THE METABOLISM OF LINOLEIC ACID IN MAMMALIAN CELLS IN CULTURE*

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The biosynthesis and transformations of the polyunsaturated fatty acids have been largely elucidated through studies with whole animals (Mead, 1961; Klenk, 1961), liver slices (Mohrhauer and Holman, 1965) and subcellular particulate preparations (Nugteren, 1962; Stoffel, 1963; Stoffel and Ach, 1964). The functions of these substances and the significance of their structural peculiarities, however, have not generally yielded to these approaches. It seems probable that these fatty acids are important as precursors of the prostaglandins (Van Dorp et al., 1964; Bergstrom et al., 1964) and that they have some function in transport, membrane integrity and certain enzyme structures, but the actual nature of these latter functions is still largely conjecture and their action in cellular metabolism in general is obscure.

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It seems obvious that some experimental subject of lower complexity than the whole animal but with the cellular structures largely intact is required to elucidate the function of polyunsaturated fatty acids in metabolism. For this purpose the mammalian cell in culture was chosen, since it has the proper order of complexity, and unlike the bacteria, appears, in at least one case (Ham, 1963), to require a source of essential fatty acid. In a study of the influence of fatty acids on the metabolism of cells in culture, the results of which will be reported elsewhere (Gerschenson et al. - submitted for publication), two types of cells were chosen, the HeLa cell, which has existed in culture through many generations, and a culture of beating newborn rat heart cells developed in This Laboratory (Harary and Farley, 1960). As a part of the study, the metabolism of linoleic acid by these cells was also investigated. The somewhat unexpected finding that, contrary to all previous studies to date on mammalian systems and unlike the heart cells, the HeLa cells appear to be unable to transform linoleic acid to arachidonic acid forms the basis of the present communication

MATERIALS AND METHODS

Cell Culture. HeLa S_3 cells were cultured on glass using normal growth medium (Harary and Farley, 1960) supplemented with 20 per cent fetal calf serum (Microbiological Associates). For a given experiment, cells were transferred to plastic Petri dishes and allowed to grow for two days on the normal medium, at which time they were rinsed twice with a balanced salt solution and incubated on the experimental medium. This medium consisted of normal growth medium minus serum plus either fatty acid-free albumin (Goodman, 1957) or albumin-bound linoleic acid (Harary et al., 1965). Linoleic acid-1-^{14}C was dissolved in ethanol (4.0 micromoles/ml. with an activity of 24.7 mc/mM) and an aliquot was added to the medium containing 0.5 moles albumin per mole of fatty acid. The mixture was left for two hours at 37° C with occasional shaking

before use. Reduced glutathione (20 mM) was added to prevent peroxide formation. The cells were incubated for the duration of the experiment at 37° C under a gas phase consisting of 5% CO_2, 95% air, and the medium was changed every two days. Growth was defined as increase in protein (Lowry et al., 1951). At the end of the experiment, the cells were removed from the Petri dishes using a rubber policeman and collected in a 13 ml. centrifuge tube. They were rinsed twice with balanced salt solution and the supernatant was poured off after centrifugation. The cells were analyzed immediately or stored at -10° under N_2 until use.

Newborn rat heart cells were cultured for two days according to the method of Harary and Farley (1963). Transfer to the experimental medium and subsequent treatment were essentially as described for the HeLa cells.

Extraction of Cells. The cells were homogenized in a ground glass hand homogenizer using 5 ml. of 2.5:1 $CHCl_3$:CH_3OH. The centrifuge tube and homogenizer were rinsed twice with 5 ml. portions of the extraction solvent and the washings combined with the original extract. The total solution was then filtered by gravity through a filter paper to remove insoluble material and the filtrate was collected in a centrifuge tube with teflon-lined screwcap and evaporated to dryness at 45° C under nitrogen. Transmethylation of the extracted lipids was accomplished by heating the extracts in the tightly closed centrifuge tubes at 90° C for one hour after addition of 1 ml. anhydrous benzene, 1 ml. of 7% BF_3-CH_3OH reagent and 2 ml. CH_3OH (Morrison and Smith, 1964). The resulting methyl esters were dissolved in 5 ml. pentane and the pentane phase, after addition of 2 ml. water, was separated, washed twice with 2 ml. portions of water, dried over anhydrous magnesium sulfate and freed of solvent at 40° C under nitrogen. The resulting methyl esters were analyzed using a Loenco Model 70 gas chromatographic apparatus equipped with 1/4 in. X 4 ft. column of 10% DEGS on Chromosorb W, 60/80

mesh, a thermal conductivity mass detector and an ionization chamber for detection of radioactivity. Chromatography was carried out at 175° C and peaks were identified by comparison with standards.

RESULTS AND DISCUSSION

In Fig. 1 are plotted the amounts and activities of the fatty acids derived from the lipids of heart cells incubated with 10^{-7} M linoleic acid-1-^{14}C. It may be noted first from the high specific activity of the

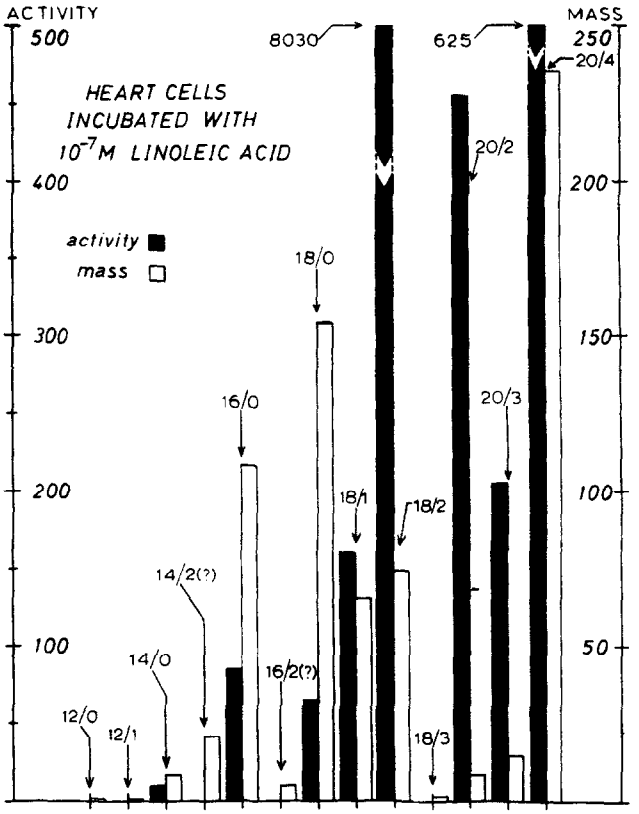

FIG. 1

Uptake of Radioactivity From Linoleic-1-^{14}C Acid By Fatty Acids of Heart Cells.

Amount (from thermal conductivity detector) and radioactivity (from ionization detector) in arbitrary units on the ordinates, plotted against chain-length and unsaturation of fatty acids on the abscissa. Heights of bars reflect actual peak heights multiplied by the relative retention time in order to represent actual area of the GLC peaks.

linoleic acid that this acid was readily absorbed by the cells and incorporated into the cellular lipids. Second, the high activity in 20:3, 20:4 and particularly a fatty acid with the retention time of 20:2 serves to indicate the ready conversion of linoleate to arachidonate probably by the pathway studied by Stoffel (1963) in subcellular particles, rather than that proposed by Mead (1961) for the whole animal (No activity is seen in 18:3). The much lower activity in 14:0, 16:0, 18:0 and 18:1, undoubtedly results from incorporation of radioactive acetate from degradation of the linoleate into these acids.

A different picture is seen in the case of the HeLa cells (Fig. 2).

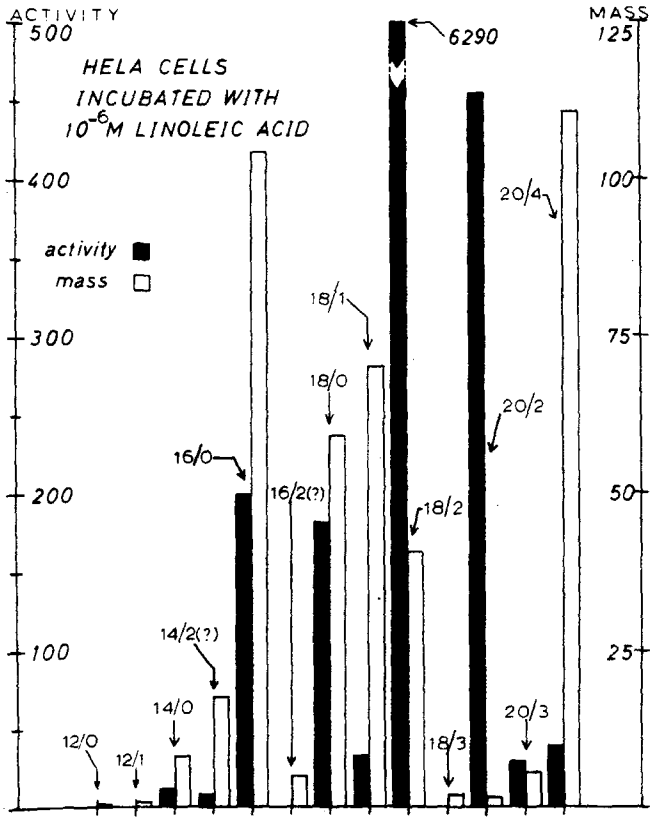

FIG. 2

Uptake of Radioactivity From Linoleic-1-^{14}C Acid By Fatty Acids of HeLa Cells.

Coordinates are the same as for Fig. 1.

The high activity of 18:2 and the trace activity in the biosynthetic acids are similar to the results with the heart cells. However, within the limits of sensitivity of the method (better than 0.1% of the linoleate activity) negligible activity appeared in 20:3 or 20:4, indicating little or no transformation of linoleate to arachidonate in these cells. Although an attempt was made to estimate values for the radioactivity of these acids for inclusion in Fig. 2, it should be emphasized that these estimates are of doubtful significance. Moreover, there is very high activity in the 20:2 although only a negligible mass peak could be detected. From the results with the heart cells it seems highly probable that this acid is $20:2^{11,14}$, the product of elongation of linoleic acid and the substrate for desaturation to $20:3^{8,11,14}$ and $20:4^{5,8,11,14}$ (arachidonate).

It seems evident from these results that although the heart cells are capable of carrying on an active metabolism of polyunsaturated fatty acids, the HeLa cells are unable to desaturate the fatty acids provided or formed in the medium although they appear to possess the machinery for elongation. The question naturally arises as to whether this apparent inability is due to the nature of the HeLa cells as cancer cells or as cells raised in culture for many generations. The fact that the heart cells can effect this transformation would hint at the former reason although these cells had been in culture only two days, and it is possible that, during the dedifferentiation resulting from longer periods in culture, the desaturation mechanism could be lost (Harary et al., 1963).

A further point of interest stems from the observation (Gerschenson et al., submitted for publication) that with HeLa cells, growth, respiratory control and oxidative phosphorylation are maintained near normality with 2×10^{-8} M linoleate nearly as well as with 10^{-8} M arachidonate. It thus appears that, at least with these cells, linoleate functions as an essential fatty acid in its own right rather than by virtue of its

transformation to arachidonic acid. The nature of this function remains the object of further study.

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