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# DISTRIBUTION OF HEPATIC PHOSPHOFRUCTOKINASE ISOZYMES IN PARENCHYMAL AND SINUSOIDAL CELLS[1,2]

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SUMMARY

The distribution profile of the isozymes of phosphofructokinase (PFK) in different cell types of rat liver is established using the techniques of electrophoresis and immunodiffusion. Agarose gel electrophoresis of the extracts of parenchymal cells, Kupffer or sinusoidal cells, and whole liver indicated that two PFK isozymes are present in whole liver and that the faster moving hepatic PFK isozyme is present only in parenchymal cells; whereas, the slower moving hepatic PFK isozyme is only in sinusoidal cells. Immunodiffusion studies using antiserum specific for the major hepatic PFK isozyme (PFK-$L_2$) revealed that PFK-$L_2$ is present only in whole liver or parenchymal cell extracts and is absent from sinusoidal cells. It is apparent that the other hepatic PFK isozyme (PFK-$L_1$) is normally found only in sinusoidal cells.

We have previously shown that two phosphofructokinase (PFK) isozymes are present in rat liver (1). The major PFK isozyme, PFK-$L_2$, was found to be immunologically, chromatographically (DEAE cellulose), and electrophoretically (starch gel) distinguishable from the minor PFK isozyme, PFK-$L_1$ (1,2). Further, PFK-$L_2$ appears to be highly regulated by small molecular weight effectors while PFK-$L_1$ is affected to a lesser extent (1,2). Subsequent studies demonstrated that although PFK-$L_1$ activity is minimally altered by nutritional and hormonal fluctuations, PFK-$L_2$ levels are markedly sensitive (3). In this communication, we present data concerning the distribution of PFK isozymes in isolated parenchymal and sinusoidal cells.

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[2]The term sinusoidal cell is the currently accepted nomenclature for Kupffer cells preparations that are isolated by the applied techniques. This nomenclature was recommended at the International Symposium on Kupffer Cells, Leiden, Holland, October, 1977.

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## METHODS

Male rats of the Wistar strain weighing 200-250 g were used in these studies and maintained as described (3). Immunodiffusion studies were performed as previously described except that petri dishes instead of microscope slides were used (2). Antiserum against PFK-$L_2$ was prepared by a published method (1). The electrophoresis was performed in 1.2% agarose (Bio-Rad) prepared in 100 mM Tris-$PO_4$, pH 8.0, 1 mM ATP, 1 mM $MgSO_4$, 10 mM NaF. Separation of the PFK isozymes were accomplished in the same buffer with a LKB Multiphor apparatus using a constant voltage of 300 V and constant current of 60 ma. PFK activity was detected using a previously described activity stain (2).

Parenchymal cells and sinusoidal cells were prepared, as previously reported (4,5). These cells were isolated from adult rats (200-250 g), quick frozen, and rapidly thawed prior to use. Both cell types or whole liver were homogenized in a stabilizing buffer (1) and centrifuged at 100,000 x g for 1 hour (1). The resulting supernate was used for the immunodiffusion or electrophoretic studies.

## RESULTS AND DISCUSSION

As can be seen in Figure 1, 2 bands of PFK activity are found for whole liver (LIV) consisting of a slowly migrating band and a faster migrating band. The faster migrating band appeared to be present only in the parenchymal cells (PAR), and the slower migrating band appeared only in the sinusoidal cell preparation (KUP). Earlier work with starch gel electrophoresis indicated that PFK-$L_2$ exhibited greater electrophoretic mobility than PFK-$L_1$ (2). Extrapolation from that data suggested that PFK-$L_2$ (faster electrophoretic mobility) was present only in parenchymal cells and that PFK-$L_1$ (slower electrophoretic mobility) was present only in the sinusoidal cells. Confirmation of this suggestion was obtained by immunodiffusion studies (Figure 2) using an antisera specific for PFK-$L_2$ (well No. 1). These data indicated a line of precipitation against a parenchymal extract (well No. 2), pure PFK-$L_2$ (well No. 3), and a whole liver extract (well No. 4). In addition, no line of precipitation is visible for the sinusoidal cell preparation (well No. 5). Further, a line of identity is clearly visible between pure PFK-$L_2$ (well No. 3) and the parenchymal extract (well No. 2). Thus, within the limits of detection by these two methods PFK-$L_2$ is present only in parenchymal cells. Further, under normal conditions PFK-$L_1$ is present only in sinu-

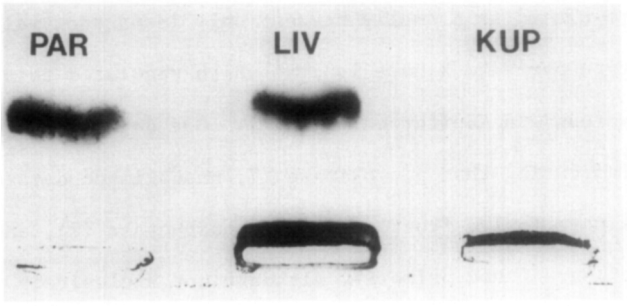


Figure 1: Agarose Electrophoresis of Whole Liver, Parenchymal Cells, and Sinusoidal Cells Extracts. The parenchymal cell extract (PAR), whole liver extract (LIV), and sinusoidal cell extract (KUP) are shown from left to right, respectively. For experimental details see the Method section.

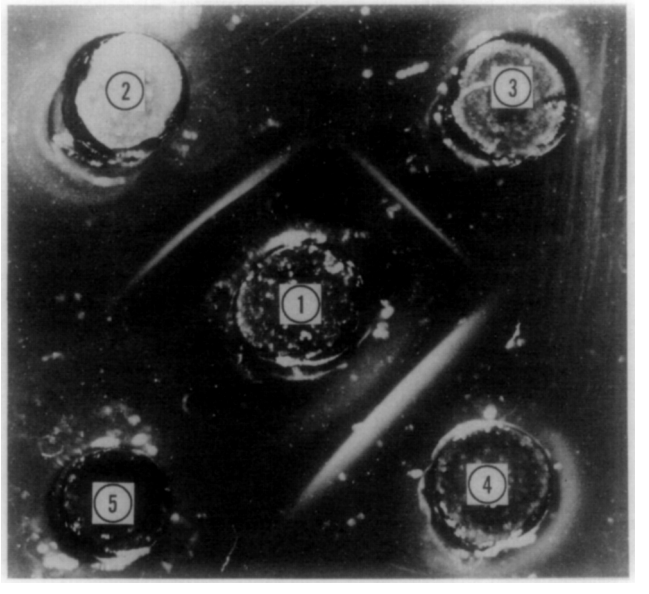


Figure 2: Immunodiffusion of Parenchymal, Purified PFK-$L_2$, Whole Liver Extracts, and Sinusoidal Cell Extracts Against PFK-$L_2$ Specific Antiserum. PFK-$L_2$ antiserum is in well No. 1. The parenchymal extract, purified PFK-$L_2$, whole liver extract, and sinusoidal cell extracts are in well No. 2, 3, 4, and 5, respectively. See Methods section for experimental details.

soidal cells. To our knowledge, this report is the first to demonstrate the distribution of PFK isozymes in the two major cell types of the liver.

Two isozymes of pyruvate kinase which is another regulatory enzyme in carbohydrate metabolism are present in the liver. L-type pyruvate kinase

which is highly regulated is normally present only in parenchymal cells; whereas, the $M_2$-type pyruvate kinase isozyme which regulated to a lesser extent is usually found in Kupffer cells (6,7). Our data in conjunction with other work indicating that glucokinase (7, unpublished data of Wagle), L-type pyruvate kinase (6,7), fructose-1,6-bisphosphatase (7), and glucose-6-phosphatase (7,8) are found primarily although not exclusively in parenchymal cells strongly suggests that parenchymal cells are the primary sites of hepatic gluconeogenesis. Further, parenchymal cells appear to be largely responsible for the hepatic adaptations to hormonal and dietary fluctuations as well as nutritional stress.

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