The specific activity of hexose 6-phosphates in liver

Kalant *et al.* (1987) incubated isolated hepatocytes with [^{14}C]glucose or [^{14}C]fructose, and measured the specific activity of glucose 6-phosphate, glucose 1-phosphate and fructose 6-phosphate in hepatocytes. They claim (their Fig. 1) that the ratios of specific activity of glucose 1-phosphate/glucose 6-phosphate and fructose 6-phosphate/glucose 6-phosphate were over 6 at 15 min of incubation, declining at 1 h to a ratio of about 3.5. On the basis of these and similar data, they claim the existence of different pools of hexose 6-phosphates in liver and compartmentation of carbohydrate metabolism. These findings, if accepted, may have a large impact on the application of tracer data to studies of hepatic carbohydrate metabolism.

In my opinion, the methods use by Kalant *et al.* (1987) are flawed, and their claims unacceptable. These investigators used commercial uniformly labelled sugars without rigorous purification. I reproduce here (Fig. 1) a figure from Katz *et al.* (1978) in which [^{14}C]glucose was incubated with isolated hepatocytes and phosphorylated intermediates isolated. When a commercial sample of [^{14}C]glucose was applied to a borate column, 0.5% of 'chromatographically pure' [^{14}C]glucose was retained on the column, which upon elution yielded a complex spectrum of peaks, as shown in the figure. The activity in this fraction was similar to or exceeded the total yield of labelled intermediates from glucose after incubation. Glucose 1-phosphate appears in this system

Fig. 1. Chromatogram of the sample of [^{14}C]glucose, 'chromatographically pure'

Upper curves, 124×10^6 c.p.m. were applied, 123×10^6 recovered in the water eluate, and 0.68×10^6 eluted with the borate gradient (see the text). The neutral fraction (glucose) was taken to dryness and rechromatographed under the same condition. Lower curve, about 25000 c.p.m. were eluted in the phosphate ester fraction, but there virtually was no activity in the glucose 6-phosphate peak around fraction 70. Reproduced with permission from Katz *et al.* (1978).

at fraction 38 (see Fig. 3 of Katz *et al.*, 1978) and is completely masked by the radioactive impurities. It is not surprising that sugars prepared via starch or sucrose formed via $^{14}\text{CO}_2$ fixation would contain such contaminants in the 99.5% pure glucose.

Not only were the sugars not freed from phosphorylated contaminants, but the total ^{14}C in the incubation medium, the mass of cells, the concentration of hexose phosphates and the actual activity recovered in the fractions was not reported by the authors. This is relevant since the yield of ^{14}C in glucose 6-phosphate is 0.1% or less and that of glucose 1-phosphate of the order of 0.01% of the ^{14}C dose. Glucose 1-phosphate was not isolated or purified as such, but was taken to equal the glucose liberated by short acid hydrolysis of a crude fraction. Most surprising is the omission to report the specific activity of the compounds relative to that of substrate glucose. We found that the specific activity of glucose 6-phosphate was about half that of [^{14}C]glucose. Thus, accepting the ratios of Kalant *et al.* (1987), the specific activities of glucose 1-phosphate and fructose 6-phosphate would exceed that of glucose, an obvious impossibility.

As is apparent from our study, rather rigorous criteria must be applied in attempting to measure the specific activity of phosphorylated compounds in liver. The methods of Kalant *et al.* (1987), as well as those of some other investigators in this area, are inadequate in this respect.

It is well established that enzyme activities in periportal and perivenous hepatocytes differ. It is possible that there is compartmentation and separate pools. However, in my opinion this has so far not been satisfactorily documented (including references cited by Kalant *et al.*, 1987). The experimental data by Kalant *et al.* (1987) are misleading and do not serve to support compartmentation of hexose 6-phosphates.

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Kalant, N., Parniak, M. & Lemieux, M. (1987) *Biochem. J.* **248**, 927-931

Katz, J., Wals, P. A. & Rognstad, R. (1978) *J. Biol. Chem.* **253**, 4530-4536

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Compartmentation of glucose 6-phosphate in hepatocytes

In the preceding letter [1], Dr. Katz has outlined a number of criticisms which he feels negate the conclusions in our recent report concerning the compartmentation of glucose 6-phosphate in rat hepatocytes [2]. We consider these criticisms unwarranted for a number of reasons.

1. In our initial work on compartmentation [3], the [^{14}C]glucose was purified before use since earlier commercial preparations were indeed contaminated with phosphorylated glycolytic intermediates; this was apparently still the case in 1978 [4]. However, such contamination appears not to be a significant factor in currently available preparations. A sample of [^{14}C]-