SC 2210

## Change from liver-type to muscle-type fructose metabolism in hepatomas

Previous studies demonstrated correlations between growth rate and certain carbohydrate metabolic alterations in liver tumors by enzymic[1] and isotope[2] technics. In the isotope investigations differentially labelled glucose was used and the results were in agreement with the enzymic indications found[3]. One of the outstanding features revealed in the study of neoplastic livers is the tendency of the liver to lose specific functions[3].

This is especially striking in rapidly-growing liver tumors where key gluconeogenic enzymes, glucose-6-phosphatase (EC 3.1.3.9)[4] and fructose-1,6-diphosphatase (EC 3.1.3.11)[5], are markedly decreased or absent. As a result of such metabolic lesions a number of characteristic features of liver carbohydrate metabolism are lost and the metabolic pattern of the hepatomas approaches that of the muscle with a capacity to produce large amounts of lactate[3,6].

The investigations of HERS and others demonstrated a clear-cut difference between the metabolic utilization of fructose in liver and in muscle[7,8]. In the liver fructose is metabolized by fructokinase (EC 2.7.1.4) and the molecule enters into glycolysis or glycogenesis by the triose phosphate stage. In muscle fructose is phosphorylated by hexokinase (EC 2.7.1.1), and it undergoes subsequent reactions as fructose 6-phosphate. HERS described the use of [1-$^{14}$C]fructose for elucidating the metabolic pathways of this hexose[9]. When [1-$^{14}$C]fructose is incubated with liver slices, the [$^{14}$C]glycogen that is formed is composed of glucose units in which the label is randomized between C-1 and C-6. However, in the isolated diaphragm muscle, [1-$^{14}$C]fructose incubation results in glycogen in which all of the $^{14}$C is located in C-1 of the hexose molecule. Since there is a definite difference between the metabolic fate of fructose in normal liver and muscle, and because several of the carbohydrate features of neoplastic liver tend to approach the metabolic pattern of the muscle, it has become of interest to assay the behavior of fructose metabolic pathways in neoplastic livers of different growth rates. These studies led to an elucidation of a definite metabolic alteration in liver tumors of rapid growth rates, whereas fructose metabolism was essentially normal in hepatomas which exhibited slow growth.

The following transplantable liver tumors were used: 5123-D, 7800 (slowly growing); 7288-C (intermediate growth rate); 3924-A, 3683 (rapidly growing). The tumors 5123-D, 7800 and 7288-C were carried in rats of the Buffalo strain; 3924-A and 3683 were transplanted in the AXC strain. For controls the livers of normal rats of Buffalo and AXC strains were used. The animals were kept in separate cages and Purina Laboratory Chow and water were available *ad libitum*.

Animals were stunned, decapitated and exsanguinated. Livers and tumors were rapidly excised. Hepatomas were carefully dissected free of necrotic, hemorrhagic and non-tumorous material. Samples were taken for glycogen assays which showed that very little glycogen was present in all examined tumors. This is in agreement with previous reports[3].

Slices of hepatoma and liver were incubated separately with 30 mM [1-$^{14}$C]fructose for 90 min in a Ringer-bicarbonate buffer, equilibrated with 95 % $O_2$–5 % $CO_2$. In all tumors the fructose uptake was approx. 50 % of that utilized by normal livers. Tissue glycogen was isolated by KOH digestion and alcohol precipitation[10]. After

hydrolysis, carrier glucose was added and the phenylosazone formed was used for radioactive assay[11]. A portion of the osazone was degraded according to LANDAU *et al.*[11] and counted.

Data given in Fig. 1 are results of an experiment comparing the metabolism of [1-$^{14}$C]fructose in normal liver and hepatoma 3683. This is shown to illustrate the pathways of fructose metabolism under the two extreme conditions. The utilization of fructose in the normal livers followed the expected normal liver pattern. On the other hand, in the rapidly-growing 3683 tumor the metabolism shifted from fructose 1-phosphate to fructose 6-phosphate, as evidenced by the fact that 96 % of the radioactivity resided in C-1 of glycogen glucose.

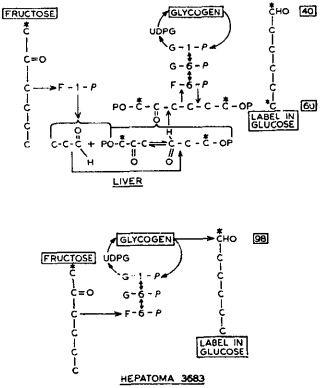


Fig. 1. Example of an experiment comparing the path of [1-$^{14}$C]fructose in normal liver and hepatoma 3683.

The per cent of the label in the mesoxaldehyde (C-1,2,3) to that observed for the phenylosazone (C-1,2,3,4,5,6) in the various preparations studied is plotted in Fig. 2. If all the radioactivity were located in C-1, 100 % of the label would be expected in the mesoxaldehyde and would be indicative of metabolism via hexokinase. In contrast, metabolism via fructokinase should result in randomization so that only 50 % of the $^{14}$C would be expected in the top three carbons. It may be noted that the fructose metabolism was the same in the livers of the two different strains of rats. It is interesting that fructose was metabolized according to the pathways of normal liver in the slow-growing hepatomas 5123-D and 7800. On the other hand, a shift of metabolic pattern takes place in the more rapidly-growing tumors and fructose is utilized to an increasing extent through the hexokinase route. In 3683, which is the most rapidly-growing tumor in the present series, this tendency culminates in a nearly exclusive hexokinase metabolism of fructose.

The presented data indicate that a normal fructose metabolism is compatible with liver neoplasia as far as slow-growing tumors are concerned. However, the fructose metabolic pattern of liver tumors changes with increasing growth rate, ex-

hibiting a tendency to decreased fructose utilization through fructokinase and with an emergence of the predominance of the hexokinase route of metabolism. These alterations are in line with previous observations[1-3] that some of the carbohydrate metabolic features of rapidly-growing hepatomas resemble those of the muscle.

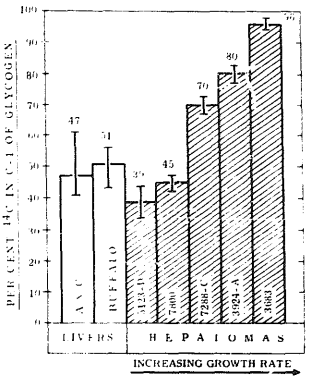


Fig. 2. Summary of fructose-metabolic studies in normal and neoplastic livers. The means and spread of values represent two or more experiments for each group. The ordinate gives the % $^{14}C$ derived from fructose 1-phosphate found in C-1 of glycogen.

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[1] G. WEBER, H. P. MORRIS, W. C. LOVE AND J. ASHMORE, *Cancer Res.*, 21 (1961) 1406.
[2] G. WEBER, G. BANERJEE AND H. P. MORRIS, *Cancer Res.*, 21 (1961) 933.
[3] G. WEBER, *Advan. Cancer Res.*, 6 (1961) 403.
[4] G. WEBER AND A. CANTERO, *Cancer Res.*, 15 (1955) 105.
[5] G. WEBER AND J. ASHMORE, *Exptl. Cell Res.*, 14 (1958) 226.
[6] J. ASHMORE, G. WEBER AND B. R. LANDAU, *Cancer Res.*, 18 (1958) 974.
[7] H. G. HERS AND T. KUSAKA, *Biochim. Biophys. Acta*, 11 (1953) 427.
[8] F. LEUTHARDT, E. TESTA AND H. P. WOLF, *Helv. Chim. Acta*, 36 (1953) 227.
[9] H. G. HERS, *J. Biol. Chem.*, 214 (1955) 373.
[10] C. A. GOOD, H. KRAMER AND M. SOMOGYI, *J. Biol. Chem.*, 100 (1933) 485.
[11] B. R. LANDAU, F. B. NESBETT AND A. B. HASTINGS, *J. Biol. Chem.*, 214 (1955) 525.

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