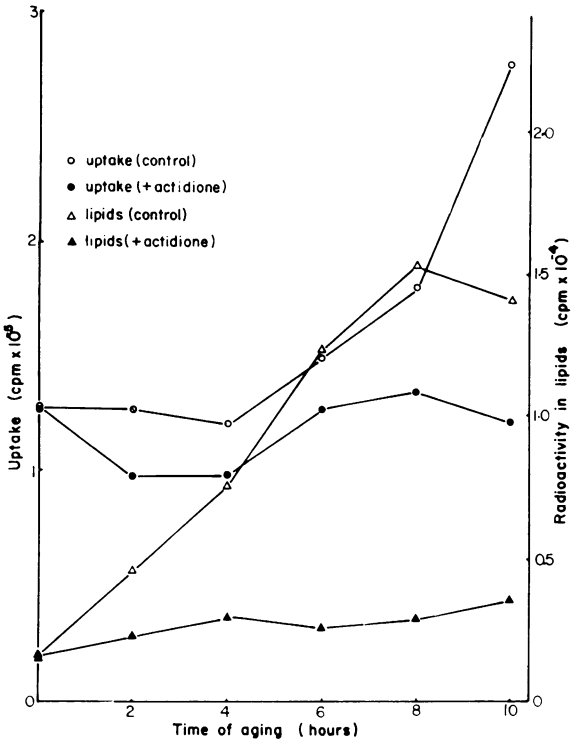

FIG. 4. Total uptake and incorporation of radioactivity into lipids by potato slices incubated for 2 hours in the presence of ethanolamine-1,2-^{14}C. Before the incubation the slices were aged for 0 to 10 hours as indicated on the abscissa. Dark symbols indicate that the slices were aged in the presence of actidione.

It appears unlikely that the incorporation of radioactive precursors into lipids is due to bacterial contamination because of the presence of chloromycetin in these experiments. Furthermore Kloit (13) has reported that bacteria are insensitive to actidione. Hence the observed inhibition of phospholipid synthesis in the presence of actidione strengthens our confidence that bacterial contamination is probably insignificant in our system.

Table I. *Incorporation of Radioactivity Into Lipids by Potato Slices Using Different Labeled Precursors and Slices of Different Ages*

The assay was done by incubating 10 slices in 1 ml $CaSO_4$/chloromycetin solution containing 0.5 μc of the radioactive phospholipid precursor for 2 hours. The incorporation is expressed as percent and calculated as follows: Radioactivity in lipids/total radioactivity taken up × 100.

Precursor	Time of aging 0	2	4	6	8	10
			hr			
Acetate-1-^{14}C	2.8	6.8	10.5	16.8	19.5	21.0
Ethanolamine-1,2-^{14}C	2.5	7.0	12.0	19.0	5.5	3.5
Choline-1,2-^{14}C	3.8	3.6	4.0	4.4	4.3	4.2
Choline-methyl-^{14}C	2.2	2.3	2.5	2.1	2.1	2.0
Methionine-methyl-^{14}C	0.4	0.55	1.1	1.0	1.2	0.72
L-Serine-u-^{14}C	0.08	0.38	0.4	0.5	0.55	0.45

Stability of the Incorporated Lipid Soluble Radioactivity. In order to exclude the possibility that the radioactivity found in the lipid fraction from slices treated with labeled choline or ethanolamine was due to an exchange reaction instead of new synthesis, the following experiments were carried out. After an initial 2 hour pulse labeling in 0.5 μc of choline-1,2-^{14}C, the slices were placed in either $CaSO_4$/chloromycetin solution or in such a solution containing unlabeled choline for 4, 10, or 22 hours before lipids were isolated. After the initial pulse the radioactivity in lipids continued to increase for another 22 hours in $CaSO_4$/chloromycetin solution (table II). However, if the chase was carried out in the presence of 0.01 M ^{12}C-choline, the lipid soluble radioactivity increased in the first 4 hours, a slight decrease was observed subsequently in the next 18 hours.

Table II. *Change of Radioactivity in Lipids After the Incubation in Either $CaSO_4$ Solution or ^{12}C-Choline Solution for Different Period of Times Following a Two Hour Pulse Labeling*

The radioactivity in lipids after pulse labeling is 23,500 cpm per 10 slices.

Incubation time	Radioactivity in lipids $CaSO_4$	^{12}C-choline
hr	*cpm*	*cpm*
4	54,200	36,600
10	80,000	28,000
22	110,200	26,000

Identification of the Labeled Compounds Inside the Slices. The radioautograph of the thin layer chromatogram of the lipids isolated from 6-hour-old slices incubated with different precursors is shown in figure 5. From radioactive choline, most of the labeling is concentrated in a single spot corresponding to standard lecithin. Several other slightly labeled lipids can also be seen. The incorporation of both methionine and serine is very low. From methionine-methyl-^{14}C, most of the radioactivity was found in lipids with R_F value higher than phosphatidylethanolamine. The major spot found in lipids isolated from serine-treated slices is phosphatidylethanolamine. Many lipids were labeled from acetate-1-^{14}C. Among them, lecithin possessed the highest radioactivity, neutral lipids, phosphatidylethanolamine, and possibly phosphatidylglycerol were labeled in a decreasing order. Lipids isolated from slices incubated with ethanolamine showed some variation depending on the storage history and condition of the tuber. Sometimes 2 successive spots with decreasing radioactivity were found right behind phosphatidylethanolamine, and sometimes lecithin was also found labeled. The labeling pattern for fresh tissue was quite similar.

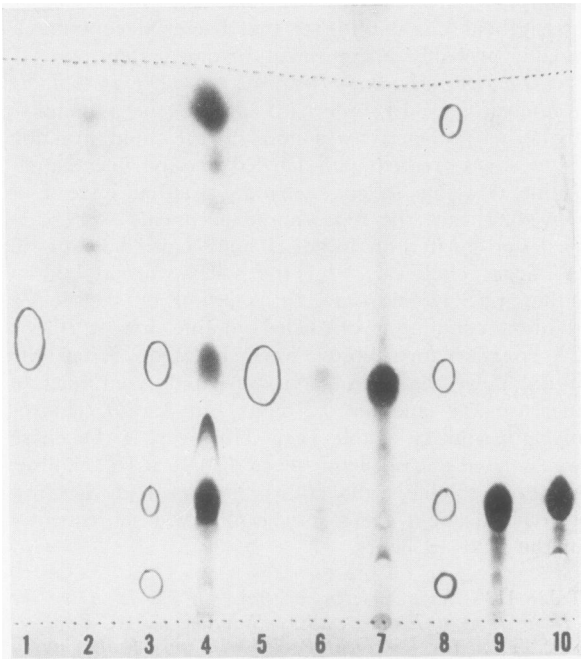

FIG. 5. Radioautograph of thin-layer chromatography of lipids isolated from 6-hour-old potato slices. In all cases, 0.5 μc of the radioactive precursor was used except for choline-1,2-^{14}C where 0.25 μc was used. One and 5 are standard phosphatidylethanolamine; 3 and 8 are mixtures of standard lysolecithin, lecithin, phosphatidylethanolamine, and cholesterol arranged in an order of increasing R_F values. Two, 4, 6, 7, 9, and 10 are the patterns of lipids isolated from slices incubated with methionine-methyl-^{14}C, acetate-1-^{14}C, L-serine-u-^{14}C, ethanolamine-1,2-^{14}C, choline-1,2-^{14}C, and choline-methyl-^{14}C respectively.

Further identification of phospholipids was obtained by paper chromatography of the nitrogenous base released by hydrolysis in 4 N HCl. Only the parent compounds were found to be labeled from both radioactive choline and radioactive ethanolamine-treated slices.

Slices which had been previously aged for 0, 4, and 8 hours, were incubated with 2 μc of methionine-methyl-^{14}C for a 4 hour period. The lipids were extracted, hydrolyzed and the free bases analyzed by paper chromatography. A sequential picture emerged which is shown in figure 6. From 0 to 4 hours, a peak was found corresponding to standard *N*-methyl-ethanolamine. From 4 to 8 or 8 to 12 hours, the 2 major peaks were found having the same R_F values as standard choline and *N*-methyl-ethanolamine. The small shoulder in front of the choline peak is possibly *N*,*N*-dimethyl-ethanolamine.

The question of whether the water soluble radioactivity inside the slices is present as choline or as one of its derivatives during the time course of phospholipid synthesis and the pulse and chase experiments was tested by the method of Sung and Johnstone (32). The aqueous extracts of potato disks were passed through Dowex 50 (H^+ form) columns. In this system, phosphorylcholine is not retained, betaine is eluted by 2 N NH_4OH. It was found that less than 5 % of the radioactivity came through the column in the water wash. Most of it was retained by the column and could be eluted out by 3 N HCl. The compound possessing radioactivity in HCl eluate was identified as choline by paper chromatography in the 2 solvent systems mentioned above.

Discussion

Results from acetate-1-^{14}C indicated that its uptake into the slices did not change significantly during aging and was actidione insensitive. The observed increase in lipid-soluble radioactivity is in agreement with the work of Willemot and Stumpf (37), who have shown that tuber slices undergo a marked increase in fatty acid synthetase activity during aging. More than half of the fatty acids thus synthesized were incorporated into phospholipids (fig 3). In light of the important role played

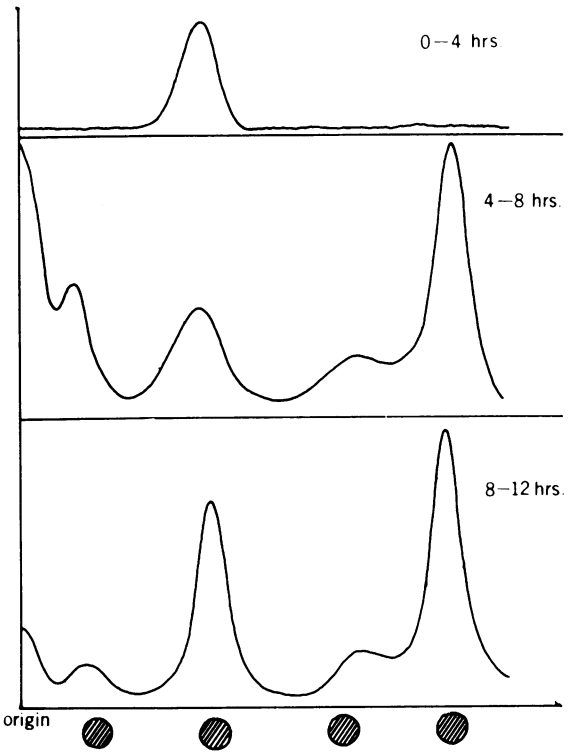

FIG. 6. Sequential studies of lipid hydrolysates from slices incubated with 2 μc of methionine-methyl-^{14}C for 4 hours. Lipids isolated were hydrolyzed in 4 N HCl and the aqueous hydrolysates were separated by paper chromatography in a solvent system of phenol:*n*-butanol: 80 % formic acid: H_2O (50:50:3:5,w/v/v/v), saturated with KCl. The standard compounds counting from the origin are ethanolamine, *N*-methyl-ethanolamine, *N*,*N*-dimethyl-ethanolamine and choline.

by phospholipids in cellular structures and processes, these findings are interesting.

The behavior of choline was completely different from that of acetate. Its uptake by the slices showed a conspicuous increase with aging and the observed increase in radioactive lipids appeared to be a reflection of this increased uptake. The protein synthesis dependence of choline uptake due to its inhibition by actidione and the observed lag period during early aging suggest that the synthesis of at least 1 enzyme may be involved in its transport across the cellular membrane.

Three different pathways have been reported for lecithin synthesis: (1) incorporation of free choline via CDP-choline (19); (2) stepwise methylation of phosphatidylethanolamine (11, 15); and (3) calcium stimulated exchange reaction between free choline and preformed lecithin. The exchange reaction found in rat liver microsomes was reported by Dils and Hubscher (14). Its *in vivo* significance is still unknown. If the radioactivity obtained during a 2 hour pulse labeling is due to such an exchange instead of *de novo* synthesis, the further 4 hour incubation in ^{12}C-choline would be long enough to dilute the choline pool to a great extent and exchange part of the labeled choline out from lecithin molecules, thus causing a decrease in the total activity in the lipid fraction. From the data shown in table II, an increase was obtained instead, indicating that the radioactivity found in lipid fraction is not from the exchange between ^{14}C-choline itself and preformed lecithin. During the chase period, the radioactivity still increases showing that the lipids formed are stable for at least 4 hours. The equilibration between different choline pools and the turnover of phospholipids possibly contribute to the decrease in radioactivity which is observed later on. It is likely that the isotope dilution inside the pool by the choline synthesized within the cell is not large, and during the chase period studied, the rate of synthesis is faster than breakdown. This is shown by the fact that in $CaSO_4$ solution the radioactivity increases even after 22 hours. It is also indicated by the table that a pool of water-soluble radioactivity was present after the pulse labeling and that this radioactivity could be incorporated into lipids later. Results from ion exchanger separation showed that the major radioactive compound in this pool is unmetabolized choline.

The methylation pathway seems to be present in potato tissue, although its contribution is very slight compared to the mechanism of incorporating free choline directly. The presence of more than 1 pathway for lecithin biosynthesis has also been reported by Sastry and Kates (29) in a plant system. One possible explanation for the existence of both mechanisms is that they are responsible for the synthesis of different lecithins. Balint *et al.* (3) found in rats that the methylation pathway was involved in the synthesis of arachidonyl-lecithin while the direct incorporation of choline accounted for the synthesis of linoleoyl-lecithin.

Judging from the results of paper and thin-layer chromatography, the major pathway for phosphatidylethanolamine synthesis is via ethanolamine itself. Although the major lipid isolated from slices incubated with L-serine-u-^{14}C is phosphatidylethanolamine, the contribution of phosphatidylserine to give phosphatidylethanolamine via a decarboxylation reaction as reported by Wilson *et al.* (38) is very slight, because the total amount of serine incorporated is very little compared to that of ethanolamine.

Although little direct information is available concerning the biosynthesis of glycerophosphatides in higher plant tissue, evidences suggest that the pathway worked out by Kennedy (19) is operative. In this pathway phosphorylcholine (phosphorylethanolamine) is the first intermediate. This compound is transformed into CDP-choline (CDP-ethanolamine) by reacting with CTP. This nucleotide derivative of choline (ethanolamine) can then react with diglyceride to form lecithin (phosphatidylethanolamine). From both the time course and the pulse-chase experiments, it appears that the amount of radioactive phosphorylcholine present is small in comparison with the choline pool, indicating that if phosphorylcholine is one of the intermediates present in the pathway for lecithin synthesis, the reaction for its formation must be rate determining. Whether the synthesis of lecithin and phosphatidylethanolamine from choline and ethanolamine directly is mediated by cytidine nucleotide can not be predicted from the data presented here. We hope that *in vitro* studies with cell free systems will shed some light on this point.

Very likely, this phospholipid synthesizing ability which is present in potato tuber tissue and which increases so markedly during the first 6 hours of aging (fig 1–4) represents the biogenesis of certain types of cellular membranes. We have undertaken a series of experiments using radioactive phospholipid precursors and density gradient separations which, we hope, will tell us what membrane systems are synthesized during the first crucial hours of aging.

Acknowledgments

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Literature Cited

1. Ansell, G. B. and J. N. Hawthorne. 1964. Phospholipids: Chemistry, Metabolism, and Function. Vol. 3. Elsevier, New York. p 244.
2. Asahi, T., Y. Honda, and I. Uritani. 1966. Increase of mitochondrial fraction in sweet potato tissue after wounding or infection with *Ceratocystis fimbriata*. Plant Physiol. 41: 1179–84.

3. BALINT, J. A., D. A. BEELER, D. A. TREBLE, AND H. L. SPITZER. 1967. Studies on the biosynthesis of hepatic and biliary lecithin. J. Lipid Res. 8: 486–93.
4. BENSON, A. A., S. FREER, AND S. F. YANG. 1965. Intern. Conf. Biochem. Probl. Lipids, 9th, Noordwijk, Netherlands, September.
5. BJORNSTAD, P. AND J. BREMER. 1966. *In vivo* studies on pathways for the biosynthesis of lecithin in the rat. J. Lipid Res. 7: 38–45.
6. BLIGH, E. G. AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–17.
7. BOEHM, J. 1887. Über die Atmung der Kartoffel. Bot. Zeitung 45: 671–75, 681–91.
8. BRANTE, G. 1949. Iodine as a means of development in paper chromatography. Nature 163: 651–52.
9. BREIDENBACH, R. W., P. CASTELFRANCO, AND C. PETERSON. 1966. Biogenesis of mitochondria in germinating peanut cotyledons. Plant Physiol. 41: 803–09.
10. BREMER, J., P. H. FIGARD, AND D. M. GREENBERG. 1960. The biosynthesis of choline and its relation to phospholipid metabolism. Biochim. Biophys. Acta 43: 477–88.
11. BREMER, J. AND D. M. GREENBERG. 1961. Methyl transfering enzyme system of microsomes in the biosynthesis of lecithin. Biochim. Biophys. Acta 46: 205–16.
12. CLICK, R. E. AND D. P. HACKETT. 1963. The role of protein and nucleic acid synthesis in the development of respiration in potato tuber slices. Proc. Natl. Acad. Sci. U.S. 50: 243–50.
13. DE KLOET, S. R. 1966. Effect of actidione on the synthesis of RNA in *Saccharomyces carlsbergensis*. Biochem. J. 99: 566–81.
14. DILS, R. R. AND G. HÜBSCHER. 1961. The effect of Ca^{2+} on the incorporation of labeled choline into rat liver microsomes. Biochim. Biophys. Acta 46: 505–13.
15. GIBSON, K. D., J. D. WILSON, AND S. UDENFRIEND. 1961. The enzymatic conversion of phospholipid ethanolamine to phospholipid choline in rat liver. J. Biol. Chem. 236: 673–79.
16. HACKETT, D. P., D. W. HAAS, S. K. GRIFFITHS, AND D. J. NIEDERPRUEM. 1960. Studies on development of cyanide-resistant respiration in potato tuber slices. Plant Physiol. 35: 8–19.
17. HACKETT, D. P. AND K. V. THIMANN. 1953. The nature of the auxin induced water uptake by potato tissue. II. The relation between respiration and water absorption. Am. J. Botany 40: 183–88.
18. KATES, M. 1954. Lecithinase system in sugar beet, spinach, cabbage, and carrot. Can. J. Biochem. Physiol. 32: 571–83.
19. KENNEDY, E. P. AND S. B. WEISS. 1956. The function of cytidine in the biosynthesis of phospholipids. J. Biol. Chem. 222: 193–214.
20. LATIES, G. G. 1957. Respiration and cellular work and the regulation of the respiration rate in plants. Surv. Biol. Progr. 3: 215–99.
21. LATIES, G. G. 1963. Control of respiratory quality and magnitude during development. In: Control Mechanisms in Respiration and Fermentation. Barbara Wright, ed. Ronald Press. p 129–55.
22. LATIES, G. G. 1964. The onset of tricarboxylic acid cycle activity with aging in potato slices. Plant Physiol. 39: 654–63.
23. LEAVER, C. J. AND J. EDELMAN. 1965. Antibiotics as a means of control of bacterial contamination of storage tissue disks. Nature 207: 1000–01.
24. LEE, S. G. AND R. M. CHASSON. 1966. Aging and the development of enhanced respiration in potato tuber tissue. Physiol. Plantarum 19: 194–98.
25. LEE, S. G. AND R. M. CHASSON. 1966. Aging and the mitochondrial development in potato tuber tissue. Physiol. Plantarum 19: 199–206.
26. LEPAGE, M. 1964. The separation and identification of plant phospholipids and glycolipids by two dimensional thin-layer chromatography. J. Chromatog. 13: 99–103.
27. MARRÉ, E., S. COCUCCI, AND E. STURANI. 1965. On the development of the ribosomal system in the endosperm of germinating castor bean seeds. Plant Physiol. 40: 1162–70.
28. MUNIER, R. 1951. Microchromatographie de partage sur papier des alcaloides et de diverser bases azotees biologiques. V. Separation des constituants azotes der phosphoaminolipides (choline, colamine, serine). Bull. Soc. Chim. Biol. 33: 862–67.
29. SASTRY, P. S. AND M. KATES. 1965. Biosynthesis of lipids in plants. I. Incorporation of orthophosphate-^{32}P and glycerophosphate-^{32}P into phosphatides of *Chlorella vulgaris* during photosynthesis. Can. J. Biochem. 43: 1445–53.
30. SPERLING, E. AND G. G. LATIES. 1963. The dependence of auxin induced growth on auxin independent metabolic changes in slices of storage tissue. Plant Physiol. 38: 546–50.
31. STEWARD, F. C., P. R. STOUT, AND C. PRESTON. 1940. The balance sheet of metabolites for potato disks showing the effects of salts and dissolved O_2 on metabolism at 23°. Plant Physiol. 15: 409–47.
32. SUNG, C. P. AND R. M. JOHNSTONE. 1965. Active transport in rat kidney cortex slices. Can J. Biochem. 43: 1111–18.
33. THIMANN, K. V., C. S. YOCUM, AND D. P. HACKETT. 1954. Terminal oxidases and growth in plant tissues. III. Terminal oxidation in potato tuber tissue. Arch. Biochem. Biophys. 53: 239–57.
34. VERLEUR, J. D. AND I. URITANI. 1965. Respiratory activity of the mitochondrial fractions isolated from healthy potato tuber and from tuber tissue incubated after cutting or infection with *Ceratocystis fimbriata*. Plant Physiol. 40: 1008–12.
35. WALTERS, L. C. AND L. S. DURE. 1966. Ribonucleic acid synthesis in germinating cotton seeds. J. Mol. Biol. 19: 1–27.
36. WILLEMOT, C. AND P. K. STUMPF. 1967. Fat metabolism in higher plants. XXXIII. Development of fatty acid synthetase during the "aging" of storage tissue slices. Can. J. Botany 45: 579–84.
37. WILLEMOT, C. AND P. K. STUMPF. 1967. Fat metabolism in higher plants. XXXIV. Development of fatty acid synthetase as a function of protein synthesis in aging potato tuber slices. Plant Physiol. 42: 391–97.
38. WILSON, J. D., K. D. GIBSON, AND S. UDENFRIEND. 1960. Studies on the conversion *in vitro* of serine to ethanolamine by rat liver and brain. J. Biol. Chem. 235: 3539–43.