A Comparison of the Properties of Fructose 1,6-Diphosphatase, and the Activities of other Key Enzymes of Carbohydrate Metabolism, in the Livers of Embryonic and Adult Rat, Sheep and Domestic Fowl

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1. The activities of some key enzymes of glycolysis and gluconeogenesis were measured in embryonic chick, sheep and rat livers. 2. In chicken the activities of hexokinase, phosphofructokinase and pyruvate kinase are low, but those of glucose 6-phosphatase and fructose diphosphatase are very high; the converse situation exists in the rat (Burch *et al.* 1963), but in sheep the activities of both phosphofructokinase and fructose diphosphatase are high, and the activities of hexokinase and glucose 6-phosphatase are low. These findings are discussed in relation to carbohydrate metabolism in these embryonic livers. 3. The regulatory properties of fructose diphosphatase from the embryonic livers of these three species were compared with the properties of the enzymes from adult animals. The inhibitions by AMP and fructose diphosphate and the effects of Mg^{2+} and pH on the activities of adult and foetal fructose diphosphatase are almost identical. 4. It is concluded that regulatory properties are characteristic of fructose diphosphatase from embryonic and adult tissue, and the importance of this in relation to enzyme development is discussed.

Studies with enzymes in embryonic tissue have been mainly confined to measuring enzyme activity changes during development, in an attempt to correlate differentiation with enzyme synthesis. Metabolic activity in embryonic tissue is under genetic control such that, at a particular time of development, specific enzyme synthesis is initiated and a particular metabolic reaction or pathway is established. It is not known, however, how the rate of enzyme synthesis is regulated during the remainder of embryonic and post-natal development or whether other metabolic control systems are also present. Certain properties of enzymes in adult animals which are considered to be of importance for metabolic control may only appear at a late stage of development. Some changes during development have been observed for a number of proteins (e.g. lactate dehydrogenase isoenzymes; see Markert & Ursprung, 1962; Kaplan & Goodfriend, 1964).

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In this investigation the properties of FDPase‡ (D-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) from adult and embryonic rat, sheep and domestic fowl livers have been examined with special reference to inhibition by excess of substrate, fructose diphosphate, and by low concentrations of AMP (Taketa & Pogell, 1965; Underwood & Newsholme, 1965b). FDPase was considered ideal for such a study as the sensitivities to AMP and substrate inhibitions appear to be constant properties of this enzyme and are not dependent upon the concentration of any other common intermediate or cofactor of metabolism (except Mg^{2+} concentration).

The rat, sheep and domestic fowl were chosen for this investigation as FDPase develops at different rates in the embryonic livers of these animals, and because the metabolic role of the liver in relation to glucose metabolism is different in these three species. FDPase activity in rat increases gradually throughout development and appears to be more important to the animal after birth than during embryonic development (Burch *et al.* 1963). In the developing chick this enzyme plays a fundamental

‡ Abbreviation: FDPase, fructose 1,6-diphosphatase.

role in provision of glucose for an embryo, which is provided with only 300–500mg. of carbohydrate, but adequate stores of protein and fat (Needham, 1931). The FDPase of the embryonic sheep appears to be related to liver glycogen metabolism rather than glucose metabolism (see the Discussion section). The experiments presented and discussed in this paper were designed to examine whether the control mechanisms in the three species might be different or develop at different stages.

MATERIALS AND METHODS

Chemicals and enzymes. These were obtained from the sources given by Underwood & Newsholme (1965a,b).

Animals. Rats were of the Wistar strain, fed *ad libitum* on a diet of commercially available rat cubes (H. C. Styles Ltd., Bewdley). Foetuses were obtained from 14–21-day-pregnant rats under pentobarbitone anaesthesia. Adult domestic fowls, 1-day-old chicks and fertile eggs of the Cobb strain were obtained from a local commercial breeder (G. T. Hook, Aston, Oxon.). Pregnant sheep of the Clan Hampshire breed were supplied by a local farmer to the Nuffield Institute for Medical Research, and the foetuses were obtained under chloralose anaesthesia. Livers of adult sheep were obtained from the local abattoir.

Age of embryos. The age of the sheep embryo was calculated from the known time of mating; that of the chick from the period of incubation of the eggs; and that of the rat by comparing the average foetal weight with the data of Stotsenburg (1915).

Removal and collection of livers. The livers of the foetal sheep were removed immediately after death, and chilled in ice until extracted, usually 10–15 min. later. As the accumulation of any appreciable amount of liver from embryonic rats or chickens is a time-consuming process, it was considered desirable to freeze the tissue as it was collected to avoid any possible loss of activity or properties. The livers from these embryos were quickly excised and frozen between aluminium blocks cooled in liquid N₂. Later they were powdered in a percussion mortar at –70°. Preliminary experiments established that neither phosphofructokinase nor FDPase was adversely affected by this procedure. Livers from the adult fowl and rat were treated in the same way for studying the properties of FDPase. Small amounts of tissue for determination of hexokinase and glucose 6-phosphatase activities were removed and kept on ice until extracted.

Preparation of extracts. The cold or powdered livers were homogenized at 0–2° in the appropriate medium in a manually operated glass Potter–Elvehjem homogenizer. The chilled adult livers were given a preliminary treatment with a Fischer mincer. Cell debris was removed by centrifugation at 600g for 10 min. at 0–2°, and the supernatant was centrifuged at 100 000g for 60 min. in a Spinco model L preparative ultracentrifuge. Most enzyme activities were assayed in the supernatant obtained from the 100 000g centrifugation, because non-specific oxidation of NADH₂ was very low and, because this extract was less opaque than the supernatant from the 600g centrifugation, larger volumes of extract could be used in the assay cuvettes without interfering in the measurement of the extinction; this was important when the total activity in the tissue

was very low. However, some enzyme activities were assayed in the 600g supernatant, namely glucose 6-phosphatase, hexokinase and glucokinase.

For the determination of phosphofructokinase, FDPase and pyruvate kinase activities, the livers were extracted in 3 vol. of a solution comprising 20 mM-tris, 5 mM-MgSO₄ and 0.1 mM-EDTA, pH 8.0. For the assay of hexokinase and glucokinase activities, the livers were extracted in 3 vol. of a medium comprising 100 mM-tris, 150 mM-KCl, 5 mM-EDTA, 10 mM-mercaptoethanol and 10 mM-MgCl₂, pH 7.6; the 600g and 100 000g supernatants were dialysed against several changes of 100 vol. of the same medium at 0–2° for 4 hr. to remove endogenous glucose. For the measurement of glucose 6-phosphatase activity the tissue was extracted in 100 mM-potassium citrate, pH 6.5.

Assay of enzymes. Phosphofructokinase (EC 2.7.1.11) was assayed by the procedure for crude extracts described by Underwood & Newsholme (1965a); the assay medium contained 1 mM-AMP to inhibit FDPase. Pyruvate kinase (EC 2.7.1.40) activity was assayed as described by Krebs & Eggleston (1965). FDPase assays were based on the method of Underwood & Newsholme (1965b), and varied as indicated in the Tables. Hexokinase (EC 2.7.1.1) and glucokinase (EC 2.7.1.2) activities were measured usually with 600g supernatant by the method of Viñuela, Salas & Sols (1963), except that the cysteine was replaced by mercaptoethanol. Glucose 6-phosphatase (EC 3.1.3.9) activity was assayed with the 600g supernatant as duplicated by Harper (1963), and the inorganic phosphate liberated was measured by the method of Allen (1940) in a Zeiss model PMQII spectrophotometer.

All enzyme-activity measurements, except that of glucose 6-phosphatase, were carried out in a Gilford recording spectrophotometer at 25°, and the results expressed as μ moles of product formed/g. wet wt. of liver/hr. All the assays were carried out at least in duplicate, and each separate extract was tested to see if the enzyme activity was linear with increasing concentrations of extract. Each replicate represents an extract from a single animal or a pooled sample from several animals (when one animal provided insufficient material).

RESULTS

Pattern of embryonic development of some key enzymes of glycolysis and gluconeogenesis

Presented in Table 1 are activities of some key enzymes of glycolysis and gluconeogenesis at various stages of development in the sheep and chicken. In embryonic sheep the low activity of hexokinase (5–10 μ moles/g. wet wt./hr.) throughout development, and the absence of any detectable glucokinase, indicate a limited capacity for glucose phosphorylation. This low activity is not due to the presence of inhibitors in the extracts, since, when a suitable amount (200 μ g.) of commercial yeast hexokinase was added to the cuvettes, the increase in the rate of NADP reduction was comparable with the rate in the absence of sheep liver extract. In view of the fairly high activity of phosphorylase in sheep liver during development (Ballard &

Table 1. *Activities of some enzymes of glycolysis and gluconeogenesis in the livers of sheep and domestic fowl during development*

Values given are the means $\pm$ s.e.m., and the numbers of replicates are given in parentheses. Each replicate represents one extract from a single animal or a pooled sample from several animals. The ages of the animals are in days before (—) or after (+) birth or hatching, as appropriate. The hexokinase activities for the sheep refer to those of the 100000g supernatants, and those of the chick to the whole homogenate. With the exception of the glucose 6-phosphatase, the other enzyme activities refer to those found in the 100000g supernatants. Maximum FDPase activity in sheep liver was measured in the presence of 100 μ M-fructose diphosphate and 5 mM-Mg²⁺, but in the chick maximum activity was measured in the presence of 25 μ M-fructose diphosphate and 1 mM-Mg²⁺. Other details are given in the text.

Species	Age (days)	Activities (μ moles of product formed/g. wet wt./hr.)				
		Hexokinase	Glucose 6-phosphatase	Phospho-fructokinase	FDPase	Pyruvate kinase
Sheep	—60	7.8 $\pm$ 0.6 (5)	7 $\pm$ 2 (3)	169 $\pm$ 22 (8)	733 $\pm$ 127 (5)	1842 $\pm$ 65 (3)
	—40	—	23 $\pm$ 8 (4)	185 $\pm$ 13 (5)	692 $\pm$ 83 (3)	1452 $\pm$ 145 (5)
	—10	5.5 $\pm$ 1.0 (4)	39 $\pm$ 6 (4)	118 $\pm$ 18 (4)	757 $\pm$ 70 (5)	968 $\pm$ 92 (5)
	Adult	5.9 $\pm$ 0.8 (3)	167 $\pm$ 14 (7)	31 $\pm$ 7 (4)	717 $\pm$ 56 (5)	828 $\pm$ 41 (4)
Fowl	—8	—	—	32 $\pm$ 8 (3)	2460 $\pm$ 82 (3)	—
	—5	—	281 $\pm$ 15 (3)	35 $\pm$ 8 (7)	2336 $\pm$ 258 (4)	222 $\pm$ 41 (3)
	—1	5.2 $\pm$ 1.1 (3)	296 $\pm$ 31 (3)	48 $\pm$ 19 (3)	2177 $\pm$ 199 (3)	215 $\pm$ 24 (3)
	+2	2.8 $\pm$ 0.5 (4)	349 $\pm$ 6 (3)	68 $\pm$ 13 (4)	2540 $\pm$ 145 (4)	243 $\pm$ 33 (4)
	+4	12.7 $\pm$ 2.4 (3)	205 $\pm$ 16 (3)	136 $\pm$ 24 (4)	1898 $\pm$ 204 (4)	365 $\pm$ 52 (4)
	+8	16.4 $\pm$ 1.9 (4)	229 $\pm$ 9 (3)	300 $\pm$ 48 (4)	1446 $\pm$ 150 (4)	720 $\pm$ 110 (3)
	Adult	25.5 $\pm$ 3.5 (3)	108 $\pm$ 9 (3)	81 $\pm$ 21 (3)	1149 $\pm$ 121 (3)	390 $\pm$ 32 (3)

Oliver, 1965), the substantial activities of phospho-fructokinase (125–175 μ moles/g./hr.), and pyruvate kinase (1000–1800 μ moles/g./hr.), may indicate a high capacity for glycogenolysis and subsequent glycolysis. The high FDPase activity (700–750 μ moles/g./hr.) in the absence of significant amounts of glucose 6-phosphatase suggests a considerable capacity for gluconeogenesis, rather than glucose release.

When the activities of these enzymes (except glucose 6-phosphatase) in the 600g and 100000g supernatants were compared, it was found that all the FDPase and pyruvate kinase, and most of the hexokinase, activity of the tissue was in the 100000g supernatant. Some 40% of the phospho-fructokinase activity sedimented during the 100000g centrifugation.

In the embryonic chick the very high glucose 6-phosphatase and FDPase activities throughout development indicate a considerable capacity for glucose formation by the liver from an early age. There was a very low activity of hexokinase and no detectable glucokinase, but hexokinase activity increases 4 days after hatching; most of this increase in activity was sedimented at 100000g. The increase in hexokinase activity thus appears to be mainly particulate hexokinase. Both phospho-fructokinase and pyruvate kinase activities rise during development; phospho-fructokinase activity increases from 35 μ moles/g./hr. at 5 days before hatching to 300 μ moles/g./hr. at 8 days after hatching, and pyruvate kinase increases from 200 to 700 μ moles/

Table 2. *Activities of phospho-fructokinase and fructose 1,6-diphosphatase in rat liver during embryonic development*

Values are given as means $\pm$ s.e.m., and the numbers of replicates (see Table 1) are given in parentheses. Maximum FDPase activity was measured in the presence of 100 μ M-fructose diphosphate and 5 mM-Mg²⁺. Other details are given in the text.

Age (days)	Wt. of liver (mg.)	Activity (μ moles of product formed/g. wet wt./hr.)	
		FDPase	Phospho-fructokinase
16–17	117 $\pm$ 17 (3)	34 $\pm$ 15 (3)	—
18–19	197 $\pm$ 7 (3)	22 $\pm$ 2 (3)	—
19–20	268 $\pm$ 21 (3)	51 $\pm$ 12 (3)	32 $\pm$ 2 (3)
20–22	318 $\pm$ 27 (4)	97 $\pm$ 16 (4)	29 $\pm$ 1 (3)
Adult			
pregnant		516 $\pm$ 52 (9)	29 $\pm$ 2 (3)
non-pregnant (females)		414 $\pm$ 19 (9)	—

g./hr. during the same period. Phospho-fructokinase and pyruvate kinase develop during this period so that the capacity to utilize carbohydrate is available after hatching, when the animal commences to feed. Glucokinase activity in the adult fowl was only 25% that of hexokinase, which contrasts with rat, in which glucokinase activity exceeds hexokinase by approximately threefold (Viñuela *et al.* 1963).

Table 3. *Effect of Mg^{2+} concentration on the activity of fructose 1,6-diphosphatase from the livers of embryonic and adult rats, sheep and fowls*

Values given are the means $\pm$ S.E.M. of at least three replicates (see Table 1). Activities were measured in the presence of 100 μM -fructose diphosphate; other details are given in the text. The embryonic age was 5 days before birth for rat, 10–60 days for sheep, and 5 days before hatching for the chick.

Concn. of Mg^{2+} (mm)	Activity (μ moles of product formed/g. wet wt./hr.)						Ratio of activities (adult/embryonic)		
	Rat		Sheep		Fowl		Rat	Sheep	Fowl
	Embryo	Adult	Embryo	Adult	Embryo	Adult			
0	0	0	0	0	0	0	0	0	0
0.25	17 $\pm$ 5	217 $\pm$ 25	—	—	441 $\pm$ 113	277 $\pm$ 45	12.8	—	0.63
0.5	33 $\pm$ 7	428 $\pm$ 45	393 $\pm$ 43	548 $\pm$ 59	1128 $\pm$ 244	588 $\pm$ 163	13.0	1.40	0.52
1.0	40 $\pm$ 6	513 $\pm$ 58	556 $\pm$ 84	818 $\pm$ 62	1441 $\pm$ 330	841 $\pm$ 93	12.9	1.47	0.58
2.5	47 $\pm$ 9	599 $\pm$ 74	629 $\pm$ 112	840 $\pm$ 79	1222 $\pm$ 240	564 $\pm$ 102	12.7	1.34	0.46
5.0	44 $\pm$ 7	570 $\pm$ 70	562 $\pm$ 109	730 $\pm$ 57	948 $\pm$ 182	486 $\pm$ 63	13.0	1.30	0.51
10.0	45 $\pm$ 7	581 $\pm$ 88	500 $\pm$ 119	694 $\pm$ 53	815 $\pm$ 261	408 $\pm$ 34	12.9	1.39	0.50
25.0	33 $\pm$ 4	422 $\pm$ 49	422 $\pm$ 43	511 $\pm$ 45	768 $\pm$ 132	340 $\pm$ 28	12.8	1.21	0.44

The phosphofructokinase activity of foetal rat liver was not very different from that of the adult (Table 2) although this activity reflects only part of the total activity as some phosphofructokinase is sedimented at 100000g. FDPase activity in the foetal liver rises from 20–30 μ moles/g./hr. at about 16–18 days of the gestation period to 97 μ moles/g./hr. just before birth, but is still low when compared with the adult activity (pregnant 516 μ moles/g./hr.; non-pregnant 414 μ moles/g./hr.). The low activity in foetal tissue is unlikely to be due to the presence of inhibitors (e.g. AMP) in these extracts since, when they are mixed with high-activity adult rat liver extracts, the activities are additive.

Properties of liver fructose 1,6-diphosphatase in embryonic and adult livers

Effect of Mg^{2+} concentration. Gomori (1943) first showed that FDPase had an absolute requirement for a cation (Mg^{2+} or Mn^{2+}). In these studies, both embryonic and adult tissue extracts have exhibited a similar requirement for Mg^{2+} (Mn^{2+} was not tested), and no FDPase activity was detected in the absence of Mg^{2+} (Table 3). There were slight differences between species in the concentration of Mg^{2+} required for maximum activity; thus in the rat 2.5–10mm- Mg^{2+} was optimum, whereas in the sheep and the fowl 1.0–2.5mm- Mg^{2+} produced the highest activities. The responses of embryonic and adult enzymes from rat, sheep or fowl to this essential cation are very similar, as is shown by the ratio of activities, adult/embryonic (Table 3).

Effect of substrate concentration. FDPase from embryonic livers of all three species studied was inhibited by high concentrations of the substrate. The optimum substrate concentration varies

between species (Table 4); thus in the rat and sheep 50–100 μM -fructose diphosphate gave the highest activity, whereas in the fowl 10–25 μM was optimum. The degree of inhibition at high substrate concentrations (1–5mm) is also different for each species. Rat and sheep were inhibited 66% and 50% respectively by 5mm-fructose diphosphate, whereas the fowl FDPase was inhibited 80% by only 1mm-fructose diphosphate. Despite these differences between species there were only minor differences between the responses of the embryonic and adult FDPase to the substrate concentration, as is shown by ratio of activities, adult/embryonic, for each species (Table 4).

Inhibition by AMP. The FDPase enzymes present in liver, kidney and muscle of various species are all inhibited by low concentrations of AMP (Taketa & Pogell, 1965; Mendicino & Vasarhely, 1963; Underwood & Newsholme, 1965b; Krebs & Woodford, 1965). In the present investigation, the activity of hepatic FDPase in all three species, both embryonic and adult, was inhibited by low concentrations of AMP. Thus, in the presence of 5mm- Mg^{2+} , 50% inhibition of embryonic FDPase activity was produced by 30, 9 and 12 μM -AMP in the rat, sheep and chick respectively (Table 4). Underwood & Newsholme (1965b) showed that AMP inhibition of rat liver FDPase was relieved by Mg^{2+} . This effect was confirmed in the present investigation and extended to include the enzymes from adult sheep and fowl livers, and from the embryonic livers of the three species. These results are presented in Table 5 as percentage inhibitions by AMP, which simplifies the comparison between embryonic and adult FDPase as a large number of results are presented. For each of the three species there was a tendency for the embryonic FDPase to

Table 4. *Effect of substrate concentration on the activity of fructose 1,6-diphosphatase from the livers of embryonic and adult rats, sheep and fowls*

Values given are the means $\pm$ s.e.m. of at least three replicates (see Table 1). Activity was measured in the presence of 5 mM-Mg²⁺. Other details are given in the text. Age of embryos was as given in Table 3.

Concn. of fructose diphosphate (μ M)	Activity (μ moles of product formed/g. wet wt./hr.)						Ratio of activities (adult/embryonic)		
	Rat		Sheep		Fowl		Rat	Sheep	Fowl
	Embryo	Adult	Embryo	Adult	Embryo	Adult			
5	—	—	—	—	1828 $\pm$ 267	510 $\pm$ 85	—	—	0.28
10	50 $\pm$ 6	—	525 $\pm$ 37	629 $\pm$ 38	2251 $\pm$ 313	674 $\pm$ 75	—	1.20	0.30
25	61 $\pm$ 4	—	685 $\pm$ 72	813 $\pm$ 73	2418 $\pm$ 308	744 $\pm$ 102	—	1.19	0.31
50	65 $\pm$ 4	614 $\pm$ 57	744 $\pm$ 52	775 $\pm$ 62	2146 $\pm$ 335	633 $\pm$ 69	9.4	1.04	0.29
75	66 $\pm$ 5	693 $\pm$ 69	700 $\pm$ 105	790 $\pm$ 95	1724 $\pm$ 42	562 $\pm$ 91	10.5	1.13	0.33
100	64 $\pm$ 6	679 $\pm$ 51	729 $\pm$ 85	767 $\pm$ 69	1604 $\pm$ 265	463 $\pm$ 55	10.6	1.05	0.29
150	—	650 $\pm$ 65	700 $\pm$ 56	698 $\pm$ 63	1198 $\pm$ 120	371 $\pm$ 59	—	1.00	0.31
200	54 $\pm$ 6	492 $\pm$ 71	634 $\pm$ 67	667 $\pm$ 33	1065 $\pm$ 237	303 $\pm$ 58	9.1	1.05	0.28
500	37 $\pm$ 5	338 $\pm$ 51	518 $\pm$ 49	591 $\pm$ 35	729 $\pm$ 164	172 $\pm$ 25	9.2	1.14	0.24
1000	27 $\pm$ 2	294 $\pm$ 46	503 $\pm$ 42	545 $\pm$ 44	554 $\pm$ 96	139 $\pm$ 24	10.5	1.08	0.25
2000	24 $\pm$ 2	—	423 $\pm$ 25	453 $\pm$ 59	—	—	—	1.07	—
5000	22 $\pm$ 1	—	372 $\pm$ 43	414 $\pm$ 39	—	—	—	1.12	—

Table 5. *Percentage inhibition by AMP at three Mg²⁺ concentrations of the fructose 1,6-diphosphatase from the livers of embryonic and adult rats, sheep and fowls*

Values are the means of at least two replicates (see Table 1). Activity was measured at pH 7.5, in the presence of 100 μ M-fructose diphosphate, 20 mM-mercaptoethanol, 50 mM-tris-HCl, 0.2 mM-NADP and coupling enzymes as described in the text. Other details are given in the text or Table 3. The minus sign indicates that AMP activates the enzyme. The 100% activities are those given in Table 3.

Species	Age	Concn. of Mg ²⁺ (mM)	Concn. of AMP (μM)	Percentage inhibition							
		0.5	1.0	2.5	5.0	10	25	50	100	200	
Rat	Embryonic	0.5	—	—	—	20	70	87	96	—	—
		5.0	—	—	—	—	9	42	87	89	—
		25	—	—	—	—	5	8	22	40	—
	Adult	0.5	—	—	—	-11	7	75	95	—	—
		5.0	—	—	—	—	9	34	71	92	—
		25	—	—	—	—	-4	2	5	28	45
Sheep	Embryonic	0.5	10	15	28	76	95	—	—	—	—
		5.0	—	-2	13	12	37	82	95	95	—
		25	—	—	—	—	10	22	60	86	92
	Adult	0.5	-3	-3	14	52	87	96	—	—	—
		5.0	—	5	2	10	23	76	88	94	—
		25	—	—	—	—	—	5	44	73	86
Fowl	Embryonic	0.5	35	54	—	96	97	—	—	—	—
		5.0	—	-5	—	15	39	95	96	—	—
		25	—	—	—	-4	-4	3	32	59	71
	Adult	0.5	—	13	32	79	95	—	—	—	—
		5.0	—	-2	—	0	22	60	82	—	—
		25	—	—	—	—	-8	-3	8	40	61

be more sensitive to AMP inhibition at 0.5 and 25 mM-Mg²⁺. The reason for this is not known, but it was not observed at 5 mM-Mg²⁺. Nevertheless the responses of the adult and embryonic FDPase to AMP inhibition were very similar. In some cases

AMP, at very low concentrations, activated FDPase (Table 5).

Effect of pH. In all three species investigated, in both adult and embryonic livers, the optimum for FDPase activity was obtained at pH 7.5

Table 6. *Effect of pH on the activity of the fructose 1,6-diphosphatase from the livers of embryonic and adult rats, sheep and fowls*

Values are the means $\pm$ S.E.M. of at least three replicates (see Table 1). Activity was measured in the presence of 50 mM-tris, 5 mM-Mg²⁺, 100 μ M-fructose diphosphate and pH as specified. Other details are given in the text or in Table 3.

pH	Activities (μ moles of product formed/g. wet wt./hr.)						Ratio of activities (adult/embryonic)		
	Rat		Sheep		Fowl		Rat	Sheep	Fowl
	Embryo	Adult	Embryo	Adult	Embryo	Adult			
7.0	31 $\pm$ 8	121 $\pm$ 22	649 $\pm$ 130	577 $\pm$ 87	453 $\pm$ 90	275 $\pm$ 138	3.9	0.89	0.61
7.5	70 $\pm$ 15	354 $\pm$ 37	724 $\pm$ 108	666 $\pm$ 73	964 $\pm$ 124	529 $\pm$ 50	5.1	0.92	0.55
8.0	50 $\pm$ 8	260 $\pm$ 35	598 $\pm$ 72	605 $\pm$ 54	877 $\pm$ 120	518 $\pm$ 70	5.2	1.01	0.59
8.5	34 $\pm$ 8	212 $\pm$ 28	454 $\pm$ 50	430 $\pm$ 56	733 $\pm$ 124	418 $\pm$ 74	6.2	0.95	0.57
9.0	27 $\pm$ 6	148 $\pm$ 31	361 $\pm$ 47	383 $\pm$ 54	646 $\pm$ 126	307 $\pm$ 38	5.5	1.06	0.48
9.5	23 $\pm$ 4	96 $\pm$ 17	318 $\pm$ 38	342 $\pm$ 41	578 $\pm$ 124	291 $\pm$ 40	4.2	1.07	0.50

(Table 6). These findings are not in agreement with the earlier work of Gomori (1943), who found a pH optimum of about 9.0, or with the work of Byrne (1964), who showed that FDPase from rat liver had two pH optima (pH 9.0 and 7.0). McGilvery (1964) showed that the pH optimum depends very markedly upon the Mg²⁺ concentration and the buffer. Thus it seems probable that the conditions of the assay are important in determining the pH optimum for this enzyme. Nevertheless the pH profiles for adult and embryonic FDPase were very similar, as shown by the ratios of activities, adult/embryonic (Table 6). The inhibition of foetal rat liver FDPase by AMP and excess of substrate is relieved by raising the pH, in a manner similar to that described for adult liver FDPase by Underwood & Newsholme (1965b).

DISCUSSION

Burch *et al.* (1963) investigated some key enzymes of glycolysis and gluconeogenesis in developing rat liver; they found relatively high activities of hexokinase, phosphofructokinase and pyruvate kinase and low activities of glucose 6-phosphatase and FDPase, suggesting that glucose degradation rather than synthesis is a major metabolic pathway for embryonic rat liver. The opposite situation exists in embryonic chick liver: the activities of hexokinase, phosphofructokinase and pyruvate kinase are relatively low, but the activities of glucose 6-phosphatase and FDPase are very high. In embryonic chick liver the formation of glucose from non-carbohydrate sources is of prime importance. After hatching, the degradative pathway develops, and at the same time there is a decrease in the enzymic capacity for glucose formation. The embryonic liver of the sheep is interesting in that, from the activity of some of these enzymes, it seems to have properties somewhere between

those of chick and rat liver: thus the activities of both phosphofructokinase and FDPase are relatively high but the activities of both hexokinase and glucose 6-phosphatase are very low. This would suggest that the activities of FDPase and phosphofructokinase are related more to glycogen than to glucose metabolism. This is supported by the high activities of both phosphorylase and glucosyl-transferase in embryonic liver of the sheep (Ballard & Oliver, 1965).

The inhibition of adult liver and kidney FDPase by AMP or excess of substrate have been discussed in relation to control of glucose formation (Taketa & Pogell, 1965; Underwood & Newsholme, 1965b); more direct evidence has been obtained for the role that AMP plays in the regulation of glucose metabolism in these tissues (Krebs, Newsholme, Speake, Gascoyne & Lund, 1964; Newsholme & Underwood, 1966; Gevers & Krebs, 1966). Taketa & Pogell (1965) proposed that AMP is an allosteric inhibitor of adult rat liver FDPase: the inhibition curve was sigmoid, the enzyme bound more than one molecule of AMP and the binding sites for AMP were spatially distinct from the substrate-binding site.

The present investigation showed that the properties of FDPase from embryonic livers were very similar to the adult liver enzymes, and that the differences between the three species studied were exceptionally small. In particular, adult and embryonic FDPase showed generally similar sensitivities to AMP and substrate inhibitions; the effect of Mg²⁺ on AMP inhibition was the same, and the effects of pH on FDPase activity and AMP inhibition of these enzymes were similar. The embryonic FDPase was obtained at an early stage of development of the liver, and, as discussed above, the function of FDPase in the embryonic tissues would appear to be quite distinct in the three species studied.

The enzyme hexokinase from muscle is inhibited

by glucose 6-phosphate (Crane & Sols, 1953), and this has been shown to be of importance in glycolytic control in the perfused rat heart (Newsholme & Randle, 1961, 1964; Regen, Davies, Morgan & Park, 1964). The work of Gaja, Loreti, Ragnotti, Guidotti & Foa (1966) on the inhibition of the hexokinase in early embryonic chick hearts by glucose 6-phosphate has not been carried out in detail, so that it is not possible to compare the sensitivity of embryonic with adult hexokinase. However, this work does suggest that this enzyme is controlled similarly to that in adult tissues. Therefore embryonic FDPase and hexokinase possess regulatory properties which are very similar to the enzyme in adult tissues. If it is permissible to generalize from these two enzymes, it would seem that regulatory properties are characteristic of both embryonic and adult tissue enzymes, and that the part of the enzyme protein which is responsible for these regulatory properties is able to function at all stages of development. If the enzyme comprises two peptides, one of which possesses the catalytic properties and the other possesses the regulatory properties, as has been shown for bacterial aspartate transcarbamoylase (Gerhart & Schachman, 1965), the synthesis of the component peptides must occur synchronously throughout embryonic development. This would be in complete contrast with some isoenzymes, e.g. of lactate dehydrogenase (Markert & Ursprung, 1962), whose component peptides appear to be synthesized at different rates during embryonic development.

It remains possible that at the very early stages of embryonic development some enzymes may not possess the regulatory properties which are characteristic of the adult enzymes; but, because of the small quantity of tissue involved at this stage, the present spectrophotometric or fluorescent assays may not be sensitive enough to investigate regulatory properties.

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