DIFFERENCES IN THE METABOLISM OF PHOSPHOLIPIDS DEPENDING ON CELL POPULATION DENSITY.

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Summary: Pulse-chase experiments in embryonic mouse fibroblasts at low and high cell population densities using radioactive phosphate and tritiated glycerol as precursors revealed a blocked turnover of phosphatidylinositol and a blocked biosynthesis of phosphatidylethanolamine in densely packed cells.

Introduction: Since the discovery of differences in the glycolipid composition of in vitro propagated normal and tumor cells by Hakomori and Murakami (1) the lipid metabolism of cells grown in tissue culture has attracted increasing attention. The observed differences in the glycolipid composition could in some cases be attributed to the population densities of the cells studied (2,3,4,5). We have shown that the level of cellular triglycerides increases when cells grown on petri dishes reach confluency or when grown in suspension culture reach saturation density (6). In this communication we will report on two reactions of phospholipid metabolism which we have found to be dependent on cell population density.

Methods and Materials: Eagle's minimum essential medium (7) used for cell culture was modified using twice the concentration of amino acids and four times the concentration of vitamins. In addition the medium contained 0,45 % glucose and 1 mM sodium pyruvate. Earle's balanced salt solution was used. The medium was fortified by addition of 10 % fetal calf serum.

Embryonic STU mouse fibroblasts (6) of the 3rd passage were seeded into 10 cm Falcon petri dishes ($1,5 \cdot 10^6$ cells/dish). Six hours after seeding, when the cells were firmly attached to the surface of the dish the medium was replaced by 8 ml of medium containing 100 µCi of carrier free ^{32}P-orthophosphate and 30 µCi of 1(3)-^{3}H-glycerol (2,5 Ci/ mM, The Radiochemical Centre, Amersham). After 12 hours this medium was removed, the cells were washed twice with fresh phosphate buffered medium containing nonradioactive glycerol

(3 drops/liter). Half the number of dishes was incubated further with 1o ml of this medium. These cells continued to grow exponentially. The other half of the plates was incubated with the same amount of medium containing 1,5 • $1o^7$ nonradioactive embryonic cells from the 3rd passage. This resulted in a densely packed layer of cells. The medium was again changed in all plates after 36 hours. At time intervals given inthe figures the cells from one dish of each of the two groups were harvested with a rubberpoliceman and the lipids were seperated as has been described (8).

Results and Discussion: The fate of the ^{32}P and the tritiated glycerol incorporated during the pulse period into the variuos phospholipids of STU cells was studied in exponentially growing cells and cells in a confluent layer over a period of up to 85 hours. Within these two groups no differences could be detected in the kinetics of the ^{32}P-decrease from phosphatidylserine and sphingomyelin. Significant differences, however, were found in the release of ^{32}P from phosphatidyl - inositol and phosphatidylethanolamine. The significance of slight differences in the decrease of the ^{32}P content of phosphatidylcholine in growing and confluent cells remains to be determined.

Figure 1a shows that the decrease of the ^{32}P content of phosphatidylinositol is significantly slower in confluent cells than in exponentially growing cells. This is most pronounced beginning 12 hours after the chase and the addition of unlabelled cells.

The time course of the $^{3}H/^{32}P$-ratio found in phosphatidylinositol is plotted in Figure 1b. This ratio increases in the cells growing exponentially but remains constant in cells at confluency. An increase in the $^{3}H/^{32}P$-ratio during the chase period of exponentially growing cells appears to be unique for phosphatidylinositol since it has not been found in the other phospholipids (8). This increase in the ratio of $^{3}H/^{32}P$ has been interpreted as a recycling of the diacylglycerol moiety of phosphatidylinositol in the biosynthesis of this lipid (8). Using different systems of resting and stimulated cells and tissues and different experimental design other investigators have reached the same conclusion (9,1o,11,12). The data presented give an explanation for our earlier results obtained in pulse labelling experiments, where the relative specific activity of ^{32}P in phosphatidylinositol was consistently higher in sparsely populated cultures as compared to densely packed cells (6).

The biphasic disappearence of ^{32}P from phosphatidylinositol

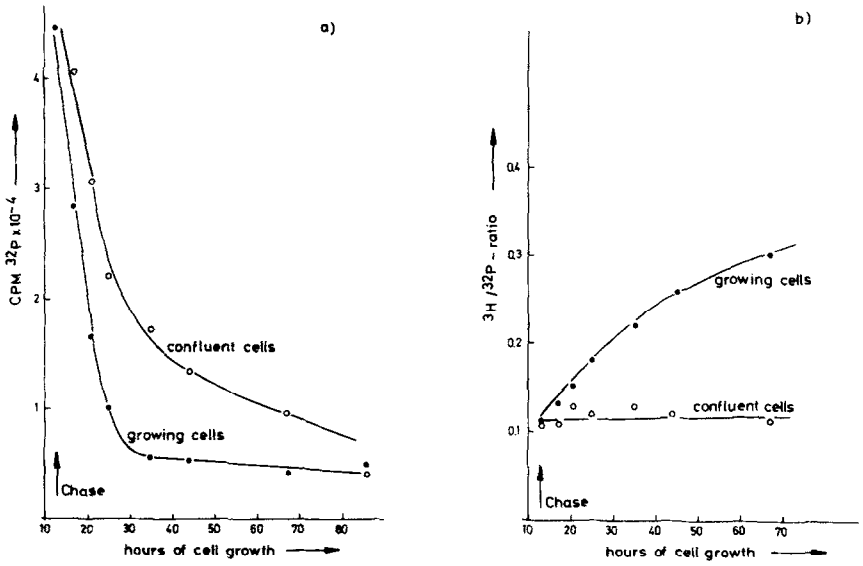

Figure 1

a. ^{32}P-release from phosphatidylinositol of exponentially growing STU cells and of densely packed cells.
b. The $^{3}H/^{32}P$-ratio of phosphatidylinositol of exponentially growing and densely packed STU cells. The lipid was labelled with tritiated glycerol and radioactive phosphate durind a 12 hours pulse period.

(8,13,14,15) has been shown to be due in part to a release of cyclic inositol phosphate (16,17). The diacylglycerol moiety can then be reutilized for the biosynthesis of phosphatidylinositol (8,9,1o,11,12). This reutilization is blocked in cells at confluency as can be deduced from the unchanged $^{3}H/^{32}P$-ratio. Furthermore the data show that the recycled diacylglycerol is derived from the rapidly occuring phosphatidylinositol breakdown. The slower decrease of the ^{32}P content of this lipid in cells at high population density indicates that the process of recycling is blocked at the level of phosphatidylinositol breakdown. A release of this block has been postulated for the action of acetylcholine which stimulates the phosphatidylinositol turnover of pancreas slices (1o). In lymphocytes stimulated by phythemagglutinin the action of the stimulating agent, resulting in an increased phosphatidylinositol turnover, was attributed to a deblocking of the diglycerol kinase (9).

The changes in the ^{32}P content of phosphatidylethanolamine in exponentially growing and confluent cells are given in Figure 2. The incorporation of ^{32}P into this lipid continues for about 3o hours

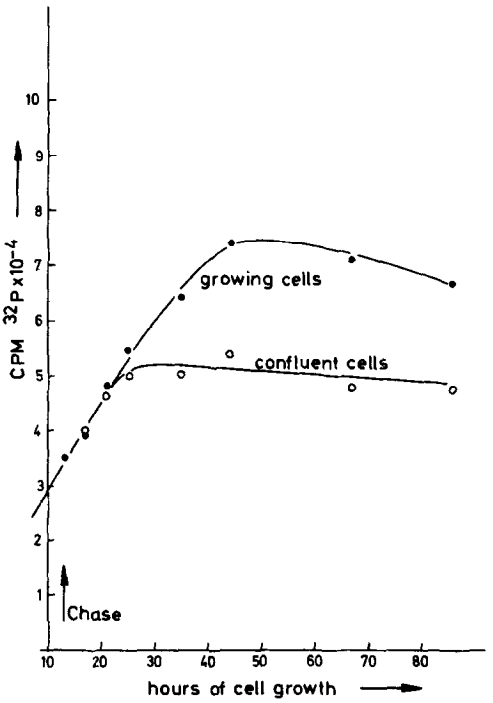

Figure 2

The ^{32}P-release from phosphatidylethanolamine of exponentially growing and densely packed STU cells.

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after the chase in exponentially growing cells. In confluent cells, on the other hand, this incorporation ceases completely already 1o hours after the chase.

The prolonged incorporation of ^{32}P into phosphatidylethanolamine observed during the chase period, which can be blocked completely by establishing close contact between cells, must proceed via a pathway different from the established (18) incorporation via CDP-ethanolamine (Diringer, Koch to be published). Preliminary experiments suggest that phosphatidylcholine is the source of the ^{32}P incorporated into phosphatidylethanolamine during the chase period as has been postulated earlier (8).

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