

Changes in Patterns of Enzymes of Carbohydrate Metabolism in the Developing Rat Liver*

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The mature liver has an unusually versatile metabolic capacity. Presumably the embryonic liver lacks many of the mature activities which develop during growth of the animal. Knowledge of the changes in specific enzyme capacities should help in understanding biochemical differentiation. Although enzymatic changes have been studied in organs of various species during development (1-5), the amount of available information on embryonic and young rat tissues is still rather limited (6-13). The rat at birth is unfinished relative to many other animals, the guinea pig, for instance. Consequently it shows some embryonic characteristics during the early postnatal days and is thus a convenient source of organs at different stages of maturation. A previous study (14) in this laboratory demonstrated marked increases in certain flavoproteins in rat liver from birth to weaning.

This paper reports the concentrations of 13 hepatic enzymes concerned with glucose and lactate metabolism during the late fetal stage and the first 21 days of life. Complex changes in enzyme patterns during development and growth were observed. The methods that were used are of sufficient sensitivity to study a variety of enzymes in individual embryonic organs, and therefore may be of more general use.

EXPERIMENTAL PROCEDURE

Animals—Pregnant Sprague-Dawley rats (Holtzman Rat Company) were fed a synthetic diet (15) from the time of arrival in the laboratory (on the 12th to 17th day of gestation) through the weaning period. Young adult male and female rats were also fed the same synthetic diet for 10 days before their tissues were analyzed.

Fetuses and pups were weighed, decapitated, and drained of blood as completely as possible. Adult rats were killed in a manner which removes most of the blood from the liver (15). Liver was rapidly dissected, weighed, frozen in liquid nitrogen, and stored at -40° or below until analyzed. A record of the liver and body weights and liver protein levels is provided in Table I.

Reagent Chemicals and Enzymes—Auxiliary enzymes were from Boehringer and Sons through California Corporation for Biochemical Research, except for isomerase, which was prepared from muscle (17), and 6-P-gluconate dehydrogenase, which was

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prepared from rat liver (18). Fructose-1-P (barium salt), dihydroxyacetone-P (cyclohexylamine salt), glyceraldehyde-P, and triose-P esters were also from Boehringer and Sons. Fructose-6-P (Boehringer), after enzymatic standardization, was used as the standard for P-glucoisomerase. The barium salts of fructose 1,6-diphosphate (Schwartz BioResearch, Inc.) and of fructose-1-P were converted to the sodium salts. Sodium salts of glucose-6-P, 6-P-gluconate, and glucose-1-P and the pyridine nucleotides were from Sigma Chemical Company. Sodium lactate was from Mallinckrodt Chemical Works; dimethylglycine from K and K Laboratories, Inc.; 2-methylimidazole from California Corporation for Biochemical Research; and 2-amino-2-methylpropanol and 2-amino-2-methylpropanediol from Fisher Scientific Company.

Preparation of Homogenates—Frozen or sometimes fresh tissues were homogenized at 0° in ground glass grinders with 10 volumes of the appropriate buffers. The homogenates were usually prepared and diluted in the same buffer except that the diluent contained 0.05% bovine serum albumin to protect enzymes when the total dilution was greater than 1:100. The buffer used for this purpose with most of the enzymes was 0.02 M Tris, pH 7.3 to 8.1. In some cases other buffers were used, but the only exceptions worth noting (and the buffers used) are for triose-P isomerase (0.1 M imidazole, pH 7.4), P-fructokinase (0.1 M Pi, pH 8), glyceraldehyde-P dehydrogenase (0.05 M Pi, pH 7.5), and hexokinase (0.02 M Pi, pH 8). The frozen samples were not homogenized until just before assay, to avoid decrease in activity of the enzymes.

General Conditions of Assay—Enzyme activities were measured with close to optimal concentrations of substrate and coenzymes and at optimal pH (Table II). Conditions were also such as to give activities proportional to time of incubation and amount of enzyme. The total dilution of tissue was very great, which presumably accounts for the fact that no evidence of interference from inhibitors or accelerators in the tissue has so far been observed. For enzymes of low activity, suitable correction was made for endogenous tissue blanks.

In general, the procedure was as follows. The appropriate complete reagent (Table II) was pipetted into tubes, 6 \times 50 mm (except in the case of glyceraldehyde-P dehydrogenase), in a rack at 0° . An aliquot of homogenate or an equal volume of buffer or standard was added. (When tissue blank determinations were necessary the substrate was omitted from the reagent.) The tubes were covered with Parafilm, transferred all together to a water bath for incubation, and then transferred back to the

TABLE I
Change in liver protein and liver and body weights
during growth

Protein was measured colorimetrically (16). Values represent the averages for the number of animals. The standard errors are also given.

Age	No. of animals	Liver weight	Body weight	Protein
		mg	g	g/kg
Fetal (-5 days)....	20	62.8 ± 2.1	0.82 ± 0.02	109 ± 8
Fetal (-2 days)....	35	222.3 ± 8.5	3.51 ± 0.05	107 ± 8
Newborn (12 hours)....	57	235 ± 4.5	5.63 ± 0.09	139 ± 3
4 days.....	39	351 ± 8	8.73 ± 0.19	157 ± 4
9 days.....	29	661 ± 14	18.9 ± 1.0	157 ± 3
15 days.....	29	1164 ± 33	34.9 ± 0.9	176 ± 3
21 days.....	24	2025 ± 85	51.0 ± 1.9	178 ± 3
Adult (M) (65 days)....	24	9400 ± 307	230.0 ± 6	184 ± 3
Adult (F) (65 days)....	24	7430 ± 199	174.0 ± 2	180 ± 5

bath at 0°. The reaction was stopped more permanently within a few minutes either by great dilution or by the addition of acid or alkali.

With the assays requiring auxiliary enzymes, the competence of these enzymes was tested by incubating a duplicate set of standards for only 2 minutes. A conversion of at least 50% of substrate to product in this time was set as a requirement. The amount of auxiliary enzymes needed was also calculated as previously described (19). In all but the last three methods of Table II, the final measurement was that of the fluorescence of DPNH or TPNH or of the derivative of DPN⁺ or TPN⁺ formed in strong alkali (20, 21). The fluorescence measurements were made in selected Pyrex tubes, 10 × 75 mm, in a Farrand model A fluorometer. Colorimetric measurements were made in a Beckman DU spectrophotometer.

Most of the substrate reagents were stored at -20° to -40° with all components present except the auxiliary enzymes and (when used) DPNH. These were added just before use. The DPNH was treated with alkali to remove traces of DPN⁺ (22).

The following are details of the methods which are not covered in Table II. For *fructose 1,6-diphosphate aldolase*, *fructose-1-P aldolase*, *glycero-P-dehydrogenase*,¹ *triose-P dehydrogenase*,¹ and *P-fructokinase* (19), after incubation, 0.1 volume of 5 N HCl was added to destroy excess DPNH and a 10-μl aliquot was transferred into 100 μl of 6.6 N NaOH in a fluorometer tube. After incubation again for 30 minutes at 38°, 1 ml of H₂O was added and the fluorescence was read. Measurement of *triose-P isomerase* in Tris buffer (23) resulted in low activity because Tris reacts with glyceraldehyde phosphate, making it unavailable to the enzyme (24). For *P-fructokinase* assays, tissue blanks, with ATP omitted from the substrate, amounted to 10 to 20% of the corrected readings. This blank was later found to be negligible if 2 mM Amytal was added to the reagent. High levels of ATP are inhibitory to *P-fructokinase* (25). It is recommended that the reagent composition given in Table I be altered by sub-

stitution of 20 mM Na₂HPO₄ for the KCl and the addition of 1 mM 5'-AMP, since P_i and 5'-AMP, among a few other compounds, overcome ATP inhibition (26).

*Glyceraldehyde-P Dehydrogenase*¹—After incubation, the reaction was arrested with 1 ml of 0.02 N NaOH, and the fluorescence of the DPNH formed was measured within 15 minutes, since some DPN⁺ fluorescence is generated on longer standing.

Lactic Dehydrogenase—After incubation, 10-μl aliquots were diluted in 1 ml of buffer containing 0.005 M NaHCO₃ and 0.05 M Na₂CO₃ for reading of the fluorescence.

Hexokinase (19)—After incubation, 10-μl aliquots were transferred into tubes at 0° containing 20 μl of a second reagent consisting of 0.1 M Tris, 0.05% bovine serum albumin, 0.05 mM TPN⁺, 2.5 mM EDTA, and sufficient 6-P-gluconate dehydrogenase to catalyze the oxidation of 50% of a low level of 6-P-gluconate (0.002 mM) in 2 minutes or less at 25°. The samples were incubated for 10 minutes at 38° and then transferred back to a bath at 0°, and 20 μl of 0.25 M Na₃PO₄-0.35 M KH₂PO₄ were added. Excess TPN⁺ was now destroyed by heating for 15 minutes at 60°, and 40-μl aliquots were transferred to fluorometer tubes containing 200 μl of 0.01% H₂O₂ in 7.7 N NaOH. After 40 minutes at 38°, 1 ml of water was added and the fluorescence was measured. TPNH was measured indirectly in this manner to increase the sensitivity (21). Samples were corrected for tissue blanks (ATP omitted), which were carried through the entire procedure.

Dilution in a solution containing EDTA prevented further hexokinase action by sequestering Mg⁺⁺. The use of 6-P-gluconate dehydrogenase increased the sensitivity of the method 2-fold, which is of some importance because of the low levels of hexokinase in liver. However, the chief purpose is to increase reliability of the assay by making sure that all of the 6-P-gluconate formed is oxidized by TPN⁺, whereas endogenous 6-P-gluconate dehydrogenase might oxidize only a variable fraction.

The approximate K_m of glucose for hexokinase in fetal liver was 1 mM. This is some 100 times that reported for brain and 10 times that reported for skeletal muscle. The determination of the K_m for hexokinase in adult liver was more difficult than in fetal liver because of the low activity, but approximately the same value was obtained.

Glucose-6-P standards, carried through the procedure, agreed with appropriate TPNH standards added later, which indicates freedom from interference from other enzymes.

Glucose-6-P Dehydrogenase (19) and *6-P-gluconate Dehydrogenase* (19)—After incubation, 20-μl aliquots were removed into 1 ml of a buffer containing 0.05 M Na₂CO₃, 0.005 M NaHCO₃, and 0.1 mM EDTA, and the fluorescence was measured. At pH 9.8 the high K_s of 6-P-gluconate dehydrogenase, combined with the high tissue dilution, prevents this endogenous enzyme from adding more than an extra 5% to the readings. In subsequent experiments the estimation of glucose-6-P dehydrogenase with the addition of purified 6-P-gluconate dehydrogenase, as previously described for retina (19), gave essentially the same activity.

P-glucoisomerase and *Glycogen Phosphorylase*—These were measured by published procedures (27) except for volume changes. In the case of the latter enzyme, cysteine apparently did not enhance the activity in liver as it did in brain, and was omitted from the reagent.

Glucose 6-Phosphatase—The procedure was essentially that of Segal and Washko (28), except for the method of P_i measurement. After incubation, 20 μl of 30% trichloroacetic acid were

¹ Details of the method are to be published by David B. McDougal, Jr.

TABLE II
Analytical conditions

For certain ages (usually before birth) larger samples were sometimes needed, as shown, because of very low enzyme activities. Standards of given concentrations were used in volumes equal to those of the tissue samples. Incubations were all for 30 minutes at 38° except for glyceraldehyde-P dehydrogenase (15 minutes at 25°), hexokinase (60 minutes at 38°), and glucose 6-phosphatase (20 minutes at 30°).

Enzyme	Buffer	Additions	Reagent volume	Sample size		Standards
				Weight	Volume	
Fructose diphosphate aldolase	Tris, 70 mM, pH 7.7	Fructose diphosphate, 15 mM; DPNH, 1 mM; nicotinamide, 20 mM; sodium Amytal, 2 mM; glycero-P dehydrogenase-triose-P dehydrogenase, 10 µg per ml	30	5	3	DPN ⁺ or triose-P, 5
Fructose-1-P aldolase	Tris, 70 mM, pH 7.7	Fructose-1-P, 10 mM; DPNH, 1 mM; nicotinamide, 20 mM; sodium Amytal, 2 mM; glycero-P dehydrogenase, 10 µg per ml	30	15 or 75	3	DPN ⁺ or dihydroxyacetone-P, 3
Glycero-P dehydrogenase	P _i , 100 mM, pH 7.3	Dihydroxyacetone-P, 5 mM; DPNH, 1 mM; nicotinamide, 20 mM; sodium Amytal, 2 mM	20	0.5-3	4	DPN ⁺ , 0.6
Triose-P isomerase	Imidazole, 100 mM; pH 7.4	Glyceraldehyde-3-P, 5 mM; DPNH, 1 mM; glycero-P dehydrogenase, 10 µg per ml	100	0.3	2	DPN ⁺ or dihydroxyacetone-P, 5
P-fructokinase	Tris, 100 mM, pH 8.3	Glucose-6-P, 2 mM; ATP, 1.8 mM; DPNH, 1 mM; MgCl ₂ , 5 mM; KCl, 100 mM; P-glucoisomerase, 15 µg per ml; fructose diphosphate aldolase, 10 µg per ml; glycero-P dehydrogenase-triose-P dehydrogenase, 10 µg per ml	30	12 or 25	5	DPN ⁺ , 1; or fructose diphosphate, 0.5
Glyceraldehyde-P dehydrogenase	Dimethylglycine, 100 mM, pH 9.0	Glyceraldehyde-3-P, 2 mM; K ₂ HPO ₄ , 30 mM; DPN ⁺ , 1 mM; EDTA, 2 mM; mercaptoethanol, 5 mM; sodium Amytal, 3.5 mM	100	0.6	1.3	DPNH, 1
Lactic dehydrogenase	2-Amino-2-methylpropanol, 100 mM, pH 9.9	Sodium lactate, 100 mM; DPN ⁺ , 20 mM	100	1.2	5	DPNH, 1
Glucose-6-P dehydrogenase	2-Amino-2-methylpropanol, 100 mM, pH 9.8	Glucose-6-P, 5 mM; TPN ⁺ , 0.4 mM	100	25	5	TPNH, 1
6-P-gluconate dehydrogenase	2-Amino-2-methylpropanediol, 100 mM, pH 8.9	6-P-gluconate, 1 mM; TPN ⁺ , 0.5 mM	100	6-12	5	TPNH, 1
Hexokinase	Tris, 75 mM, pH 8.3	Glucose, 10 mM; ATP, 5 mM; MgCl ₂ , 5 mM; TPN ⁺ , 1 mM; Na ₂ HPO ₄ , 30 mM; glucose-6-P dehydrogenase, 0.25 µg per ml	30	5	5	Glucose-6-P, 0.3; or TPNH, 0.3
P-glucoisomerase	Tris, 100 mM, pH 8.0	Glucose-6-P, 90 mM	50	40	5	Fructose-6-P, 50
Glycogen phosphorylase	Collidine, 50 mM, pH 6.7	Glucose-1-P, 30 mM; glycogen, 0.5%; 5'-adenylic acid, 1 mM; NaF, 50 mM	100	200	20	P _i , 10
Glucose 6-phosphatase	Sodium cacodylate, 150 mM, pH 6.4	Glucose-6-P, 10 mM	100	250 or 500	5 or 10	P _i , 10

added and the samples were centrifuged. Aliquots of 35 µl of supernatant fluid were transferred into 1 ml of a solution of 0.15% ammonium molybdate and 3% ascorbic acid in 0.7 N H₂SO₄ (29). After heating for 20 minutes at 38°, the tubes were cooled to room temperature and the optical density was measured at 820 m μ . Endogenous P_i was measured, in order to make suitable correction, by adding homogenate to extra reagent blanks after the addition of trichloroacetic acid. The high sensitivity of the phosphate method, $\epsilon = 25,000$, permitted measurement of the phosphatase in individual fetal tissues, which had not been possible by a less sensitive procedure (30).

RESULTS

The 13 enzymes measured are known to participate in at least eight systems or functions: glucose utilization (hexokinase), glycogen breakdown (phosphorylase), glycolysis (P-glucoisomerase, P-fructokinase, fructose diphosphate aldolase, triose-P isomerase, glyceraldehyde-P dehydrogenase, lactic dehydrogenase), free glucose formation (glucose 6-phosphatase), lactate utilization (lactic dehydrogenase, glyceraldehyde-P dehydrogenase, triose-P isomerase, and possibly fructose-1-P aldolase), glucose-6-P oxidation through the pentose shunt (glucose 6-P dehydrogenase, 6-P-gluconate dehydrogenase), glycero-P forma-

TABLE III
Enzymes of carbohydrate metabolism in fetal and adult rat liver

Enzyme activities are expressed as moles per kg of protein per hour and represent the average of triplicate determinations on 6 to 10 rats, occasionally more. Standard errors are shown in italics.

Enzyme	Fetal				Adult (65) days				Adult rabbit brain	
	-7 to -5 days		-4 to -2 days		Female		Male			
	moles/kg protein/hr									
Hexokinase.....	1.73	<i>0.07</i>			0.33	<i>0.01</i>	0.44	<i>0.02</i>	10 ^a	
Phosphoglucoisomerase.....			57	<i>3</i>	55	<i>0.3</i>	63	<i>0.4</i>	100 ^b	
Phosphofructokinase.....	4.79	<i>0.13</i>			1.38	<i>0.05</i>	1.56	<i>0.06</i>	16 ^b	
Fructose diphosphate aldolase.....	11.3	<i>0.3</i>	15.2	<i>1.1</i>	6.1	<i>0.1</i>	6.6	<i>0.2</i>	9 ^b	
Triosephosphate isomerase.....	1014	<i>11</i>	1090	<i>50</i>	849	<i>14</i>	735	<i>12</i>	2500 ^c	
Glyceraldehyde phosphate dehydrogenase.....	52.4	<i>2.6</i>	72.6	<i>2.9</i>	52.6	<i>2.2</i>	52.0	<i>1.2</i>	325 ^c	
Lactic dehydrogenase.....			74	<i>2</i>	76	<i>2</i>	95	<i>2</i>	32 ^d	
Fructose 1-phosphate aldolase.....	0.54	<i>0.01</i>		1.7	0.2	3.6	0.1	3.9	0.1	
Glycerophosphate dehydrogenase.....				5.8	<i>0.2</i>	51.2	<i>1.4</i>	52.8	<i>1.3</i>	
Phosphorylase.....	5.3	<i>0.4</i>				7.9	<i>0.3</i>	10.6	<i>0.3</i>	
Glucose 6-phosphate dehydrogenase.....	2.5	<i>0.1</i>				2.3	<i>0.1</i>	1.7	<i>0.1</i>	
6-Phosphogluconate dehydrogenase.....	3.8	<i>0.1</i>				5.3	<i>0.2</i>	3.4	<i>0.1</i>	
Glucose 6-phosphatase.....	0.20	<i>0.01</i>				5.1	<i>0.2</i>	5.8	<i>0.2</i>	

^a From Lowry (31).

^b From McDougal *et al.* (32).

^c D. B. McDougal, Jr., R. T. Schimke, E. M. Jones, and E. Touhill, unpublished data.

^d From Robins *et al.* (33).

^e From Buell *et al.* (27).

tion, and the glycero-P shuttle (glycero-P dehydrogenase). At least three of these enzymes (triose-P isomerase, glyceraldehyde-P dehydrogenase, and lactic dehydrogenase) are common to both glycolysis and lactate utilization.

The changes in the levels of the 13 enzymes with growth and development will be presented in relationship to these eight functions. For comparison, the levels of 12 of these enzymes are recorded for brain, an organ characterized by a high capacity for glycolysis and glycogenolysis (Table III). This is desirable, since a comparison of the absolute activity levels of different enzymes, measured under optimal conditions, will not necessarily be a true indication of the relative activities of these enzymes in the cell.

Over-all Changes from Fetal Age of -5 Days to the Adult (Table III)—There is seen to occur a 5-fold decrease in hepatic hexokinase but a doubling of phosphorylase, suggestive of a greatly decreased capacity for glucose use but an increase in capacity for glycogen use. The adult level of hepatic hexokinase is about 4% of the level in brain. The combination of an increase in phosphorylase and glucose 6-phosphatase with a decrease in P-fructokinase and fructose diphosphate aldolase is in keeping with an increased capacity for conversion of glycogen to free glucose and with a decreased capability for conversion of glycogen to lactate. An inconsistency with this interpretation might appear to be the high level of P-glucoisomerase in the adult. However, if there is to be any P-glucoisomerase there may need to be an ample supply if the glucose-6-P level is to be precisely and quickly adjustable.

The three enzymes of the Embden-Meyerhof pathway below aldolase (triose-P isomerase, glyceraldehyde-P dehydrogenase, and lactic dehydrogenase) all are high in the adult, presumably in relation to the capability of the adult liver for lactic acid uptake rather than formation. The 7-fold increase in fruc-

tose-1-P aldolase from -5 days to 65 days suggests that it may be involved in the conversion of lactate to glucose or glycogen (see "Discussion").

The ratios between adult brain and liver enzyme levels along the glycolytic pathway are: hexokinase, 25; P-fructokinase, 12; fructose diphosphate aldolase, 1.4; triose-P isomerase, 3; glyceraldehyde-P dehydrogenase, 6; and lactic dehydrogenase, 0.4.

Glycero-P dehydrogenase increases 10-fold during maturation and almost attains the level of lactic dehydrogenase. The differences in hepatic glucose-6-P dehydrogenase and 6-P-gluconate dehydrogenase between fetus and adult are not striking, although intermediate changes are of interest (see below).

There are a number of modest but statistically significant differences between the adult male and female livers. The enzymes necessary to produce glucose-6-P from glucose or glycogen (hexokinase and phosphorylase) are a third higher in the male than the female. The rest of the measured enzymes of the Embden-Meyerhof pathway concur within 15% of each other except for lactic dehydrogenase, which is 20% higher in the male. In contrast, the two enzymes of the oxidative shunt are one-third lower in the male, so that the ratio of glucose-6-P dehydrogenase to hexokinase is 7 in the female and 4 in the male.

Intermediate Ages—The changes observed during the developmental period are complicated and do not follow any simple relationship to age (Figs. 1 to 4). Except for P-glucoisomerase, the enzymes needed to convert glucose to triose-P fall more or less together. Fructose diphosphate aldolase and P-fructokinase parallel each other from -3 days to +4 days, at which time the adult level is approximated. Hexokinase falls more slowly and does not reach the adult level until weaning. The high level of P-glucoisomerase at birth was observed by Weber and Cantero (7); the fall in fructose diphosphate aldolase after birth was reported by Stave (4).

The other three enzymes of the glycolytic pathway that were measured rise temporarily during development to 50% above the adult level; yet the peak for glyceraldehyde-P dehydrogenase and triose-P isomerase occurs at or just before birth, and the peak of lactic dehydrogenase is at 9 days after birth (in confirmation of Boxer and Shonk (34)). Possibly the early rise in the former two enzymes is related to glycolysis and is compensatory for the falling capacity of P-fructokinase, whereas the rise in lactic dehydrogenase is related to its role in lactate uptake and should be compared to the changes in glucose 6-phosphatase, fructose-1-P aldolase, and even glycero-P dehydrogenase. Of these three enzymes that are conceivably related to lactate utilization, glycero-P dehydrogenase and glucose 6-phosphatase have reached 50% of the adult level at birth, and fructose-1-P aldolase is at the 75% level. All three are at the adult level by 9 days; however, glucose 6-phosphatase goes through a transient maximum

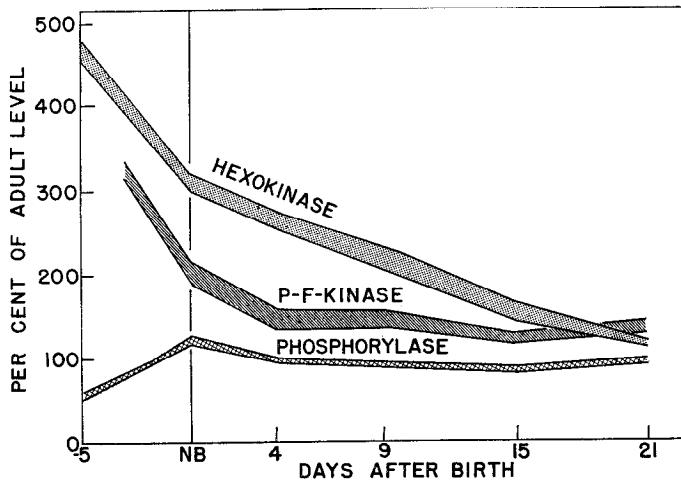


FIG. 1. Changes with age in hexokinase, P-fructokinase (*P-F-kinase*), and phosphorylase of rat liver. The average enzyme levels in male and female adult liver are equal to 100%. The levels are calculated on the basis of protein and expressed as moles per kg of protein per hour. The width of the lines represents \pm one standard error of the mean.

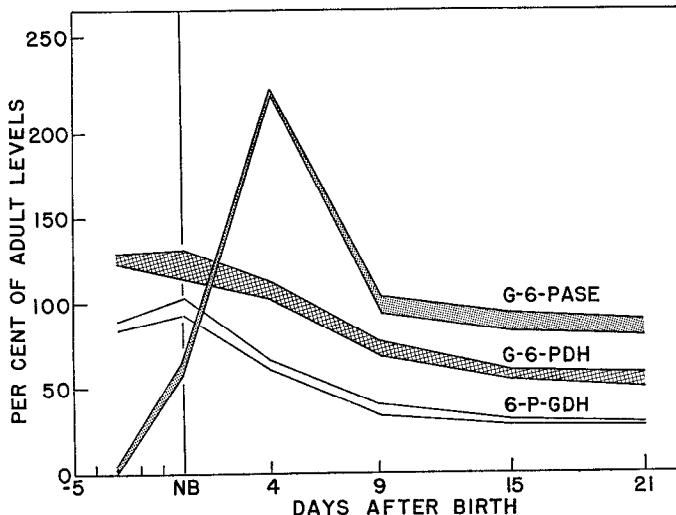


FIG. 2. Changes with age in glucose 6-phosphatase (*G-6-Pase*), glucose-6-P dehydrogenase (*G-6-PDH*), and 6-P-gluconate dehydrogenase (*6-P-GDH*) of rat liver. The data are expressed as in Fig. 1.

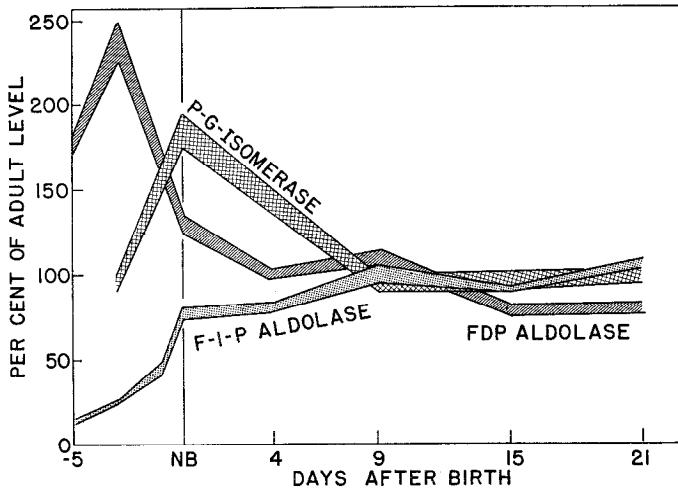


FIG. 3. Changes with age in *P*-glucoisomerase (*P-G-isomerase*), fructose diphosphate aldolase, and fructose-1-P aldolase of rat liver from 5 days before to 21 days after birth. The data are expressed as in Fig. 1.

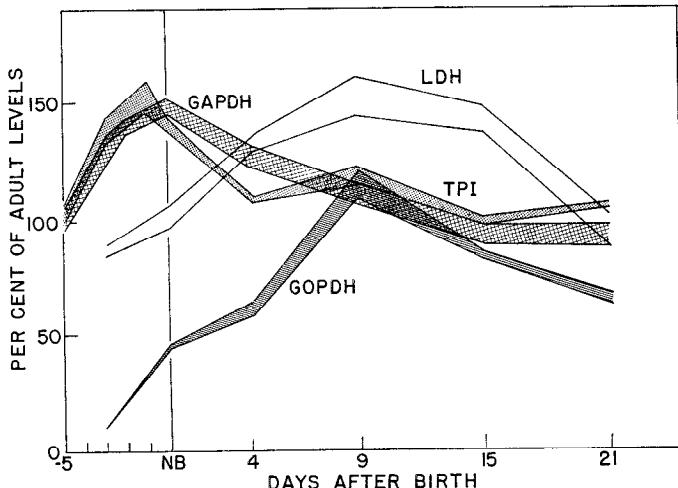


FIG. 4. Changes with age in triose-P isomerase (*TPI*), glycero-P dehydrogenase (*GOPDH*), glyceraldehyde-P dehydrogenase (*GAPDH*), and lactic dehydrogenase (*LDH*) of rat liver. The data are expressed as in Fig. 1.

at 4 days which is more than twice the adult concentration. Boxer and Shonk (34) observed in rat liver changes in glycero-P dehydrogenase similar to those reported here, but the absolute activities were only 10 or 20% as high. They also found no difference between livers of fetal and newborn animals. Others have found very low or zero values for glucose 6-phosphatase in fetal liver (7, 35-38), and their results differ from present findings chiefly in that the peak values were found at birth rather than 4 days later.

To judge from the level of phosphorylase, the hepatic capacity for glycogenolysis reaches the adult level before birth, and subsequent changes are not remarkable.

Very different behavior is seen in regard to the two shunt enzymes, both of which are at or near the adult level before and at the time of birth and are depressed throughout the 21-day nursing period. This is not unexpected since these enzymes are known to be increased by a diet high in carbohydrate (39, 40), whereas milk has a high fat content. The ratio between 6-P-

gluconate dehydrogenase and glucose-6-P dehydrogenase varies considerably throughout growth. It is 1.7 at birth, falls to 1.1 at weaning, and then rises to 2 or over in the adult. The decrease in glucose-6-P dehydrogenase after birth has been reported by several groups (4, 5, 7), but Nemeth and Dickerman (6) found no change in 6-P-gluconate dehydrogenase in guinea pig liver after birth.

DISCUSSION

Adult liver is characterized by low hexokinase, P-fructokinase, and glycolytic capacity. Burk (41) and others (42) have reported that anaerobic glycolysis in livers of different species falls progressively with maturation. In rat liver slices, anaerobic glycolysis at 5 days before birth (39), expressed as moles of lactic acid produced per kg (dry weight) per hour, was 0.4. It fell to 0.36 at 2 days before birth, 0.29 at 8 hours after birth, 0.1 at 1 day of age, and 0.045, the adult rat liver value, at 9 days. This decrease in anaerobic glycolysis is astonishingly parallel to the decreases in hexokinase and phosphofructokinase of developing rat liver, particularly the latter (Fig. 1). Burk cites evidence from histological studies and from experiments with marrow cells and avian blood cells to indicate that the high glycolytic metabolism of embryonic livers is due to their erythropoietic function rather than to true liver cells. Without direct measurements on the immature blood cells of embryonic liver, separate from liver cells, it is not possible to tell whether or not this is the true explanation.

In contrast to the two kinases of the Embden-Meyerhof pathway, fructose diphosphate aldolase falls only moderately during development of the liver. The other four members of the pathway are not greatly different at -5 days of age and in the adult. If these changes signify a capacity for conversion of lactate to glucose rather than the reverse, it might be that some of the enzymes have different kinetic properties in adult and fetus. Flexner *et al.* (43) have shown that in guinea pig and mouse liver, the lactic dehydrogenases differ in kind during the course of development. Certain fetal liver lactic dehydrogenases are lost during the change to adult liver. Presumably similar changes take place in rat liver lactic dehydrogenases during development. The shape of the curve for total activity in the developing mouse liver from birth to 21 days of age (43) is very similar to that obtained with rat liver.

Glucose 6-phosphatase, glycero-P dehydrogenase, and fructose-1-P aldolase are extremely low in the late fetal liver. *Glucose 6-phosphatase* has been the most widely investigated of these enzymes. Its relation to glycogen storage disease, in which it is abnormally low or lacking as described by Cori and Cori (44), emphasizes the importance of glucose 6-phosphatase formation in young liver. In fetal rat liver, storage of glycogen occurs, particularly toward the end of gestation. This happens in all species of animals examined, as reviewed by Shelley (45). There is a precipitous decrease in glycogen of rat liver at birth, with a low level characteristic of the first neonatal days. The present enzyme measurements show that the phosphorylase needed for this glycogenolysis is available at birth and that the glucose 6-phosphatase required for release of free glucose increases 60-fold between -3 and +4 days. After the 4th day, rat liver glycogen rises again (45). This probably cannot be attributed to the corresponding decrease in glucose 6-phosphatase, but the decline may reflect less need for rapid glucose release.

Factors which promote the appearance of glucose 6-phosphatase in fetal liver are conjectural. It seems probable that the supply of glucose to the fetus from the maternal circulation is sufficient so that there would be no need for glucose 6-phosphatase in the fetus. Yet when the demand to supply glucose to the blood appears after birth, the fetal liver has already prepared to meet it. Freedland and Harper find an increase in rat liver glucose 6-phosphatase in response to a decrease in dietary glucose intake. They consider this change a rapid adaptation and a synthesis *de novo* of the enzyme (46). Perhaps there is a dual control for this enzyme, a basic constitutive level characteristic of differentiated liver, plus an incremental level dependent on the mean glucose-6-P level. This might explain the peak value observed at 4 days.

Glycero-P dehydrogenase undergoes an extremely rapid rise in activity between -5 and +9 days of age. The ratio of lactic dehydrogenase to glycero-P dehydrogenase for fetal rat liver 3 days before term is 15; for newborn, 4; and for adult, 1.6. These ratios change in the same direction reported by Boxer and Shonk (34), but differ in magnitude. Although the adult liver is very active in synthesizing phospholipids, it seems doubtful that this synthesis would demand the very high glycero-P dehydrogenase activity observed. Instead, it seems likely that this enzyme serves a metabolic function, perhaps in lactate utilization. By competing with lactic dehydrogenase for DPNH it would favor pyruvate formation from lactate. Bücher and Klingenberg have discussed the possible role of the "glycerophosphate shuttle" as a mechanism for transferring electrons from the extramitochondrial space into the mitochondria (47).

The emergence of *fructose-1-P aldolase* and its increase in fetal liver is in direct contrast to the behavior of fructose diphosphate aldolase. The ratio of the diphosphate aldolase to the 1-phosphate aldolase in the fetus at -5 days is 21, and in the adult it is 1.6. That these enzymes show different patterns of change during development constitutes good evidence for the existence of two different aldolases in liver. On the basis of immunological behavior, Blostein (48) gives further evidence of difference between them. Peanasky and Lardy (49) found crystalline aldolase from bovine liver to act 2.4 times faster on fructose diphosphate than on fructose-1-P. Impure preparations from rat liver (50) exhibit both activities and a similar ratio. Fetal liver aldolases may be quite different from those of adult liver or muscle.

The total activity of fructose-1-P aldolase in the whole liver increases 1600-fold in the period from -5 to 65 days. The usefulness of this development is not clear. Ochoa, Cori, and Cori (51) reported that addition of fructose-1-P to liver homogenates in the absence of fluoride gives rise to glucose. Stein, Cori, and Cori (52) suggested that liver contains a mutase system for the conversion of fructose-1-P to hexose-6-P. If this is the case, the increase of liver fructose-1-P aldolase during growth might furnish a capability for synthesizing glucose from dihydroxyacetone phosphate and glyceraldehyde. The report by Hers and Joassin (53) that fructose-1-P aldolase is low in livers of infants intolerant to fructose suggests an important function for this enzyme.

SUMMARY

Thirteen enzymes involved in carbohydrate metabolism have been measured in the livers of rats at -5, -3, 0, 4, 9, 14, 21, and 65 days of age, during which time the liver increases from 60 to 8400 mg in weight.

Hexokinase, phosphofructokinase, and fructose 1,6-diphosphate aldolase decrease to 20, 30, and 40%, respectively, of prenatal values. Fructose 1-phosphate aldolase and glycerophosphate dehydrogenase each increase 10-fold, and glucose 6-phosphatase increases 30-fold from the fetal to the adult stage. Glycogen phosphorylase doubles during this time. The other enzymes show smaller over-all changes, but all undergo marked changes in level at one time or another during maturation of the liver. The course of rise or fall of the enzymes which are more drastically affected varies from one enzyme to another.

The complex patterns of changes in levels of these enzymes are consistent with a moderate capacity for glycolysis in the fetal liver with a transition, starting before birth, to a capability for utilizing lactic acid and for the conversion of both lactate and glycogen to free glucose.

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