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RELATIONS BETWEEN FATTY ACID SYNTHESIS, PYRUVATE CONCENTRATION AND CELL CONCENTRATION OF SUSPENSIONS OF ISOLATED RAT HEPATOCYTES

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Abstract—1. The cell concentration of suspensions of isolated rat hepatocytes affects both the rate of pyruvate accumulation in the incubation medium and the rate of fatty acid synthesis.

2. At low cell concentrations pyruvate accumulation is directly related to the cell concentration but levels off at higher concentrations even when maximum pyruvate concentrations in the medium are not yet reached.

3. The rate of fatty acid synthesis in the 30–60-min incubation interval is proportional to the cell concentration. In contrast, the rate of fatty acid synthesis during the 0–30-min incubation period decreases with increasing cell concentrations and subsequently becomes independent of the cell concentration.

INTRODUCTION

The rate of fatty acid synthesis by suspensions of isolated rat hepatocytes has a lag phase of 15 to 30 min (Harris, 1975; Beynen *et al.*, 1979). It has been suggested that this lag phase reflects the time required for accumulation in the medium of pyruvate and lactate to concentrations that maximally support lipogenesis (Harris, 1975). This suggestion is substantiated by the observation that the lag in fatty acid synthesis is largely abolished when pyruvate and/or lactate are added to the incubation medium (Harris, 1975; McGarry *et al.*, 1978; Beynen *et al.*, 1982). The rate of pyruvate production by hepatocytes is enhanced with increasing quantities of cells incubated (Harris, 1975). Likewise, the rate of fatty acid synthesis is positively associated with the concentration of cells (Harris, 1975). Moreover, under various incubation conditions fatty acid synthesis in isolated rat hepatocytes is directly related to the pyruvate concentration in the medium (Harris, 1975). Thus, it appears that high rates of fatty acid synthesis depend on rapid accumulation of pyruvate. Evidence is presented in this study, however, that in isolated rat hepatocytes a direct relationship between pyruvate concentration and rate of fatty acid synthesis is only observed under specific experimental conditions.

METHODS AND MATERIALS

Hepatocytes were obtained from male Wistar rats (200–250 g), which had free access to water and were meal-fed a stock, pelleted diet between 4 and 7 a.m. The animals were sacrificed at 9 a.m. Liver cells were isolated according to Seglen (1976), with modifications described previously (Beynen *et al.*, 1979). After isolation the cells (approx. 750 mg cellular protein) were suspended in about 25 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4), supplemented with 1.0% bovine serum albumin (defatted and dialysed) and 20 mM glucose. Two ml of this stock suspension was added to a 25-ml Erlenmeyer flask containing 2.0 ml of Krebs–Henseleit buffer, supplemented with 6.0% bovine serum albumin. In other incubations part of the stock suspension of cells was replaced by Krebs–Henseleit bicarbonate buffer containing 1.0% bovine serum albumin and 20 mM glucose. Incubations were carried out in a metabolic shaker (90 strokes/min) at 37°C under an atmosphere of 95% O_2 and 5% CO_2. To monitor rates of fatty acid synthesis, [^{3}H]H_2O (0.3 mCi/ml) was added to the incubation vessels.

Cellular protein was determined according to Lowry *et al.* (1951), using bovine serum albumin as a standard. 1 mg of cellular protein was found to equal 6.7 mg of wet weight of cells. Fatty acids were extracted according to Harris (1975). Neutralized perchloric-acid extracts of aliquots of hepatocyte incubations were analysed for pyruvate by the method of Hohorst *et al.* (1959). Sources of chemicals have been described in a previous paper (Beynen *et al.*, 1979).

RESULTS AND DISCUSSION

Upon incubation for 30 min the pyruvate concentration in the hepatocyte suspension increases with cell concentrations up to about 8 mg cellular protein/ml, but levels off with higher cell concentrations (Fig. 1). This indicates that the pyruvate concentration of the hepatocyte suspension reaches a certain maximum more rapidly with higher cell concentrations. This would have been anticipated since pyruvate produced within the hepatocyte equilibrates with the external medium (Woods and Krebs, 1971; Crabb *et al.*, 1976) and thus the rate of accumulation of pyruvate in the medium should depend on the quantity of cells incubated. Figure 1 also shows that at higher cell concentrations (above 8 mg of protein/ml) pyruvate levels continue to increase in the 30–60-min incubation interval.

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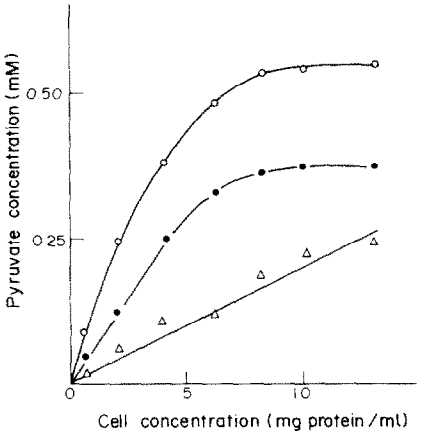

Fig. 1. Effect of cell quantity on the concentration of pyruvate in suspensions of isolated hepatocytes. Closed circles: pyruvate levels after 30 min of incubation; open circles: pyruvate levels after 60 min of incubation; triangles: pyruvate levels at $t = 0$ min. Experimental data are shown for one hepatocyte preparation. The results have been reproduced with other cell preparations. The absolute values on the axises, however, varied for different cell preparations.

The rate of fatty acid synthesis was measured by the incorporation of ^3H from tritiated water, and expressed as nmoles ^3H incorporated into fatty acids per mg cellular protein per 30 min.

In the 0–30-min incubation period fatty acid synthesis by the hepatocytes is inversely related with the cell concentration of the suspension in the range 0–5 mg of protein/ml. At higher cell concentrations no relationship between fatty acid synthesis and cell concentration is observed in this incubation period (Fig. 2). In keeping with results published by Harris (1975), fatty acid synthesis is directly related to the cell concentration in the 30–60-min incubation interval (Fig. 2). For the lower cell concentrations, in the 30–60-min incubation interval a linear relationship exists between the mean pyruvate concentration of the cell suspension and the rate of fatty acid synthesis. At cell concentrations higher than about 8 mg of protein/ml, fatty acid synthesis is not correlated with the pyruvate concentration of the incubation medium (Fig. 3).

The earlier observed direct relationship between pyruvate levels and fatty acid synthesis by hepatocytes (Harris, 1975) implies that fatty acid synthesis is controlled by substrate availability. This may not be true under all conditions. We have demonstrated that insulin stimulates fatty acid synthesis by isolated hepatocytes but slightly lowers pyruvate concentrations (Beynen *et al.*, 1980). The latter effect is most likely the result of the observed activation of pyruvate dehydrogenase by this hormone (Vaartjes *et al.*, 1980). Likewise, dichloroacetate has been shown to increase both pyruvate dehydrogenase activity and the rate of fatty acid synthesis in isolated hepatocytes, but the compound caused a fall in the pyruvate concentration of the cell suspension (Harris *et al.*, 1979). Therefore, it can be argued that the flux through glycolysis is more important in determining the rate of fatty acid synthesis than the pyruvate concentration of the incubation medium. Pyruvate generation, expressed as nmoles pyruvate produced per mg of cell protein per min increases with increasing hepatocyte concentrations up to about 6 mg protein/ml, both in the 0–30 and 30–60-min incubation interval (Fig. 4). However, in the same range of cell concentrations, the rate of fatty acid synthesis decreases and increases with increasing cell concen-

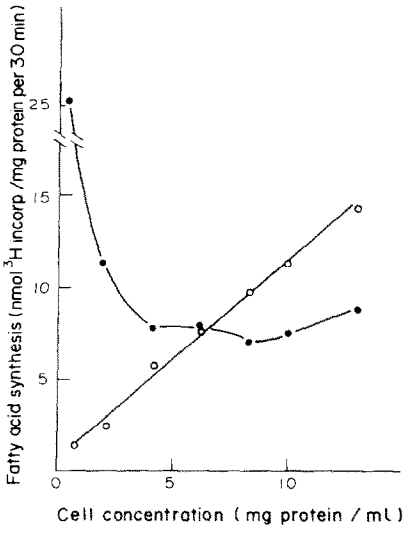

Fig. 2. Relationship between cell concentration and fatty acid synthesis in suspensions of isolated hepatocytes. Closed symbols: 0–30 min incubations; open symbols: 30–60 min incubations. Experimental data are reported for one hepatocyte preparation. The results have been reproduced with other cell preparations. The absolute values on the axises, however, varied for different cell preparations.

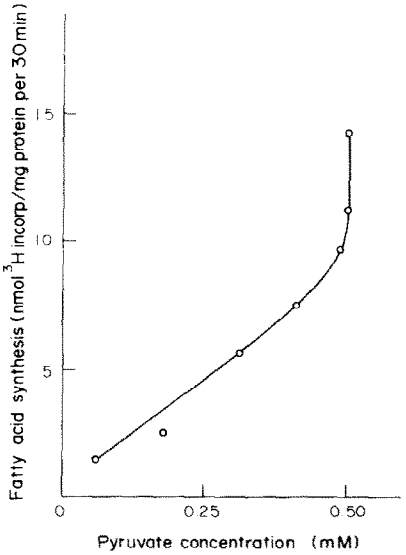

Fig. 3. Relationship between pyruvate levels and the rate of fatty acid synthesis in the 30–60-min incubation interval. Pyruvate levels are the means of the values at 30 and 60 min of the incubation period; the rate of fatty acid synthesis was measured in the 30–60-min incubation period. Data are taken from Figs 1 and 2.

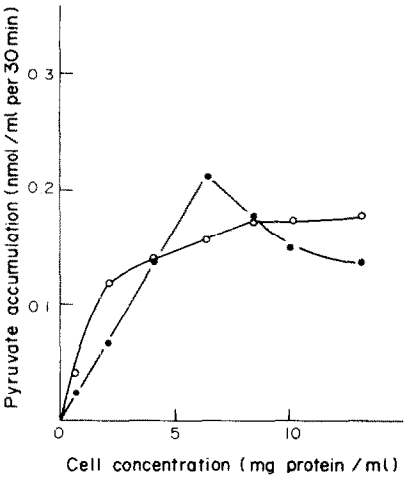

Fig. 4. Relationship between pyruvate accumulation in the hepatocyte suspension and the cell concentration. Closed circles: pyruvate accumulation in the 0–30-min incubation period; open circles: pyruvate accumulation in the 30–60-min incubation period. Data are taken from Fig. 1.

trations in the 0–30 and 30–60-min incubation period, respectively (Fig. 2). It appears therefore, that the relation between pyruvate flux and the rate of fatty acid synthesis is equivocal.

The well-documented (Harris, 1975; Beynen *et al.*, 1979) lag phase in fatty acid synthesis appears to exist only at cell concentrations higher than about 6 mg of cellular protein/ml. At these cell concentrations the rate of fatty acid synthesis in the 30–60-min incubation period is higher than that in the 0–30-min incubation period (cf. Fig. 2). However, at lower cell concentrations the opposite phenomenon was observed: the rate of fatty acid synthesis is lower in the 30–60-min incubation period than in the 0–30-min interval (cf. Fig. 2).

It is clear from the present study that reported observations such as the direct relationship between hepatic pyruvate levels and fatty acid synthesis (Harris, 1975; Walli, 1978) and the lag phase in fatty acid synthesis (Harris, 1975; Beynen *et al.*, 1979) are restricted to a relatively narrow range of cell concentrations, and also depend on the incubation interval. No satisfactory explanation can as yet be offered for the observed disproportionalities between quantity of cells incubated, the accumulation of pyruvate and the rate of fatty acid synthesis in the first 30 min of incubation.

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REFERENCES

Beynen A. C., Vaartjes W. J. and Geelen M. J. H. (1979) Opposite effects of insulin and glucagon in acute hormonal control of lipogenesis. *Diabetes* **28,** 828–835.

Beynen A. C., Geelen M. J. H. and Van den Bergh S. G. (1980) Short-term control of hepatic lipogenesis by insulin. *Trends Biochem. Sci.* **5,** 288–290.

Beynen A. C., Buechler K. F., Van der Molen A. J. and Geelen M. J. H. (1982) The effects of lactate and acetate on fatty acid and cholesterol biosynthesis by isolated rat hepatocytes. *Int. J. Biochem.* **14,** 165–169.

Crabb D. W., Mapes J. P., Boersma R. W. and Harris R. A. (1976) Effect of dichloroacetate on carbohydrate and lipid metabolism of isolated hepatocytes. *Archs Biochem. Biophys.* **173,** 658–665.

Harris R. A. (1975) Studies on the inhibition of hepatic lipogenesis by N^6,$O^{2'}$-dibutyryl-adenosine-3′,5′-monophosphate. *Archs. Biochem. Biophys.* **169,** 168–180.

Harris R. A., Mapes J. P., Ochs R. S., Crabb D. W. and Stropes L. (1979) Hormonal control of hepatic lipogenesis. In *Hormones and Energy Metabolism* (Edited by Klachko D. M., Anderson R. R. and Heimberg M.) Vol. 3, pp. 17–42. Plenum Press, New York.

Hohorst H. J., Kreutz F. H. and Bücher T. (1959) Ueber Metabolitgehalte und Metabolit-Konzentrationen in der Leber der Ratte. *Biochem. Z.* **332,** 18–46.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

McGarry J. D., Takabayashi Y. and Foster D. W. (1978) The role of malonyl-CoA in the coordination of fatty acid synthesis and oxidation in isolated rat hepatocytes. *J. biol. Chem.* **253,** 8294–8300.

Seglen P. O. (1976) Preparation of isolated rat liver cells. *Meth. Cell Biol.* **13,** 29–83.

Vaartjes W. J., Beynen A. C., Geelen M. J. H. and Van den Bergh S. G. (1980) Respiratory functions of mitochondria prepared from isolated hepatocytes treated with insulin. *Abstr. Commun. 13th Meet. Fedn Eur. Biochem. Socs*, Jerusalem, p. 99.

Walli R. (1978) Interrelation of aerobic glycolysis and lipogenesis in isolated perfused liver of well-fed rats. *Biochim. biophys. Acta* **539,** 62–80.

Woods H. F. and Krebs H. A. (1971) Lactate production by the perfused liver. *Biochem. J.* **125,** 129–139.