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[1] Lochaya, S., Hamilton, J. C., and Mayer, J., *Nature*, **197**, 182 (1963).
[2] Leboeuf, B., Lochaya, S., Leboeuf, N., Wood, jun., F. C., Mayer, J., and Cahill, jun., G. F., *Amer. J. Physiol.*, **201**, 19 (1961).
[3] Marshall, N. B., and Engel, F. L., *J. Lipid Res.*, **1**, 339 (1959–60).
[4] Christophe, J., Jeanrenaud, B., Mayer, J., and Renold, A. E., *J. Biol. Chem.*, **236**, 642 (1961).
[5] Bates, M. W., Nauss, S. F., Hagman, N. C., and Mayer, J., *Amer. J. Physiol.*, **180**, 301, (1955).

Anaerobic Production of α-Glycerolphosphate by Cell-free Preparations of Rat Hepatomas and the Effect of Potassium Cyanide

In a previous communication[1] we described a stimulatory effect of potassium cyanide on the soluble α-glycerolphosphate dehydrogenase of the post-mitochondrial fraction obtained from rat liver and hepatomas. In the present note evidence is presented for a similar effect of potassium cyanide on the production of α-glycerolphosphate by cell-free preparations of various hepatomas.

Post-mitochondrial fractions from rat liver and three transplanted rat hepatomas were prepared and incubated anaerobically with glucose-6-phosphate under the conditions mentioned in the legend of Table 1. α-Glycerolphosphate was measured by the conventional enzymatic method[2]. Table 1 shows the glycerolphosphate production per mg of protein of the hepatoma preparations to be of the same order as that of liver preparations. Addition of potassium cyanide (5×10^{-4} M) resulted in a rise of the glycerolphosphate production of 20–30, 50–60 and 130–250 per cent by preparations of hepatomas *BY* 448, *BY* 252 and *BY* 484, respectively; no stimulation was observed with liver. It should be noted that in the presence of potassium cyanide the glycerolphosphate production by *BY* 484 preparations surpassed that of liver on a protein basis and equalled the latter on a fresh weight basis. α-Glycerolphosphate dehydrogenase activity[1] amounting to 140–200 mμmoles DPNH oxidized/mg protein/min in the absence of potassium cyanide has been found in the post-mitochondrial fractions of *BY* 484 transplants studied recently, with potassium cyanide showing increased enzyme activities of up to seven-fold; these activities are of the order shown by normal liver[1]. The highest enzyme activities ever observed in the other hepatoma preparations in the absence of potassium cyanide amounted to 30–70 mμmoles DPNH oxidized/mg protein/min, zero activity being the more common finding[1]. It may be of interest that the biological (unpublished results) and some other biochemical[3,4] properties of the *BY* 484 hepatoma differ from those of the other two hepatomas.

In each experiment a concomitant one was run in the presence of potassium fluoride (10^{-2} M) in order to inhibit glycolysis so that more DPNH would become available for dihydroxyacetonephosphate reduction. The results of these experiments, which are shown in Table 1, show that the increase of α-glycerolphosphate obtained in the presence of potassium fluoride was frequently more pronounced in the case of the hepatoma than of the liver preparations. Thus, in most experiments the glycerolphosphate production/mg protein with potassium fluoride present was higher in the hepatoma than in the liver preparations. Potassium fluoride was without effect on the α-glycerolphosphate dehydrogenase in the enzymatic assay[1]. The results may indicate that in the absence of potassium fluoride, when active lactic acid formation

Table 1. Effect of Potassium Cyanide and Potassium Fluoride on α-Glycerolphosphate Formation by Rat Liver and Hepatoma Preparations

Tissue (mg protein)	KCN	μmoles glycerolphosphate KF Absent	KF Present
Hepatomas:			
BY 252 (29; 19·8)	−	2·0; 3·0	8·2; 10·0
	+	3·2; 4·6	8·9; 15·6
BY 448 (29; 31·6)	−	4·0; 3·9	7·9; 16·4
	+	5·2; 4·6	9·3; 16·4
BY 484 (28·3; 37)	−	2·3; 6·1	15·1; 25·3
	+	8·5; 14·1	18·6; 25·6
Liver (85; 68·4)	−	10·4; 9·3	26·1; 23·2
	+	10·5; 9·7	29·2; 23·0

Tissues were homogenized in 3 parts of 0·154 M KCl. After centrifuging at 0° for 10 min at 5,000*g*, 1·2 ml. of the supernatant, corresponding to 400 mg of fresh weight of tissue, was pipetted into large Warburg vessels containing 60 μmoles glucose-6-phosphate and potassium phosphate buffer of *p*H 7·4 (2·4 mM), $KHCO_3$ (25 mM), $MgCl_2$ (5 mM), nicotinamide (40 mM), DPN (0·5 mM), ATP (1 mM). Total volume 6·4 ml. KCN (0·5 mM) and/or KF (10 mM) present as indicated. Incubation during 30 min at 37° with 95 per cent nitrogen and 5 per cent carbon dioxide as gas phase. Reaction was stopped by addition of 1 ml. 6 N perchloric acid. After centrifuging, 5 ml. of the supernatant was brought to *p*H 9 with KOH, after standing in ice for 1 h the potassium perchlorate was centrifuged down and an aliquot of the supernatant used for the enzymatic determination of α-glycerolphosphate according to Bublitz and Kennedy (ref. 2). Endogenous α-glycerolphosphate was measured at zero time and subtracted.

proceeds, relatively less DPNH is available for the α-glycerolphosphate dehydrogenase of the hepatomas than for that of liver, but that the hepatomas show at least as much enzyme activity per mg of post-mitochondrial protein (not per mg fresh weight) as the liver, if not more (especially in the case of *BY* 484).

The stimulatory effect of potassium cyanide on glycerolphosphate production by the hepatoma preparations was less pronounced in the presence than in the absence of potassium fluoride, and sometimes lacking altogether. Neither KCN nor KF inhibited the very small disappearance of added glycerolphosphate in the post-mitochondrial fractions incubated in the absence of co-factors.

Whatever the precise mechanism of action of potassium cyanide in these and previous[1] experiments may be, the present results do show that, under suitable conditions, certain rat hepatomas may be capable of forming significant amounts of α-glycerolphosphate, not less than that produced by liver. This finding stands in marked contrast to the results published by Boxer *et al.*[5–7].

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[1] Emmelot, P., and Bos, C. J., *Biochim. Biophys. Acta*, **59**, 495 (1962).
[2] Bublitz, C., and Kennedy, E. P., *J. Biol. Chem.*, **211**, 951 (1954).
[3] Emmelot, P., and Bos, C. J., *Brit. J. Cancer*, **15**, 373 (1961).
[4] Emmelot, P., *Cancer Res.*, **22**, 38 (1962).
[5] Boxer, G. E., and Devlin, T. M., *Science*, **134**, 1495 (1961).
[6] Ciaccio, E. I., Keller, D. L., and Boxer, G. E., *Biochim. Biophys. Acta*, **37**, 191 (1960).
[7] Boxer, G. E., and Shonk, C. E., *Cancer Res.*, **20**, 85 (1960).

Synthesis of Lipoproteins by Rat Intestinal Mucosa

In the transport and absorption of long-chain fatty acids and mono-glycerides by the intestinal mucosa, an obligatory step is the intracellular re-esterification to triglycerides[1,2]. The triglyceride molecules afterwards appear in the lymph and plasma in the form of chylomicrons, or so-called primary fat particles. In addition to triglyceride these particles contain proteins (lipoproteins), cholesterol esters, and phospholipid[3,4]. Considerable debate still exists as to the mechanism and site of chylomicron formation as well as the nature and source of the proteins which coat the particles. Rodbell, Fredrickson and Ono[5], in investigating the incorporation of amino-acids into proteins in the dog, concluded that the intestine was a possible source of the proteins of the chylomicron. This communication describes studies