The Distribution of Enzyme and Isoenzyme Activities between Parenchymal and Haematopoietic Cells in the Liver of the Foetal Guinea Pig

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The hepatocyte and haematopoietic cell contents of the liver of the foetal guinea pig were measured over the latter half of gestation. Hepatocytes represented about 30% of liver volume at mid-gestation and this increased to 70–80% by term; cell volume remained fairly constant until 5–7 days before term, then more than doubled. Haematopoietic cells represented about 5% of liver volume at mid-gestation and this progressively fell to <1% by term. At 75% of gestation hepatocytes and haematopoietic cells were prepared from perfused foetal livers by collagenase digestion. Enzyme activity of the hepatocyte was, without exception, similar to that of the whole liver. In general, enzyme activity in the haematopoietic cells was similar to that in erythrocytes, with relatively low values for aldolase, glycerol 3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, lactate dehydrogenase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, isocitrate dehydrogenase, 'malic' enzyme, glutamate dehydrogenase and aspartate aminotransferase. The haematopoietic cell contribution to total enzyme activity in the foetal liver was usually much less than 10% and could thus not account for the major changes in hepatic enzyme activity over the latter half of gestation. Hepatocytes contained hexokinase isoenzymes I and III, aldolase isoenzymes A and B and pyruvate kinase isoenzymes 1, 2 and 4. The haematopoietic cells contained hexokinase isoenzyme I and two additional bands of activity with slightly greater mobility, aldolase isoenzyme A and pyruvate kinase isoenzymes 2 and 4.

During foetal development the liver is a major site of erythropoiesis, and thus in addition to the parenchymal cells it contains many haematopoietic cells (Bloom, 1938; Jones, 1959; Ackerman *et al.*, 1961; Sorenson, 1963; Zamboni, 1965; Metcalf & Moore, 1971). The contribution of these cells to the total liver mass varies substantially from species to species and usually falls dramatically during the course of development (Parat, 1923; Bloom, 1938; Sorenson, 1963; Oliver *et al.*, 1963; Zamboni, 1965). With such large changes in the cell composition of the developing liver it is difficult to interpret the origin of changes in enzyme and isoenzyme activity without a knowledge of the activities within the cell types. For instance, previous studies have indicated that some isoenzymes peculiar to the foetal liver may originate exclusively from the haematopoietic cells (Rutter, 1969; Faulkner & Jones, 1975a,c, 1976a).

In the present study haematopoietic cells and hepatocytes were prepared from the foetal guinea-pig liver. Although the presence of isoenzymes peculiar to haematopoietic cells has been confirmed, and these

cells have a substantially different enzyme composition from hepatocytes, their contribution to liver enzyme activities at about two-thirds of gestation has been found to be small.

Methods

Cell preparation

The livers of 49–52-day foetal guinea pigs were perfused through the umbilical vein, at about 20 cm water pressure and a flow rate of 3–4 ml/min per g, with a recirculating medium as described by Berry & Friend (1969). The liver was perfused for 5–10 min with bicarbonate buffer (Krebs & Henseleit, 1932) containing 1 mM-glucose and no albumin or Ca^{2+} ; this perfusate was then discarded. The liver was then perfused with 100 ml of the same buffer, to which was added 10 mg of collagenase (grade II, Boehringer Corp. Ltd., Lewes, East Sussex, U.K.). This was recirculated for 20 min. The liver was then chopped, suspended in 20 ml of the above medium containing no collagenase and filtered through 100 μm nylon mesh. A crude hepatocyte preparation was separated by centrifuging at 50g for 1 min, then the erythropoietic cells were recovered from the supernatant by

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centrifuging at 200g for 4 min. Both pellets were washed once with buffer, cell volumes were measured, and then they were homogenized in 1 vol. (hepatocytes) or 4 vol. (erythropoietic cells) of 50 mM-Tris/HCl, pH 8.0, containing 1 mM-EDTA and 10 mM-MgCl₂. This was sonicated (3 × 10 s, about 150 W), then centrifuged at 100 000g for 1 h.

Determination of enzyme activity

Most of the methods for the determination of enzyme activity have been previously described (Faulkner & Jones, 1975a,b; Jones & Ashton, 1976a,b; Faulkner & Jones, 1976a). Aspartate aminotransferase activity was assayed as described by Bergmeyer & Bernt (1963) and leucine aminotransferase activity was assayed as described by Aki & Ichihara (1971). Glutamate dehydrogenase, aspartate aminotransferase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities were assayed after freezing and thawing the sonicated cell preparation.

For the resolution of pyruvate kinase and hexokinase into their constituent isoenzymes, sonicated cell extracts were subjected to electrophoresis in starch gel and enzyme activity was determined as previously described (Faulkner & Jones, 1975a, 1976a). Isoenzymes of aldolase were separated and enzyme activity was determined by a method similar to that described by Masters (1968).

Determination of protein

Protein concentration was determined, after precipitation with 0.4 M-HClO₄, by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V, Sigma Chemical Co. Ltd., Poole, Dorset, U.K.) as standard.

Microscopy and cell counting

For optical microscopy, foetal livers or cell preparations were fixed with Susa's fixative and stained with Haematoxylin and Eosin (G. T. Gurr, Ltd., London S.W.6, U.K.). Parenchymal and haematopoietic cell numbers were counted in a minimum of 12 areas from 5 µm sections of liver. By treating the hepatocytes as spheres and the haematopoietic cells as ellipsoids, surface area and volume were determined by planimetry.

For electron microscopy the livers were fixed with 3% glutaraldehyde in 0.1 M-sodium phosphate, pH 7.4, post-fixed in 1% OsO₄, then, after dehydration and embedding in epoxy resin, post-stained in methanolic uranyl acetate and lead citrate (Reynolds, 1963). A Philips 301 electron microscope was used.

Expression of results

A unit of enzyme activity is that required to produce 1 µmol of product/min at 37°C. Results are expressed

as means ± 1 S.E.M., the number of observations being given in parentheses. Significance was determined by using a paired Student's *t* test.

Results

Changes in cell size and number in the foetal liver

Between 30 and 50 days the number of hepatocytes in the liver of the foetal guinea pig increased from a mean of $4.6 \times 10^5 \pm 0.31 \times 10^5$ (6) per mm³ at 33–40 days to a mean of $7.33 \times 10^5 \pm 0.46 \times 10^5$ (12) per mm³ at 48–52 days ($P < 0.001$). The number then fell to a mean of $4.5 \times 10^5 \pm 0.47 \times 10^5$ (13) per mm³ by 60–65 days ($P < 0.001$) (Fig. 1). This was associated with no change in cell volume until 60 days, after which this rose ($P < 0.001$) from a mean of 779 ± 39 (31) µm³ to 1855 ± 202 (13) µm³ (Fig. 1). These changes in cell number and cell volume were responsible for a progressive increase in the contribution of hepatocytes to liver volume, rising from about 30% at 33 days to about 70% at 60–65 days (Fig. 1).

The number of haematopoietic cells in the 33–40-day liver, mean $1.43 \times 10^6 \pm 0.03 \times 10^6$ (6) per mm³, was much higher than the number of hepatocytes. During development the number of haematopoietic cells declined, particularly between 50 and 60 days,

Fig. 1. Volume (c), number (a) and fractional volume (b) of hepatocytes in the liver of the foetal guinea pig. Fractional volume refers to the proportion of the liver occupied by this cell type.

so that at 60–65 days the mean number of $2.87 \times 10^5 \pm 0.61 \times 10^5$ (13) per mm^3 was less than for the hepatocytes. Cell volume at a mean of 34.7 ± 1.1 (44) μm^3 remained fairly constant (Fig. 2). Despite their large number the haematopoietic cells represent only a small volume of the liver, with a value of about 5% at 33–40 days, falling to a value of about 1% or less at 60–65 days (Fig. 2).

Enzyme activities in hepatocytes and haematopoietic cells from the foetal liver

It is not possible to perfuse the foetal guinea-pig liver much before 45 days, and after about 57 days high concentrations of glycogen and triacylglycerol (Jones, 1976; Faulkner & Jones, 1976b) and the low haematopoietic cell content make the isolation of hepatocytes and haematopoietic cells difficult. Thus livers of 48–52-day foetuses were used for cell preparation, this being a time when many enzyme activities can be detected in the foetal liver.

Fig. 2. Volume (c), number (a) and fractional volume (b) of haematopoietic cells in the liver of the foetal guinea pig. Fractional volume refers to the proportion of the liver occupied by this cell type.

Table 1. Enzyme activities in hepatocytes and haematopoietic cells prepared from livers of 48–52-day foetal guinea pigs. Mean values $\pm$ S.E.M. are shown with the numbers of measurements in parentheses.

	Activity (units/g of protein)		
	Haematopoietic cells	Hepatocytes	Total homogenate
Hexokinase (total) (EC 2.7.1.1)	10.1 ± 1.5 (8)	10.6 ± 1.0 (8)	11.0 ± 0.72 (6)
Hexokinase (soluble)	4.8 ± 0.5 (8)	6.6 ± 0.5 (8)	5.7 ± 0.4 (6)
Hexokinase (bound)	5.6 ± 1.3 (8)	5.2 ± 1.0 (7)	5.5 ± 0.7 (6)
Phosphoglucose isomerase (EC 5.3.1.9)	323 ± 56 (8)	676 ± 46 (8)	612 ± 44 (6)
Phosphofructokinase (EC 2.7.1.11)	21 ± 3.2 (6)	25 ± 3.5 (6)	23 ± 5.7 (3)
Aldolase (EC 4.1.2.13)	23 ± 3.2 (8)	138 ± 23 (8)	114 ± 21.6 (6)
Triose phosphate isomerase (EC 5.3.1.1)	888 ± 138 (8)	3374 ± 263 (8)	3025 ± 372 (6)
Glycerol 3-phosphate dehydrogenase (EC 1.1.1.8)	51 ± 14.7 (4)	281 ± 52 (4)	234 ± 46 (4)
Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12)	112 ± 18 (4)	237 ± 52 (4)	19.2 ± 4.1 (4)
Phosphoglycerate kinase (EC 2.7.2.3)	401 ± 71 (4)	532 ± 110 (4)	549 ± 97 (4)
Phosphoglycerate mutase (EC 2.7.5.3)	156 ± 14 (5)	750 ± 170 (5)	602 ± 95 (5)
Enolase (EC 4.2.1.11)	76 ± 21 (5)	330 ± 59 (5)	261 ± 47 (5)
Pyruvate kinase (EC 2.7.1.40)	105 ± 11 (8)	218 ± 22 (8)	193 ± 23 (6)
Lactate dehydrogenase (EC 1.1.1.27)	390 ± 106 (8)	974 ± 131 (8)	917 ± 61 (6)
Pyruvate carboxylase (EC 6.4.1.1)	0.3 ± 0.19 (5)	1.0 ± 0.7 (5)	0.9 ± 0.6 (5)
Phosphoenolpyruvate carboxykinase (EC 4.1.1.32)	4.5 ± 2.3 (6)	16.8 ± 5.5 (6)	14.4 ± 6.5 (6)
Fructose 1,6-bisphosphatase (EC 3.1.3.11)	22.6 ± 5.7 (8)	155 ± 5.9 (8)	114 ± 4.4 (6)
Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)	26.5 ± 2.7 (8)	52.3 ± 7.4 (8)	49 ± 4.6 (6)
6-Phosphogluconate dehydrogenase (EC 1.1.1.44)	19.4 ± 2.0 (4)	34.1 ± 5.2 (4)	28.6 ± 3.2 (4)
Isocitrate dehydrogenase (EC 1.1.1.42)	65 ± 6.4 (8)	275 ± 33.3 (8)	259 ± 17.4 (6)
'Malic' enzyme (EC 1.1.1.40)	4 ± 0.32 (8)	11.0 ± 0.67 (8)	9.7 ± 0.63 (6)
ATP citrate lyase (EC 4.1.3.8)	4 ± 0.9 (4)	10.4 ± 1.6 (4)	7.2 ± 1.6 (4)
Glutamate dehydrogenase (EC 1.4.1.3)	183 ± 37 (8)	1128 ± 244 (8)	1261 ± 198 (6)
Aspartate aminotransferase (EC 2.6.1.1)	179 ± 24.7 (8)	875 ± 134 (8)	993 ± 105 (6)
Leucine aminotransferase (EC 2.6.1.6)	3.1 ± 0.23 (4)	4.5 ± 0.16 (4)	3.8 ± 0.28 (4)

In the cell preparation it was possible to obtain an haematopoietic cell preparation essentially free of hepatocytes, but not an hepatocyte preparation free of haematopoietic cells (i.e. about 7–16% of the total remaining with the hepatocytes). However, as the maximum contribution of haematopoietic cell volume in the liver at this age is small (i.e. about 10% of hepatocyte volume) the haematopoietic cell contamination of the hepatocyte preparation is unlikely to have more than a small effect on total enzyme activity in this fraction.

The mean protein concentrations for hepatocytes and haematopoietic cells in preparations from 48–52-day foetal livers were respectively 141 ± 15 (8) and 188 ± 23 (8) mg/g of cells.

The enzyme activities per unit weight of protein in the haematopoietic cells and hepatocytes are given in Table 1. In many cases these activities are similar, but the activities of aldolase, glycerol 3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, lactate dehydrogenase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, isocitrate dehydrogenase, 'malic' enzyme, glutamate dehydrogenase and aspartate aminotransferase were substantially lower in the haematopoietic cells. In no case was the value higher than in the hepatocyte fraction.

After allowing for the slightly higher protein concentration of the haematopoietic cells the differences per g of cells became marginally less, but qualitatively they remained the same (Table 2). Of particular note are the low activities of aldolase, glycerol 3-phosphate dehydrogenase, enolase, the gluconeogenic enzymes, glutamate dehydrogenase and aspartate aminotransferase in the haematopoietic cells.

Because of the low haematopoietic cell volumes in the 48–52-day foetal guinea-pig liver and the lower activities of many enzymes in these cells, their contribution to total liver enzyme activity is small (Table 3). Only for hexokinase, phosphofructokinase and phosphoglycerate kinase does the contribution approach 10% of that of the hepatocytes.

Isoenzyme distribution between hepatocytes and haematopoietic cells

In the liver of the foetal guinea pig at about 50 days there are four bands of hexokinase activity after separation with starch-gel electrophoresis. There was a fast-moving activity, hexokinase III, a slow-moving activity, hexokinase I, and two bands of activity, not seen in the adult liver, that lay between hexokinase I and II (Faulkner & Jones, 1976a). The total cell suspension and the hepatocytes from the present

Table 2. *Comparative enzyme composition of hepatocytes and haematopoietic cells from livers of 48–52-day foetal guinea pigs*
Values were calculated from enzyme activity per g of cell protein $\times$ protein concentration. Mean values $\pm$ S.E.M. are shown with the numbers of measurements in parentheses.

	Activity (units/g of cells)	
	Haematopoietic cells	Hepatocytes
Hexokinase (total)	1.94 ± 0.21 (8)	1.5 ± 0.16 (8)
Hexokinase (soluble)	0.89 ± 0.11 (8)	0.96 ± 0.08 (8)
Hexokinase (bound)	1.1 ± 0.3 (8)	0.74 ± 0.15 (7)
Phosphoglucose isomerase	63 ± 11.1 (8)	97.5 ± 13.9 (8)
Phosphofructokinase	3.8 ± 0.6 (6)	3.6 ± 0.75 (6)
Aldolase	4.2 ± 0.6 (8)	19.7 ± 3.6 (8)
Triose phosphate isomerase	171 ± 31 (8)	486 ± 35 (8)
Glycerol 3-phosphate dehydrogenase	9.3 ± 3.6 (4)	39.8 ± 7.6 (4)
Glyceraldehyde 3-phosphate dehydrogenase	21 ± 1.5 (4)	34.7 ± 6.9 (4)
Phosphoglycerate kinase	74 ± 9.5 (4)	75 ± 19.4 (4)
Phosphoglycerate mutase	30 ± 3.6 (5)	107 ± 32.4 (5)
Enolase	13.7 ± 1.5 (5)	46.5 ± 11.4 (5)
Pyruvate kinase	20.1 ± 2.3 (8)	31.4 ± 4.2 (8)
Lactate dehydrogenase	71.4 ± 15.1 (8)	138 ± 17.1 (8)
Pyruvate carboxylase	0.051 ± 0.044 (5)	0.15 ± 0.1 (5)
Phosphoenolpyruvate carboxykinase	0.80 ± 0.30 (6)	2.55 ± 0.6 (6)
Fructose 1,6-bisphosphatase	4.2 ± 0.75 (8)	22.7 ± 1.4 (8)
Glucose 6-phosphate dehydrogenase	4.8 ± 0.5 (8)	7.4 ± 1.0 (8)
6-Phosphogluconate dehydrogenase	3.5 ± 0.75 (4)	4.8 ± 0.9 (4)
Isocitrate dehydrogenase	12.5 ± 1.1 (8)	39.6 ± 5.4 (8)
'Malic' enzyme	0.75 ± 0.05 (8)	1.6 ± 0.12 (8)
ATP citrate lyase	0.75 ± 0.09 (4)	1.5 ± 0.16 (4)
Glutamate dehydrogenase	31 ± 6.3 (8)	160 ± 33.9 (8)
Aspartate aminotransferase	33.2 ± 5.9 (8)	132 ± 18.9 (8)
Leucine aminotransferase	0.53 ± 0.05 (4)	0.63 ± 0.05 (4)

Table 3. Contribution of haematopoietic cells and hepatocytes to liver enzyme activities in 48–52-day foetal guinea pigs Activity was calculated as (activity/g of cells) × cell fractional volume of liver; the percentages are the sum of haematopoietic cell + hepatocyte activity. Mean values ± s.e.m. are shown with the numbers of measurements in parentheses.

	Activity			
	Haematopoietic cells		Hepatocytes	
	(units/g of liver)	(% of total)	(units/g of liver)	(% of total)
Hexokinase (total)	0.097 ± 0.01 (8)	9.7	0.90 ± 0.1 (8)	90.3
Hexokinase (soluble)	0.045 ± 0.005 (8)	7.0	0.60 ± 0.05 (8)	93
Hexokinase (bound)	0.055 ± 0.015 (8)	10.7	0.46 ± 0.09 (8)	89.3
Phosphoglucose isomerase	3.2 ± 0.55 (8)	5.1	60 ± 8.6 (8)	94.9
Phosphofructokinase	0.19 ± 0.03 (6)	7.9	2.2 ± 0.47 (6)	92.1
Aldolase	0.21 ± 0.03 (8)	1.7	12.2 ± 2.2 (8)	98.3
Triose phosphate isomerase	8.6 ± 1.5 (8)	2.8	301 ± 21.7 (8)	97.2
Glycerol 3-phosphate dehydrogenase	0.47 ± 0.18 (4)	1.9	24.6 ± 4.7 (4)	88.1
Glyceraldehyde 3-phosphate dehydrogenase	1.05 ± 0.08 (4)	4.7	21.5 ± 4.3 (4)	95.3
Phosphoglycerate kinase	3.7 ± 0.48 (4)	7.4	46.5 ± 12.0 (4)	92.6
Phosphoglycerate mutase	1.5 ± 0.18 (5)	2.2	66.3 ± 20 (5)	97.8
Enolase	0.69 ± 0.08 (5)	2.3	28.8 ± 7.1 (5)	97.7
Pyruvate kinase	1.05 ± 0.11 (8)	5.1	19.5 ± 2.6 (8)	94.9
Lactate dehydrogenase	3.57 ± 0.76 (8)	4.0	86 ± 10.6 (8)	96
Pyruvate carboxylase	0.0025 ± 0.002 (5)	2.7	0.09 ± 0.06 (5)	97.3
Phosphoenolpyruvate carboxykinase	0.04 ± 0.015 (6)	2.4	1.6 ± 0.4 (6)	97.6
Fructose 1,6-bisphosphatase	0.21 ± 0.038 (8)	1.5	14.1 ± 0.9 (8)	98.5
Glucose 6-phosphate dehydrogenase	0.24 ± 0.025 (8)	5.0	4.6 ± 0.6 (8)	95
6-Phosphogluconate dehydrogenase	0.18 ± 0.038 (4)	5.7	3.0 ± 0.56 (4)	94.3
Isocitrate dehydrogenase	0.63 ± 0.055 (8)	2.5	24.6 ± 3.3 (8)	97.5
'Malic' enzyme	0.04 ± 0.005 (8)	3.8	1.0 ± 0.07 (8)	96.2
ATP citrate lyase	0.04 ± 0.005 (4)	4.1	0.93 ± 0.1 (4)	95.9
Glutamate dehydrogenase	1.55 ± 0.32 (8)	1.5	99 ± 21 (8)	98.5
Aspartate aminotransferase	1.66 ± 0.3 (8)	2.0	82 ± 11.7 (8)	98
Leucine aminotransferase	0.026 ± 0.0025 (4)	6.3	0.39 ± 0.03 (4)	93.7

Fig. 3. Starch-gel electrophoresis of hexokinase (HK) isoenzymes from cells prepared from the liver of the 50-day foetal guinea pig

Horizontal electrophoresis of 100000g supernatants was performed at 14 V/cm (10mA/cm²) and 0–2°C for 8 h as described in the Methods section. T, Total cell preparation; E, haematopoietic cells; H, hepatocyte-enriched fraction; O, origin.

experiments showed a similar isoenzyme pattern (Fig. 3). The haematopoietic cells showed a pattern similar to foetal blood (Faulkner & Jones, 1976a),

Fig. 4. Starch-gel electrophoresis of aldolase (ALD) isoenzymes from cells prepared from the liver of the 50-day foetal guinea pig

Other details are as for Fig. 3. The broken line indicates traces of enzyme activity.

with hexokinase I together with the two further bands of activity just above that isoenzyme (Fig. 3).

During development there is a progressive appearance in the foetal liver of the isoenzyme aldolase B and decline in the proportion of aldolase A activity (Masters, 1968; Rutter, 1969). In the guinea pig this

is manifest as a progressive increase in the activity of aldolase towards fructose 1-phosphate (Masters, 1968). In the 50-day foetal liver aldolase B activity was detected in both the total cell preparation and in the hepatocytes. No aldolase B activity was detected in the haematopoietic cells (Fig. 4). All cell preparations contained aldolase A activity, and a further band of activity with mobility just above that of isoenzyme A, although this was present as only trace activity in the haematopoietic cells (Fig. 4).

The liver of the foetal guinea pig contains the isoenzymes 1 and 4 of pyruvate kinase predominantly, together with some isoenzyme 2 that disappears after 55 days (Faulkner & Jones, 1975a). The total cell preparation and the hepatocytes from 48–52-day foetal guinea-pig livers also had the three isoenzymes 1, 2 and 4, with traces of pyruvate kinase isoenzyme 3 (Fig. 5). The haematopoietic cells were essentially similar to the foetal erythrocytes (Faulkner & Jones, 1975a), with isoenzymes 2 and 4; traces of isoenzyme 3 were also found with the haematopoietic cells (Fig. 5).

Microscopic structure of the foetal guinea-pig liver

Haematopoietic cells in the foetal liver exist in clusters. At about 50 days the majority have a large nucleus with dense chromatin and little cytoplasm. The cytosol appears relatively structureless, with little evidence of an endoplasmic reticulum and very few mitochondria (Plate 1a).

The foetal parenchymal cell at about 50 days has a dense-staining nucleus, but a much larger cytoplasmic/nuclear ratio than in the haematopoietic cell. Its cytosol is very heterogeneous, with many mitochondria, much smooth endoplasmic reticulum and some glycogen granules and lipid droplets. The majority of the coarse endoplasmic reticulum is located around the mitochondria (Plate 1b).

Fig. 5. Starch-gel electrophoresis of pyruvate kinase (PK) isoenzymes from cells prepared from the liver of the 50-day foetal guinea pig

Details are as for Fig. 3. Broken lines indicate traces of enzyme activity.

Discussion

The enzyme composition of the hepatocyte preparation from the liver of the foetal guinea pig was similar to that previously reported for whole liver (Faulkner & Jones, 1975a,b, 1976a; Jones & Ashton, 1976a,b). It is of interest that hexokinase activity extracted from the whole liver at about 50 days is almost exclusively in the soluble form (Faulkner & Jones, 1976a), whereas during the preparation of hepatocytes the proportion of bound enzyme increased dramatically. Hepatocytes from the liver of the 18–19-day foetal rat have been prepared by using lysozyme (Hommes *et al.*, 1971). The activities of hexokinase and phosphoglucose isomerase are much lower and of triose phosphate isomerase about 20-fold higher in foetal guinea-pig hepatocytes than in those from foetal rats; the other glycolytic enzymes are present in similar activities.

The enzyme activities in the haematopoietic cells were similar to those reported for erythrocytes obtained from adult or newborn man (Bartels, 1967; Rapoport, 1968). This is consistent with these cells being largely erythropoietic in the foetal liver (Metcalf & Moore, 1971).

We have previously suggested (Faulkner & Jones, 1976a) that the two hexokinase isoenzymes with mobilities slightly greater than hexokinase I were probably of haematopoietic cell origin. The present data are consistent with that and demonstrate that these cells contain isoenzyme I. Thus the presence in the hepatocyte-enriched fraction of isoenzymes between hexokinase I and III is probably due to contamination with haematopoietic cells. It is clear also that much of the aldolase A activity that is detected in the foetal guinea-pig liver is probably derived from the haematopoietic tissue. Thus the fall in aldolase A and the rise in aldolase B activity that occur both in the foetal rat and guinea-pig livers during development (Masters, 1968; Rutter, 1969) may result from the decline in the number of haematopoietic cells in, and the increase in the hepatocyte content of, the developing liver. Pyruvate kinase isoenzyme 2, which was found in the foetal liver and haematopoietic cells, has been previously identified as that present in erythrocytes (Faulkner & Jones, 1975a). Thus the present results indicate that much if not all of this isoenzyme is located in the haematopoietic tissue of the foetal liver. The haematopoietic cells also contained pyruvate kinase isoenzyme 4. However, as about 40% of the foetal liver pyruvate kinase activity is in this form (Faulkner & Jones, 1975a) and only 5% of the total enzyme activity is located in the haematopoietic cells, most of the isoenzyme 4 must be located in the hepatocytes.

In summary, the present data suggest that from about 40 days to term little if any of the total enzyme

(a)

(b)

EXPLANATION OF PLATE I

Electron micrograph of the liver of the foetal guinea pig

(a) Haematopoietic cells; (b) parenchymal cell. Each horizontal bar represents 2 μ m.

activity changes seen in the whole liver of the foetal guinea pig are likely to originate from the haematopoietic cells. From 50 days on the contribution of these cells is likely to be small. They do, however, contribute isoenzyme activities that are more usually associated with erythrocytes.

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