The Metabolism of Glucose 6-Phosphate in Developing Mammalian Tissues

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Two marked changes in the metabolism of glucose 6-phosphate are known to occur in the liver during mammalian development before maturation. First, hepatic glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) appears late in gestation and increases rapidly after birth in several species (Nemeth, 1954; Weber & Cantero, 1955; Kretchmer, 1959; Coquoin-Carnot & Roux, 1960*a*; Dawkins, 1961). Secondly, hepatic glucokinase (a phosphotransferase of the type EC 2.7.1.2) develops after the birth of the animal (Walker, 1962, 1963*b*). A study of the several enzymes concerned in the further metabolism of glucose 6-phosphate has therefore been made to determine whether the development of the glucose 6-phosphatase and glucokinase have marked effects on the other enzymes. The use of the guinea pig has also enabled the development of the glucose 6-phosphatase to be followed not only in the liver and kidney but also in the intestinal mucosa (Ginsburg & Hers, 1960); some properties of this enzyme at various stages of development have been determined to ensure that the large changes are due to the same enzyme.

A preliminary communication (Lea & Walker, 1962) included a report on this work.

MATERIALS AND METHODS

Animals. The type of guinea pig used, breeding procedure, diet and methods of estimating gestational age were as described by Walker (1963*a*).

Chemicals. The glucose 6-phosphate (barium salt) used in the routine assays of glucose 6-phosphatase activity was obtained from British Drug Houses Ltd. and converted into the sodium salt (Swanson, 1955). The glucose 6-phosphate (sodium salt) for the kinetic studies, 6-phosphogluconate (sodium salt), NADP⁺ (sodium salt) and glucose 6-phosphate dehydrogenase (type V, from yeast) (EC 1.1.1.49) were obtained from Sigma Chemical Co. (through G. T. Gurr Ltd., London, S.W. 6). *N*-Phenyl-*p*-phenylenediamine monohydrochloride was obtained from Kodak Ltd., Liverpool. All other chemicals were of the best quality obtained from British Drug Houses Ltd.

Tissue extracts. Young and adult animals were killed by dislocation of the cervical vertebrae; foetal animals were removed from the dead gravid females as quickly as possible and killed by the same method. The livers were removed, chilled in ice-cold homogenizing medium (0.15M-

potassium chloride containing 8 ml. of 0.02 M-potassium bicarbonate/l.; LePage, 1948), weighed and homogenized in 9 vol. of the same medium in a Potter-Elvehjem-type homogenizer (glass tube with Teflon pestle) for 1 min. at 2000 rev./min. The cell supernatant fraction (which was used for all enzyme assays except that for glucose 6-phosphatase) was prepared by centrifugation of the homogenate at 100 000*g* for 30 min. in the Spinco model L preparative ultracentrifuge (rotor no. 40). Kidney and intestinal mucosa tissue were obtained in a similar manner and homogenates and supernatant fractions prepared by analogous procedures.

Enzyme-assay procedures. All estimations of enzyme activity were performed at 30° under optimum conditions giving zero-order kinetics. This was frequently checked by performing assays with two enzyme concentrations and two incubation periods. Checks were also made for each enzyme to ensure that the same optimum conditions held for foetal tissues as for adult tissues.

Routine determinations of glucose 6-phosphatase were performed on whole homogenates at pH 6.5 according to the method of Swanson (1950), inorganic phosphate being determined by the method of Fiske & Subbarow (1925). Kinetic studies on glucose 6-phosphatase were performed on microsomal preparations. These were made by first removing the nuclear and mitochondrial fractions at 15 000*g* for 10 min. in the MSE Major centrifuge (with high-speed attachment and 4 × 25 ml. head) and then sedimenting the microsomal fraction at 100 000*g* for 30 min. The microsomal fraction was washed once in homogenizing medium, recentrifuged at 100 000*g* for 30 min., and resuspended in a volume of medium corresponding to half the volume of the original homogenate. Convenient samples of this suspension were employed in the system described by Hass & Byrne (1960). The glucose 6-phosphate concentrations employed ranged from 1.5 to 20 mM, and the effect of two concentrations of glucose inhibitor (0.06 and 0.12M) was studied. The concentration of the solution of glucose 6-phosphate was checked with glucose 6-phosphate dehydrogenase and NADP⁺. Inorganic phosphate was determined by the micro-method of Dryer, Tames & Routh (1957).

Phosphoglucomutase (EC 2.7.5.1) activity was followed by the decrease in acid-labile phosphate (Najjar, 1955).

Phosphoglucose-isomerase (EC 5.3.1.9) activity was determined by the method of Glock, McLean & Whitehead (1956), except that the method of Roe, Epstein & Goldstein (1949) was used for the estimations of fructose 6-phosphate, with sucrose as a fructose standard. Several different values appear in the literature for the relative colours given by fructose and fructose 6-phosphate. The colours produced by the two substances were taken to be identical for the

present purposes (cf. Dische, 1951; Kahana, Lowry, Schulz, Passonneau & Crawford, 1960).

Glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase (EC 1.1.1.44) activities were assayed by the methods of Glock & McLean (1953), except that the optimum pH for glucose 6-phosphate-dehydrogenase activity was found to be 8.9–9.2 in foetal and adult guinea-pig liver. About 90% of the optimum activity was found at pH 10.1, which was the optimum pH for these tissues recorded by Nemeth & Dickerman (1960). The two methods given by Glock & McLean (1953) for the assay of glucose 6-phosphate-dehydrogenase activity gave identical results in the guinea-pig tissues examined and, for routine use, the method in which excess of 6-phosphogluconate dehydrogenase is added was employed.

Expression of enzyme activities. The need for an adequate basis for the expression of enzyme activities in studies on metabolic adaptation has been emphasized by Weber (1963). Several factors make it impossible to use the most desirable characteristics in the present study. Liver tissue shows only slight changes in the dry wt.:wet wt. ratio during development (Vilée & Hagerman, 1958; Vilée, Hagerman, Holmberg, Lind & Vilée, 1958), but the nature of the dry weight changes considerably, e.g. there is the preparturition accumulation of glycogen (Shelley, 1961) and of fat (Imrie & Graham, 1920; Rähä, 1961). Expression of enzymic activity per wet wt. of tissue, per dry wt. of tissue, or per unit of nitrogen or DNA phosphorus are all open to the objection that a large change in one cell constituent could result in an apparent change in enzyme activity when, in fact, no change had occurred. Activity/average cell is a more acceptable basis. Unfortunately, it was found that the use of a cellularity determination method such as that of Weber & Cantero (1957) with the nuclear stain orcein cannot be applied to foetal and newborn tissues because of the contribution of nucleated erythrocytes and leucocytes which give rise to high cell counts until some time after birth (see also Weber & Cantero, 1955, for the rat) and cannot be adequately removed or counted differentially. For these reasons activities are here expressed per g. wet wt. of tissue, which is a measure of 'enzyme concentration' (Harper & Young, 1959), per 100 mg. of nitrogen (determined by a micro-Kjeldahl procedure) and as activity of whole liver/100 g. body wt. The last-mentioned representation allows for any changes in the liver wt.:body wt. ratio due to changes in either factor and is an adequate means of assessing the physiological significance of the hepatic enzyme activity to the whole animal.

RESULTS

Development of glucose 6-phosphatase. In the rat (Kretchmer, 1959; Coquoin-Carnot & Roux, 1960a, b), glucose 6-phosphatase activity shows a peak 1–2 days after birth in the liver, but in the kidney the activity rises gradually during late gestation and early extra-uterine life, reaching adult levels about 2 weeks after parturition. In contrast, the activities in the three guinea-pig tissues, liver, kidney and intestinal mucosa (Figs. 1–3), all showed peaks in the progress curves 1–2 days after birth. Dawkins (1961) demonstrated

in the rabbit that birth itself in some way acts as the stimulus to the great increase in glucose 6-phosphatase activity. The present results indicate, however, that in the guinea pig, which is born at a stage of physical development well in advance of that of the rat at birth, the activity of glucose 6-

Fig. 1. Development of glucose 6-phosphatase in the guinea-pig liver. Experimental details are given in the text. T, Term; A, adult (over 4 months old).

Fig. 2. Development of glucose 6-phosphatase in the guinea-pig kidney. Details are as given in Fig. 1.

phosphatase in all those tissues is rapidly approaching adult levels immediately before birth (when expressed per g. wet wt. of tissue). The large increases that follow birth might be considered as a special stimulation of activity superimposed on a gradual increase.

Properties of glucose 6-phosphatase during development. The rapid changes in glucose 6-phosphatase activity after birth are greater than those observed in other conditions in the adult animal, e.g. in diabetes (Ashmore, Hastings & Nesbett, 1954; Langdon & Weakley, 1955; Ashmore, Hastings, Nesbett & Renold, 1956; Harper, 1959). Though Hass & Byrne (1960) showed that the properties of the enzyme in normal and diabetic livers are similar (cf. Segal, Washko & Lee, 1958; Segal & Washko, 1959), and Freedland (1962) found many properties of rat-liver and rat-kidney glucose 6-phosphatase to be identical, it was considered important to establish that the large changes near birth were due to changes in one and the same enzyme. Several characteristics of the enzyme at different stages of development in guinea-pig liver, kidney and intestinal mucosa were therefore compared. First, it was found that at least 85% of the total glucose 6-phosphatase activity of whole homogenates of each of the three tissues was present in the washed microsomal fraction, prepared from tissue samples at three different stages. These were foetal animals of gestational age 64–67 days, newborn animals about 2 days old and normal adult guinea pigs (at least 4 months old). Secondly, no significant change in phosphatase activity (measured at pH 6.5) during development was found in whole homogenates of the three tissues with substrates such as glucose 1-phosphate (in the absence of added Mg^{2+} ions and

cysteine, so that phosphoglucomutase activity was negligible) and sodium β -glycerophosphate (initial concentration 20 mM). Up to a gestational age of about 57 days the phosphatase activity was greater with sodium β -glycerophosphate than with glucose 6-phosphate as substrate. After this time, phosphatase activity at pH 6.5 towards glucose 6-phosphate was the greater. The ratio of the activity with sodium β -glycerophosphate to that with glucose 6-phosphate after birth was always less than 0.12, 0.30 and 0.65 for liver, kidney and intestinal mucosa respectively. Thirdly, the lability of the enzyme at pH 5 in whole homogenates of the same three tissues from a foetus of gestational age 61 days was determined. The decreases in glucose 6-phosphatase activity after 15 min. at pH 5 measured at pH 6.5 were in a typical experiment 67.8, 69.4 and 55.6% for liver, kidney and intestinal-mucosa homogenates respectively. When sodium β -glycerophosphate was used as substrate the corresponding decreases in phosphatase activity were 9.7, 4.4 and 17.5%. These results correlate with the known lability of glucose 6-phosphatase at pH 5 (de Duve, Berthet, Hers & Dupret, 1949). The slightly different results with intestinal mucosa, compared with liver and kidney, is almost certainly due to the high non-specific phosphatase activity of this tissue. Lastly, the kinetic parameters of the enzyme in all three tissues at various stages of development were determined by following the general procedure of Hass & Byrne (1960). By using washed microsomes from a normal male adult guinea pig, the effect of substrate (glucose 6-phosphate) concentration on reaction rate and the nature of the inhibitory effect of glucose was determined, and the results were in essential agreement with those of workers who used rat liver. The inhibition by glucose was not explicable in terms of the standard types of competitive, non-competitive or mixed inhibition (Dixon & Webb, 1958), but were consistent with the treatment by Hass & Byrne (1960).

Fig. 4 shows typical reciprocal plots for microsomal glucose 6-phosphatase from a 2-day-old guinea pig, i.e. at the stage of maximum activity. By using the equation and the calculation procedure of Hass & Byrne (1960), the parameters for a total of 13 tissue samples were obtained (Table 1). There were no significant differences between the values for the kinetic parameters K_m and K_i for the enzyme present in any of the three tissues or at different developmental stages for any one tissue. The values of K_i (glucose) for the six liver samples (111 ± 7 mM) were significantly lower than the corresponding value for the seven results for kidney and intestinal mucosa combined (158 ± 14 mM) (means $\pm$ S.E.M.; $P < 0.01$). On pooling all these results the mean ($\pm$ S.E.M.) values for K_m , K_i

Fig. 3. Development of glucose 6-phosphatase in the guinea-pig intestinal mucosa. Details are as given in Fig. 1.

(glucose) and K_s were 5.1 ± 0.2 mM, 137 ± 10 mM and 2.5 ± 0.2 mM respectively. These compare with 5.9 mM, 78 mM and 0.86 mM for the same respective constants in normal rat liver (Hass & Byrne, 1960).

Activities of phosphoglucomutase, phosphoglucose isomerase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the developing liver. The results are presented in Table 2. Both

Fig. 4. Reciprocal plots showing the influence of the initial glucose 6-phosphate concentration on microsomal glucose 6-phosphatase of liver from a 2-day-old guinea pig and the effect of glucose on the reaction rate. The reaction mixture (total vol. 2.5 ml.) contained 1.0 ml. of buffer (0.1M with respect to succinate, maleate and tris, adjusted with 0.2N-NaOH to pH 6.4), glucose 6-phosphate (1.5–20 mM), 0.1 ml. of microsomal suspension, and no added glucose (○), 0.06M-glucose (△), or 0.12M-glucose (□). Incubations were performed at 30°. Further experimental details are given in the text.

phosphoglucomutase and phosphoglucose isomerase showed considerable increases in activity after birth both per g. wet wt. and per 100 mg. of nitrogen. The increases are significant ($P < 0.001$), and that for phosphoglucomutase is to be contrasted with the absence of changes recorded by Nemeth, Insull & Flexner (1954). They are similar to the increases observed by Kornfeld & Brown (1963) for two other enzymes concerned in glycogen metabolism, α -glucan-UDP glucosyltransferase (EC 2.4.1.11) and UDP-glucose pyrophosphorylase (EC 2.7.7.9).

The initially high but decreasing level of glucose 6-phosphate-dehydrogenase activity in foetal guinea-pig livers confirms the results of Nemeth & Dickerman (1960) and differs from the pattern in the rat (Weber & Cantero, 1957), where values of 25–75 % of adult glucose 6-phosphate-dehydrogenase activity were recorded in foetal rat liver. We have confirmed this finding (the enzyme activity in the supernatant fraction of a homogenate of pooled livers from a litter of 20-day-old foetal rats was 36 % of a similar preparation of the maternal liver). The activity of 6-phosphogluconate dehydrogenase remained remarkably constant throughout the whole time period studied, and was always higher on an absolute basis than that of glucose 6-phosphate dehydrogenase. Glucose 6-phosphate dehydrogenase may thus limit the oxidation of glucose 6-phosphate through the pentose phosphate cycle in liver tissue. Because of this 6-phosphogluconate is unlikely to accumulate in liver tissue, so that it is doubtful whether this intermediate inhibits phosphoglucose isomerase *in vivo* (Parr, 1956).

Overall pattern of enzymes metabolizing glucose 6-phosphate in liver tissue during development, and comparison with kidney tissue. The results given thus far indicated that the appearance and marked

Table 1. *Properties of the glucose 6-phosphatase present in guinea-pig liver, kidney and intestinal mucosa at various stages of development*

The determinations were carried out at pH 6.4 and at 30°. Experimental details are given in the text. Adult animals were at least 4 months old.

Tissue	Age of animal	K_m (mM)	K_i (glucose) (mM)	K_s (mM)
Liver	Foetus (gestational age 66 days)	4.1	96	2.1
	Newborn (3 days)	4.9	93	2.8
	Newborn (3 days)	4.9	121	1.8
	Young (male) (1 month)	6.3	106	3.9
	Adult (male)	5.0	145	3.2
	Adult (male)	6.4	106	1.9
Kidney	Newborn (1.5 days)	4.6	137	2.5
	Newborn (3 days)	4.6	122	3.3
	Adult (male)	5.4	195	3.0
	Adult (male)	4.6	205	2.4
Intestinal mucosa	Newborn (2 days)	4.2	145	2.3
	Adult	5.6	150	2.0
	Adult	5.8	155	1.0

increase in glucose 6-phosphatase activity near birth was not associated with any marked change in the activity of other enzymes acting on glucose 6-phosphate. However, it was decided to see how the activities of the several enzymes varied when their activities are expressed in terms of the body weight which the liver must serve (because of the known role of the liver in the adult animal in the homeostatic control of carbohydrate metabolism). Because of the considerable variation in the number of determinations made at the various developmental stages, only the mean activities for each group are recorded (Table 3). In general, the pattern of results is similar to that per g. wet wt. of tissue, and the maximum activity of the pentose phosphate-cycle enzymes is low compared with the potential ability to convert glucose 6-phosphate into glucose 1-phosphate and fructose 6-phosphate and also into glucose (except during the earlier stages of gestation).

To ascertain whether the changes in the liver are unique, the enzymic activities were also measured in guinea-pig kidney (Table 4). The gestational ages of the three fetuses used for most of these determinations were 46, 57 and 60 days. Phosphoglucomutase activity was lower in the kidney than in the liver, whereas the activities of the two dehydrogenases were higher. The pattern of glucose 6-phosphatase development has been given above (Fig. 2), and the only other significant change was an increase in phosphoglucose-isomerase activity

both per g. wet wt. of tissue ($P < 0.01$) and per 100 mg. of nitrogen ($0.01 < P < 0.02$). There is again a species difference here, for Weber (1961) found the activities of the two dehydrogenases were lower in the kidney than in the liver in the rat. The present results show that the reverse is the case in the guinea pig.

DISCUSSION

Except for the value for K_i (glucose) of hepatic glucose 6-phosphatase as compared with those for the enzyme in kidney and intestinal mucosa, the properties of the enzyme in these three tissues were similar, and gave no grounds for considering them as anything but one and the same enzyme. It cannot be ascertained from the present results whether or not the noted exception is real (i.e. due to modifications in enzyme structure such as are found in isoenzymes), or due to an artifact of a physical nature, such as might result from the use of washed microsomes and not a soluble more-purified enzyme, or due to the presence of an inhibitor in the enzyme preparation. However, it is now established that the glucose 6-phosphatase has the same properties at various stages of development, including the phase of high activity in the early neonatal period. The high activities at this time are not due to mechanisms such as release from microsomes into the soluble fraction. The progress curves of glucose 6-phosphatase develop-

Table 2. *Enzymic activities in developing guinea-pig liver tissue*

The activities are expressed as μ moles of substrate metabolized/min. either per g. wet wt. of tissue or 100 mg. of nitrogen, as indicated. Results are given as means $\pm$ S.D., with the numbers of determinations in parentheses. Experimental details are given in the text. Adult animals were at least 4 months old.

Age of animals	Phosphoglucomutase activity		Phosphoglucose-isomerase activity	
	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)
Foetuses (gestational age 34-47 days)	44.5 $\pm$ 6.3 (4)	172 $\pm$ 25 (4)	34.2 $\pm$ 5.8 (4)	131 $\pm$ 13 (4)
Foetuses (gestational age 48-54 days)	45.6 $\pm$ 17.6 (5)	191 $\pm$ 49 (5)	27.9 $\pm$ 8.0 (5)	118 $\pm$ 33 (5)
Foetuses (gestational age 55-61 days)	55.5 $\pm$ 12.1 (6)	247 $\pm$ 53 (6)	43.4 $\pm$ 3.9 (6)	193 $\pm$ 14 (6)
Foetuses (gestational age 62-68 days)	61.6 $\pm$ 6.0 (5)	273 $\pm$ 40 (5)	35.9 $\pm$ 8.3 (5)	158 $\pm$ 33 (5)
Newborn (0-3 days)	73.7 $\pm$ 16.2 (4)	313 $\pm$ 39 (4)	74.5 $\pm$ 35.0 (4)	344 $\pm$ 123 (4)
Newborn (4-10 days)	99.6 $\pm$ 23.7 (3)	346 $\pm$ 35 (3)	104.5 $\pm$ 21.0 (3)	379 $\pm$ 119 (3)
Adults	92.3 $\pm$ 19.4 (8)	372 $\pm$ 92 (8)	84.6 $\pm$ 18.4 (8)	341 $\pm$ 69 (8)

Age of animals	Glucose 6-phosphate-dehydrogenase activity		6-Phosphogluconate-dehydrogenase activity	
	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)
Foetuses (gestational age 34-47 days)	1.54 $\pm$ 0.43 (3)	6.40 $\pm$ 3.40 (3)	1.83 $\pm$ 0.33 (3)	7.49 $\pm$ 2.88 (3)
Foetuses (gestational age 48-54 days)	1.75 $\pm$ 0.16 (5)	7.42 $\pm$ 0.90 (5)	2.00 $\pm$ 0.26 (5)	8.49 $\pm$ 1.10 (5)
Foetuses (gestational age 55-61 days)	1.30 $\pm$ 0.16 (6)	5.64 $\pm$ 1.00 (6)	1.87 $\pm$ 0.24 (4)	8.18 $\pm$ 1.12 (4)
Foetuses (gestational age 62-68 days)	1.32 $\pm$ 0.29 (5)	5.81 $\pm$ 1.20 (5)	1.75 $\pm$ 0.34 (5)	7.71 $\pm$ 1.46 (5)
Newborn (0-3 days)	0.70 $\pm$ 0.16 (3)	3.04 $\pm$ 0.54 (3)	1.46 $\pm$ 0.41 (3)	6.40 $\pm$ 1.23 (3)
Newborn (4-10 days)	0.50 $\pm$ 0.21 (3)	1.70 $\pm$ 1.17 (3)	1.70 $\pm$ 0.25 (3)	6.20 $\pm$ 2.01 (3)
Adults	0.39 $\pm$ 0.09 (8)	1.56 $\pm$ 0.56 (8)	2.00 $\pm$ 0.45 (6)	8.00 $\pm$ 1.59 (6)

Table 3. *Mean enzymic activities in developing and adult guinea-pig liver tissue*

The activities are expressed as μ moles of substrate metabolized/min./100 g. body wt. (individual animal or foetus), and also as percentages of the activity of the particular enzyme in the adult animal. Experimental details are given in the text. Adult animals were at least 4 months old.

Age of animals	Glucose 6-phosphatase activity		Phosphoglucose-mutase activity		Phosphoglucose-isomerase activity	
	(μ moles/min./100 g. body wt.)	(% of activity in adult)	(μ moles/min./100 g. body wt.)	(% of activity in adult)	(μ moles/min./100 g. body wt.)	(% of activity in adult)
Foetuses (gestational age 34-47 days)	—	—	262	60	201	50
Foetuses (gestational age 48-54 days)	—	—	288	66	176	44
Foetuses (gestational age 55-61 days)	8.0	15	266	61	200	50
Foetuses (gestational age 62-68 days)	13.1	25	290	66	169	42
Newborn (0-3 days)	79.8	154	321	73	325	81
Newborn (4-10 days)	56.5	109	333	76	349	87
Adults	51.9	100	437	100	400	100

Age of animals	Glucose 6-phosphate-dehydrogenase activity		6-Phosphoglucose-dehydrogenase activity	
	(μ moles/min./100 g. body wt.)	(% of activity in adult)	(μ moles/min./100 g. body wt.)	(% of activity in adult)
Foetuses (gestational age 34-47 days)	9.1	505	10.8	114
Foetuses (gestational age 48-54 days)	11.0	611	12.7	134
Foetuses (gestational age 55-61 days)	6.0	333	8.6	90
Foetuses (gestational age 62-68 days)	6.2	344	8.2	86
Newborn (0-3 days)	3.1	172	6.4	67
Newborn (4-10 days)	1.7	94	5.7	60
Adults	1.8	100	9.5	100

Table 4. *Enzymic activities in developing guinea-pig kidney tissue*

The activities are expressed as μ moles of substrate metabolized/min. either per g. wet wt. of tissue or per 100 mg. of nitrogen, as indicated. Results are given as means $\pm$ s.d., with the numbers of determinations in parentheses. Experimental details are given in the text. Adult animals were at least 4 months old.

Age of animals	Phosphoglucose-mutase activity		Phosphoglucose-isomerase activity	
	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)
Foetuses	26.5 $\pm$ 10.3 (3)	143 $\pm$ 39 (3)	34.1 $\pm$ 11.2 (6)	187 $\pm$ 42 (6)
Newborn (0-10 days)	33.7 $\pm$ 3.6 (4)	151 $\pm$ 16 (4)	55.1 $\pm$ 14.1 (4)	246 $\pm$ 60 (4)
Adults	34.2 $\pm$ 1.1 (3)	139 $\pm$ 13 (3)	67.1 $\pm$ 11.6 (6)	275 $\pm$ 55 (6)

Age of animals	Glucose 6-phosphate-dehydrogenase activity		6-Phosphoglucose-dehydrogenase activity	
	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)	(μ moles/min./g. wet wt.)	(μ moles/min./100 mg. of N)
Foetuses	1.78 $\pm$ 0.39 (3)	9.78 $\pm$ 0.65 (3)	2.60 $\pm$ 0.75 (3)	14.2 $\pm$ 2.1 (3)
Newborn (0-10 days)	1.85 $\pm$ 0.42 (4)	8.29 $\pm$ 1.83 (4)	2.98 $\pm$ 0.24 (4)	13.2 $\pm$ 0.7 (4)
Adults	2.07 $\pm$ 0.35 (3)	8.35 $\pm$ 0.93 (3)	3.74 $\pm$ 0.18 (3)	15.1 $\pm$ 0.3 (3)

ment are therefore considered to reflect the amount of enzyme present at any stage.

The fact that these progress patterns are very similar for all three guinea-pig tissues suggests that the factors that control the expression of the initial genetically-based ability to synthesize the enzyme are the same or very similar in the three tissues. The developmental pattern recorded is different from that in the rat (Kretchmer, 1959;

Coquoin-Carnot & Roux, 1960b), for in that species no post-parturition peak in activity in the kidney is observed. This species difference emphasizes that it is dangerous to make generalizations on the control of development from studies on one species only. With glucose 6-phosphatase, it is reasonable to correlate the post-parturition increase in enzymic activity with the rapid fall in tissue glycogen that occurs immediately after birth (Shelley, 1961;

Nemeth, 1954; Coquoin-Carnot & Roux, 1960*a, b*). It is not known whether the fall in glycogen is a direct result of the increase in glucose 6-phosphatase activity or whether the increase in glucose 6-phosphatase is the result of increased glycogenolysis. Some other mechanism of adaptation may operate. Further, it is not yet established whether the large increase in glucose 6-phosphatase is a stimulation of synthesis of the enzyme or a decrease in its rate of degradation. The former would seem more likely in view of the observations (Kvam & Parks, 1960*a, b*) that ethionine and 8-azaguanine inhibit the increase in enzyme activity induced by hydrocortisone in the adult animal.

Changes in the activities of enzymes metabolizing glucose 6-phosphate in the liver of adult animals correlate well with overall metabolic adaptations (see Weber, 1963, for review). The variations recorded in the present work on developing animals may reflect changes in emphasis on various metabolic pathways during hepatic maturation. Burch *et al.* (1963) have interpreted changes in a wider range of enzymes in developing rat liver in such terms. Decreases in the pentose phosphate-cycle dehydrogenases during the nursing period were discussed in terms of the high fat content of milk. Such changes were much less marked in the present study. The guinea pig is born at a comparatively later stage of development and commences eating solid food much earlier than does the neonatal rat.

The increases in stored glycogen that occur in late gestation (Shelley, 1961; Kornfeld & Brown, 1963) and the rapid decrease after birth suggest that the glycogen stores are not significantly utilized until the demands of extra-uterine life commence. The observations reported above indicate that, apart from glucose 6-phosphatase, the foetal and neonatal liver contains all the enzymes necessary for the normal functioning of the metabolic pathways diverging from glucose 6-phosphate that are present in the adult liver. Insufficient quantitative information is yet available on the magnitude of the immediate nutritional demands of the newborn animal and the nature of the hormonal changes that must inevitably occur during development (see, for example, Jost, 1961) to assess the control mechanisms involved. The results of Kornfeld & Brown (1963) on the enzymes directly involved in glycogen synthesis and degradation, together with the present study (which may be considered to be complementary), indicate that the enzyme activities measured under conditions of optimum pH, substrate concentration etc. cannot alone explain the gross changes in carbohydrate metabolism that occur. The concentration of glucose 6-phosphate itself will affect the rates of the various reactions and this may vary considerably as both glucose 6-phosphatase and glucokinase

develop and the relative contributions of hexokinase and glucokinase to glucose 6-phosphate formation vary (Walker & Rao, 1964).

The existence of species differences in enzyme-development patterns implies that changes in enzyme concentration may not reflect changes in emphasis on certain pathways. The correlation of glucose 6-phosphate-dehydrogenase activity with the pentose phosphate cycle, as is reasonable in many situations (Weber, 1963), becomes less convincing in the light of the different developmental patterns for the enzyme in the rat and guinea pig. The increases in NADPH concentration between foetal liver and newborn liver (Nemeth & Dickerman, 1960) and then to adult liver (Räihä, 1961) reflect not only the rate of NADPH production, mainly by the two dehydrogenases, but also its rate of utilization in such reductive processes as fat biosynthesis, marked changes in which occur near birth (Villev & Hagerman, 1958).

SUMMARY

1. Glucose 6-phosphatase development before and after birth follows a similar pattern in guinea-pig liver, kidney and intestinal mucosa.

2. The properties and kinetic characteristics of glucose 6-phosphatase show no change during development, so that the large increases in activity after birth are due to increased enzyme concentration.

3. The activities of phosphoglucomutase, phosphoglucose isomerase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in developing liver and kidney show, on comparison, a number of distinct changes in the liver. The activities are sufficient to permit glucose 6-phosphate metabolism by the known metabolic pathways, but the changes reveal no means of control of glycogen metabolism during development.

4. There are limitations to the value of activity determinations for glucose 6-phosphate-metabolizing enzymes in the developmental period.

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REFERENCES

- Ashmore, J., Hastings, A. B. & Nesbitt, F. B. (1954). *Proc. nat. Acad. Sci., Wash.*, **40**, 673.
- Ashmore, J., Hastings, A. B., Nesbitt, F. B. & Renold, A. E. (1956). *J. biol. Chem.* **218**, 77.
- Burch, H. B., Lowry, O. H., Kuhlman, A. M., Skerjance, J., Diamant, E. J., Lowry, S. R. & Dippe, P. V. (1963). *J. biol. Chem.* **238**, 2267.
- Coquoin-Carnot, M. & Roux, J. M. (1960*a*). *C.R. Soc. Biol., Paris*, **154**, 1763.

- Coquoin-Carnot, M. & Roux, J. M. (1960b). *Bull. Soc. Chim. biol., Paris*, **42**, 237.
- Dawkins, M. J. R. (1961). *Nature, Lond.*, **191**, 72.
- de Duve, C., Berthet, J., Hers, H. G. & Dupret, L. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 1242.
- Dische, Z. (1951). In *Symposium on Phosphorus Metabolism*, vol. 1, p. 171. Ed. by McElroy, W. D. & Glass, B. Baltimore: The John Hopkins Press.
- Dixon, M. & Webb, E. C. (1958). *Enzymes*, p. 181. London: Longmans, Green and Co. Ltd.
- Dryer, R. L., Tames, A. R. & Routh, J. I. (1957). *J. biol. Chem.* **225**, 177.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Freedland, R. A. (1962). *Biochim. biophys. Acta*, **62**, 427.
- Ginsburg, V. & Hers, H. G. (1960). *Biochim. biophys. Acta*, **38**, 427.
- Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
- Glock, G. E., McLean, P. & Whitehead, J. K. (1956). *Biochem. J.* **63**, 520.
- Harper, A. E. (1959). *Biochem. J.* **71**, 702.
- Harper, A. E. & Young, F. G. (1959). *Biochem. J.* **71**, 696.
- Hass, L. F. & Byrne, W. L. (1960). *J. Amer. chem. Soc.* **82**, 947.
- Imrie, C. G. & Graham, S. G. (1920). *J. biol. Chem.* **44**, 243.
- Jost, A. (1961). *Harvey Lect.* p. 201.
- Kahana, S. E., Lowry, O. H., Schulz, D. W., Passonneau, J. V. & Crawford, E. J. (1960). *J. biol. Chem.* **235**, 2178.
- Kornfeld, R. & Brown, D. H. (1963). *J. biol. Chem.* **238**, 1604.
- Kretchmer, N. (1959). *Pediatrics, Springfield*, **23**, 606.
- Kvam, D. C. & Parks, R. E. (1960a). *Amer. J. Physiol.* **198**, 21.
- Kvam, D. C. & Parks, R. E. (1960b). *J. biol. Chem.* **235**, 2893.
- Langdon, R. G. & Weakley, D. R. (1955). *J. biol. Chem.* **214**, 167.
- Lea, M. A. & Walker, D. G. (1962). *Biochem. J.* **85**, 30p.
- LePage, G. A. (1948). *J. biol. Chem.* **176**, 1009.
- Najjar, V. A. (1955). In *Methods in Enzymology*, vol. 1, p. 294. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Nemeth, A. M. (1954). *J. biol. Chem.* **208**, 773.
- Nemeth, A. M. & Dickerman, H. (1960). *J. biol. Chem.* **235**, 1761.
- Nemeth, A. M., Insull, W. & Flexner, L. B. (1954). *J. biol. Chem.* **208**, 765.
- Parr, C. W. (1956). *Nature, Lond.*, **178**, 1401.
- Räihä, N. C. R. (1961). *Amer. J. Physiol.* **201**, 961.
- Roe, J. H., Epstein, J. H. & Goldstein, N. T. (1949). *J. biol. Chem.* **178**, 839.
- Segal, H. L. & Washko, M. E. (1959). *Fed. Proc.* **18**, 321.
- Segal, H. L., Washko, M. E. & Lee, C. W. (1958). *Science*, **128**, 251.
- Shelley, H. J. (1961). *Brit. med. Bull.* **17**, 137.
- Swanson, M. A. (1950). *J. biol. Chem.* **184**, 647.
- Swanson, M. A. (1955). In *Methods in Enzymology*, vol. 2, p. 541. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Villee, C. A. & Hagerman, D. D. (1958). *Amer. J. Physiol.* **194**, 457.
- Villee, C. A., Hagerman, D. D., Holmberg, N., Lind, J. & Villee, D. B. (1958). *Pediatrics, Springfield*, **22**, 953.
- Walker, D. G. (1962). *Biochem. J.* **84**, 118p.
- Walker, D. G. (1963a). *Biochem. J.* **87**, 576.
- Walker, D. G. (1963b). *Biochim. biophys. Acta*, **77**, 209.
- Walker, D. G. & Rao, S. (1964). *Biochem. J.* **90**, 360.
- Weber, G. (1961). *Proc. Soc. exp. Biol., N.Y.*, **108**, 631.
- Weber, G. (1963). *Advanc. Enzyme Regulation*, **1**, 1.
- Weber, G. & Cantero, A. (1955). *Cancer Res.* **15**, 679.
- Weber, G. & Cantero, A. (1957). *Endocrinology*, **61**, 701.

Biochem. J. (1964), **91**, 424

The Concentrations of Cobalt, Copper, Iron and Zinc in some Normal Human Tissues as Determined by Neutron-Activation Analysis

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In recent years there had been much interest in the concentrations of trace metals occurring in human and animal tissues and in the manner in which these concentrations may alter in malignant and other diseases. For most trace metals there are well-established chemical methods of analysis of suitably high sensitivity. However, some metals, e.g. cobalt, occur in such low concentrations in biological materials that their determination by conventional methods of analysis is difficult and uncertain, and consequently relatively little is

known about their concentrations in normal and pathological tissues.

Thermal-neutron-activation analysis, which has been shown to be a useful technique in the general field of trace-element studies (Atkins & Smales, 1959; Bowen & Cawse, 1959), is potentially a very valuable technique for the determination of cobalt at the concentrations existing in human tissues. Even without the use of the highest available neutron fluxes, activation analysis affords for cobalt a far greater sensitivity than that