THE EFFECT OF LIPIDS ON ENZYME LEVELS IN BEATING RAT HEART CELLS*

Atsuko Fujimoto and Isaac Harary

Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics and Nuclear Medicine, and Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California

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During the incubation period of beating rat heart cells in culture there occurs a loss of beating and a shift from lipid to carbohydrate oxidation (Fujimoto and Harary, 1964). At the same time there also occurs a sharp drop in malic and isocitric dehydrogenase and an increase in glucose 6-phosphate dehydrogenase activities, which may be correlated with a decrease in oxygen uptake. A similar loss in calcium activated adenosine triphosphatase (Ca-ATPase), may be correlated with the observed cessation of beating (Harary et al, 1964; Kuramitsu and Harary, 1964).

The shift in metabolism and loss in ability to oxidize palmitate (Fujimoto and Harary, 1964), together with the loss of function, indicated that lipids play a central role in the maintenance of heart cell function. Many observations indicate that lipids are an important fuel in heart respiration (Bing, 1965). Support for the functional role of lipids also derives from the observation that fatty acids and other lipids can restore beating in cells which have stopped beating in a lipid deficient medium (Harary, 1964; Harary et al, In Press).

Investigation of the mechanism of the lipid effect has centered

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about three main areas: 1) lipids as a specific energy source; 2) lipids in membrane function; and 3) lipids as they may affect protein synthesis. The indications are that lipids are not a specific energy source for beating since ATP synthesized from any metabolic source may be used (Harary and Slater, 1965). This report deals with the third area listed and concerns the effect of serum lipids on the levels of some of the enzymes which change in culture.

MATERIALS AND METHODS

Rat heart cells were incubated in culture using a medium which substituted bovine albumin (500 mg/l) or bovine albumin plus serum lipids, for the whole serum, in complete growth medium (Harary and Farley, 1963). Fetuin (2g/l) was added to the medium only for the attachment of cells to the culture dishes and the attachment medium was replaced by the experimental medium after 48 hrs. The cells were incubated with changes of medium once every two days for eight days. At intervals, cells were harvested by scraping, and Ca-ATPase, malic dehydrogenase, glucose 6-phosphate dehydrogenase and protein were determined (Fujimoto and Harary, 1964; Kuramitsu and Harary, 1964). Bovine albumin was purchased from Mann Research Laboratories and prepared free of fatty acids (Goodman, 1957). Fetuin was prepared from fetal calf serum by the method of Fisher (Personal Communication). Serum lipid was extracted from 1:1 mixture of human serum and fetal calf serum using cold chloroform-methanol (Harary et al, In Press). Cell growth was measured by protein determination.

RESULTS

The activity of Ca-ATPase and malic dehydrogenase fell, with time in culture, in cells incubated in a medium which contained lipid-free bovine serum albumin instead of whole serum, (Table I). The cells incubated in this medium did not grow or divide. In the presence of serum lipids, the fall in activity was partially prevented. On the other hand, the level of glucose 6-phosphate dehydrogenase, which rises in growing cells losing

TABLE I

Effect of Serum Lipids on Enzyme Changes in Cultured Heart Cells.

Days in Culture	Enzyme Activity (Unit/Mg. Prot.)					
	Ca-ATPase		Malic Dehydrogenase		Glucose 6-Phosphate Dehydrogenase	
	Albumin	Albumin + Serum	Albumin	Albumin + Serum	Albumin	Albumin + Serum
1	--	--	25,500	25,500	58.5	58.5
2	2.53	2.50	--	--	--	--
3	--	--	17,400	19,000	203	143
5	0.89	1.95	--	--	--	--
6	--	--	11,900	18,000	197	150
7	0.50	1.17	--	--	--	--
8	--	--	8,750	14,400	--	--

See text for conditions

their function (Kuramitsu and Harary, 1964), also increased in the albumin medium. The presence of serum lipids slowed this increase. In two other experiments the ratio of the specific activities, malic dehydrogenase to glucose 6-phosphate dehydrogenase, at the end of seven days, was 170 and 150 in cells grown in lipid supplemented media, and 67 and 30 in lipid deficient cells.

In the absence of lipids the beating stopped in 4 to 5 days, while in the presence of lipids the beating was maintained to the end of the experiment, 7 to 8 days. These experiments demonstrate that serum lipids maintain beating while at the same time partially preventing enzyme changes associated with the loss of function.

It was of interest to see if lipids could reverse the changes initiated by the lipid deficient medium. Cells were incubated in a medium with lipid free albumin. After five days in this medium the specific activities of malic dehydrogenase fell from 25,500 to 11,500 and glucose 6-phosphate

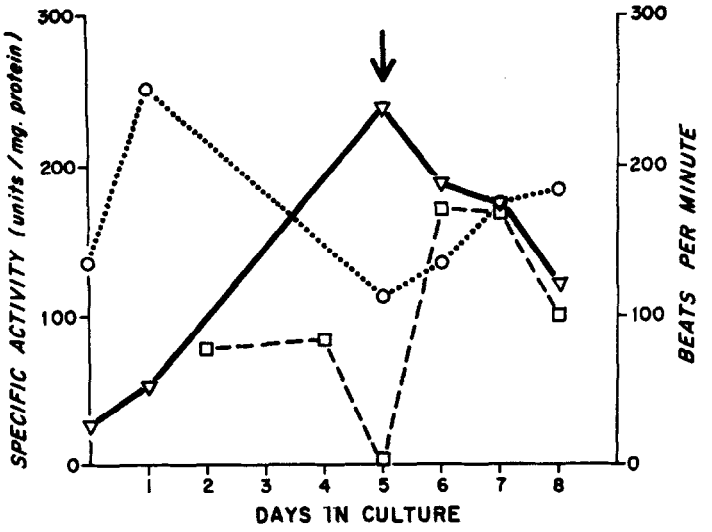

Fig. 1 Enzyme changes in lipid deficient and lipid supplemented media. The cells were cultured in growth medium with purified lipid free albumin. Malic dehydrogenase O•••O (specific activity X 10^{-2}) and glucose 6-phosphate dehydrogenase ▽——▽ were determined at the outset and on the second and fifth day. On the fifth day the beating □– – –□ stopped. At this time indicated by the arrow, the medium was changed to one supplemented with serum lipids at a concentration equivalent to that used in the complete growth medium which contains 20% serum.

dehydrogenase went from 54 to 242, (Figure I). At the same time the beating of these cells ceased. At this time the medium was changed to one containing serum lipids plus albumin and the cells were incubated for three more days. The beating was restored within 24 hrs., and on the 6th, 7th and 8th days, cells were collected and the enzyme activities determined. Malic dehydrogenase increased smoothly from 11,500 on the 5th day to 18,400 on the 8th day. Glucose 6-phosphate dehydrogenase decreased from 242 on the 5th day to 129 on the 8th day. These changes, resulting from the addition of serum lipid, are in the direction of restoring the levels of the enzymes to the original value in the freshly isolated heart cells.

DISCUSSION

The previous observations (Harary et al, 1964; Kuramitsu and Harary,

1964) linking enzyme changes with "dedifferentiation," or loss of function, were made in growing heart cell cultures. The present observations, demonstrating the same loss in Ca-ATPase and malic dehydrogenase and increase in glucose 6-phosphate dehydrogenase, were made in non-growing cultures. Thus, the changes observed may be attributed to the changes in the heart cells, which make up over 90% of the cell population, and not to a change in population due to cell division and overgrowth.

Serum lipids and fatty acids added to the albumin medium do not stimulate growth but reinitiate beating (Harary et al, In Press). The mechanism of this action is not known. The results reported here indicate that lipids in some way may maintain the enzyme activities characteristic of heart cells. We are exploring several possibilities at this time. Lipids may affect the beating by the maintenance of the structural integrity of the cell membranes. In this way they may be instrumental in the control of entry of the substances present in the medium and thus control the make-up of the internal chemical environment of the beating cell. Such a role would link lipids to the determination of enzyme activity through the possible control of substrate levels, which may act as inducers for protein synthesis. On the other hand, lipids may affect beating by acting as substrates for conversion to substances, which may play a direct role in determining the number and kind of enzymes synthesized. The process of enzyme induction and repression, which has been elucidated for bacterial systems, has been demonstrated to be operative in mammalian cell cultures. A clear example has been reported by Schimke (1964) in Hela cells. The synthesis of argininosuccinate synthetase, argininosuccinase, and arginase were shown to be affected by the level of arginine in the growth medium. In most cases the reported enzyme changes may be attributed to the presence of an inducer which is directly related to the reaction catalyzed by the enzyme. This is not true for the changes reported here. Lipids are not substrates or products of the enzymes measured. It is, however,

possible that the kind of energy metabolism of the cell, with its concomitant and possibly unique distribution of metabolic intermediates, may create a chemical environment necessary for the synthesis of heart proteins. Such a general phenomenon is similar to that discussed by Magasanik (1961) and summarized by the phrase "catabolite repression". This is a generalization of what was known as the glucose effect, the inhibition of certain unrelated enzymes by glucose. The lipid effect reported here may also be the result of a general effect on the internal chemical environment and may be an example of catabolite repression.

Fatty acids and other lipids are in some way related to the beating of heart cells in culture (Harary et al, In Press). The observations reported here open the way to a more detailed molecular study of the relation of lipids to heart cell specific function.

SUMMARY

Non-dividing beating rat heart cells, incubated in a lipid free medium, showed a decrease in malic dehydrogenase and Ca-ATPase and an increase in glucose 6-phosphate dehydrogenase. These changes were associated with a cessation of beating. Serum lipids, added to the medium, prevented the enzyme changes and maintained the beating. Serum lipids also restored the beating and reversed the enzyme changes which were caused by the lipid deficiency.

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