The Adaptive Behaviour of Isoenzyme Forms of Rat Liver Alanine Aminotransferases during Development

By KEITH SNELL and DERYCK G. WALKER

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

(Received 2 February 1972)

1. The activities of the mitochondrial and cytosol isoenzyme forms of L-alanine-glyoxylate and L-alanine-2-oxoglutarate aminotransferases were determined in rat liver during foetal and neonatal development. 2. The mitochondrial glyoxylate aminotransferase activity begins to develop in late-foetal liver, increases rapidly at birth to a peak during suckling and then decreases at weaning to the adult value. 3. The cytosol glyoxylate aminotransferase and the mitochondrial and cytosol 2-oxoglutarate aminotransferase activities first appear prenatally, increase further after birth and then rise to the adult values during weaning. 4. In foetal liver the mitochondrial glyoxylate aminotransferase and the cytosol 2-oxoglutarate aminotransferase activities are increased after injection *in utero* of glucagon, dibutyryl cyclic AMP (6-*N*,2'-*O*-dibutyryl-adenosine 3':5'-cyclic monophosphate) or thyroxine. The cytosol glyoxylate aminotransferase and the mitochondrial 2-oxoglutarate aminotransferase activities are increased after injection *in utero* of cortisol or thyroxine. 5. After birth the further normal increases in the mitochondrial and cytosol 2-oxoglutarate aminotransferase activities can be hastened by cortisol injection, whereas the increase in cytosol glyoxylate aminotransferase activity requires cortisol treatment together with the intragastric administration of casein. 6. The results are discussed with reference to the metabolic patterns and the changes in regulatory stimuli (hormonal and dietary) that occur during the period of development.

The mammalian liver undergoes a series of metabolic adaptations during development from the foetus to the adult in response to changes in nutritional status and the hormonal environment. Many of these adaptations are reflected by changes in enzyme activities (Walker, 1968, 1971; Rih, 1971; Greengard, 1971) and it has been possible to identify the dietary and hormonal factors responsible for the adaptive behaviour of some of these enzymes during development (see, e.g., Walker, 1971; Walker & Eaton, 1967; Vernon & Walker, 1968*b*; Greengard, 1970, 1971; Lockwood *et al.*, 1970).

Both L-alanine-glyoxylate aminotransferase (no EC number) (Rowell *et al.*, 1972) and L-alanine-2-oxoglutarate aminotransferase (EC 2.6.1.2) (Swick *et al.*, 1965) occur in two isoenzyme forms, located in the cytosol and mitochondria. Previous work on the development of these enzyme activities did not attempt to differentiate between the different isoenzyme forms (Rowell *et al.*, 1969*b*; Vernon *et al.*, 1968; Yeung & Oliver, 1967). The present paper describes the changes in these isoenzyme activities in developing rat liver and the regulation of the changes effected by dietary and hormonal modifications during development. Preliminary reports have been given (Snell & Walker, 1971*a,b*).

Experimental

Materials

Adenosine 3':5'-cyclic monophosphate (cyclic AMP) and its 6-*N*,2'-*O*-dibutyryl derivative (dibutyryl cyclic AMP), NADH and rabbit muscle lactate dehydrogenase (EC 1.1.1.27) were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.; L-alanine, glyoxylic acid, L-thyroxine (sodium salt) and casein (Hammarsten quality) were from British Drug Houses Ltd., Poole, Dorset, U.K.; glucagon was from Eli Lilly and Co. Ltd., Basingstoke, Hants., U.K.; cortisol acetate was from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; 2-oxoglutaric acid was from Mann Research Laboratories Inc., New York, N. Y., U.S.A.

Animals

The rats were a Wistar albino strain fed *ad libitum* on a pelleted diet (Thompson Rat Cubes; Heygate and Sons, Bugbrook Mills, Northants., U.K.), which contains (manufacturers' values) 3.5% of oil, 21.5% of protein and 5% of fibre. Weanling rats were removed from their mothers when 30-32 days old, but no attempt was made to restrict their access to the mother's food before this time. The results for the

development curves (Figs. 1–4) were from neonatal animals removed at random from litters. The date of conception of pregnant rats was determined by the appearance of vaginal plugs (after twice-daily inspections) and foetal ages were assessed from the date of conception, the normal gestation period being 22 days. All adult animals were males weighing 200–250 g.

Surgical procedure

The prenatal hormone injections were performed *in utero*. The pregnant mother was lightly anaesthetized with an ether–air mixture and a small incision was made in the abdominal wall. The uterus was partially removed and fetuses were injected intraperitoneally from an Agla syringe fitted with a gauge-30 needle directly through the transparent uterine wall with various agents dissolved or suspended in 50 μ l or less of 0.9% NaCl. Fetuses in one uterine horn were injected with the test substance and those in the other horn received 0.9% NaCl and served as controls. The uterus was replaced inside the animal, the peritoneal wall was sutured, and the skin was closed with wound clips. Anaesthesia was discontinued and the animal usually recovered in a few minutes; the whole operation was completed within 10 min. During the interval between the injections and the caesarian delivery of the fetuses (after 5 h), the mother was kept in an ambient temperature of about 26°C.

Hormone doses

Hormonal agents were in general administered intraperitoneally to unanaesthetized animals (except for foetal animals; see above). Hormonal agents were dissolved or suspended in 0.9% NaCl, except for glucagon, which was dissolved in the vehicle supplied by the manufacturer. The doses of glucagon, cyclic AMP, dibutyryl cyclic AMP, cortisol acetate and thyroxine were 0.05, 0.5, 0.5, 0.125 and 0.003 mg/foetus respectively. The doses for postnatal rats are given in the appropriate legends to the Tables.

Dietary experiments

The procedure adopted for weaning neonatal pups earlier than normal at 15 days on to normal manufactured rat diets has been described (Vernon & Walker, 1968b).

For the experiments involving the administration of casein intragastrically to 12-day-old rats, the animals were removed from their mothers at intervals during the day (09.30, 12.30, 15.30 and 18.00 h) for intubation (Walker & Eaton, 1967) and were returned to the mother between these times and overnight. A slurry of 15% (w/v) casein (Hammarsten

quality) in water was administered at a dose of 0.5 ml/rat per intubation. The rats were observed to continue suckling when returned to the mother and showed weight increases comparable with those of untreated control littermates.

Assay procedures

Homogenates (10%, w/v) of liver tissue were prepared in 0.4 M-sucrose with a Potter-type homogenizer equipped with a Teflon pestle, and were centrifuged at 100000g in an MSE Superspeed 40 centrifuge for 1 h to prepare the cytosol and particulate fractions. Particulate fractions were resuspended in 0.1 M-potassium phosphate buffer, pH 7.4, and were assayed directly (see below) for the mitochondrial aminotransferase isoenzyme activities (Rowell *et al.*, 1972; Swick *et al.*, 1965). Preliminary experiments had shown that no increase in these activities occurred after sonication or mechanical disruption with an Ultra-Turrax macerator (Janke und Kunkel K. G., Staufen i. Br., Germany) of the particulate fractions under conditions that were found to expose latent glutamate dehydrogenase (EC 1.4.1.2) activity completely.

The assay procedures for L-alanine–glyoxylate and L-alanine–2-oxoglutarate aminotransferase activities were based on those outlined by Rowell *et al.* (1969a,b). Incubations contained 24 μ mol of L-alanine, 24 μ mol of glyoxylic acid or 2-oxoglutaric acid (previously neutralized with 2 M-NaOH), 60 μ mol of 0.1 M-potassium phosphate buffer, pH 7.4, and enzyme preparation in a total volume of 1.2 ml and were made at 37°C under N₂. Portions (0.2 ml) were removed at zero time and after appropriate time-intervals (up to 15 min) and were deproteinized with equal volumes of 10% (w/v) trichloroacetic acid.

Pyruvate was measured in 0.1 ml portions of the protein-free supernatants by the addition of 0.1 ml of 100 mM-tris–HCl buffer, pH 8.4, followed by 8 ml of a batch reaction mixture that contained 9.6 mmol of tris–HCl buffer, pH 8.4, 4.5 μ mol of NADH and 180 units of lactate dehydrogenase in a volume of 24 ml. Extinctions were measured at 340 nm after 5 min. Enzyme activities were linear with respect to incubation time and tissue concentration over the ranges employed and suitable control incubations showed negligible non-enzymic pyruvate formation. All enzyme activities are expressed as μ mol of pyruvate formed/min per g wet wt. of liver.

Results

Aminotransferase developmental patterns

L-Alanine–glyoxylate aminotransferase activity, measured in unfractionated liver homogenates, begins to develop in the late foetal period, rises to a

peak during the neonatal period and then, at about the time of weaning, begins to fall to the adult value (Fig. 1). This pattern of development was observed previously in a different strain of rats (Rowell *et al.*, 1969b). The activity is the sum of that of two isoenzyme forms that differ with respect to subcellular location, pH optimum and affinity for glyoxylate (Rowell *et al.*, 1972). The development of these isoenzyme forms, which are located in the mitochondria and the cytosol, is shown in Fig. 2. The predominant, mitochondrial isoenzyme begins to develop in foetal liver and shows a peak of activity in the neonatal period before it decreases to the adult value (Fig. 2a). The cytosol isoenzyme, however, shows a biphasic change of activity with age (Fig. 2b). Activity begins to develop in the late foetal period and reaches a plateau (about 40% of the adult activity) soon after birth. A further rise to the adult value begins at about 15 days *post partum*. These results provide further evidence that the L-alanine-glyoxylate aminotransferase activities assayed in the cytosol and mitochondria are due to different molecular species of the enzyme.

The pattern of development of L-alanine-2-oxoglutarate aminotransferase in unfractionated rat liver homogenates (Fig. 3) was similar to that described previously (Rowell *et al.*, 1969b; Yeung & Oliver, 1967). This activity is also the sum of that of two isoenzyme forms that are located in the cytosol and mitochondria (Swick *et al.*, 1965; Katunuma *et al.*, 1962). The patterns of develop-

ment of these two isoenzyme activities (Fig. 4) are similar and reflect the pattern shown by the whole homogenate activity. However, the absolute activity of the mitochondrial isoenzyme greatly exceeds that of the cytosol isoenzyme in foetal liver, whereas in adult liver the opposite occurs. The pattern of development observed for the cytosol isoenzyme agrees with that found by other workers (Yeung & Oliver, 1967; Vernon *et al.*, 1968; Augenfeld & Fritz, 1970).

Fig. 1. Activity of L-alanine-glyoxylate aminotransferase in whole liver homogenates as a function of age

Each point and vertical bar represents the mean $\pm$ S.E.M. of four to eight determinations. Each determination was on the liver from a single animal (postnatal) or on the pooled liver from two animals (foetal). Activity was determined as described in the text and is expressed as $\mu\text{mol/min per g}$ of liver. B, birth; A, adult.

Fig. 2. Activity of L-alanine-glyoxylate aminotransferase in (a) the particulate fraction and (b) the cytosol fraction of liver homogenates as a function of age

Points are the means $\pm$ S.E.M. of six to ten determinations (○) or a single determination on the pooled liver from two littermates (●). Each determination before birth was on the pooled liver from two animals. Activity was determined as described in the text and is expressed as $\mu\text{mol/min per g}$ of liver. B, birth; A, adult.

Regulation of aminotransferase isoenzymes in adult rat liver by glucagon

It has been shown that L-alanine-glyoxylate aminotransferase activity in whole homogenates of adult rat liver is stimulated by injection of glucagon or cyclic AMP *in vivo* (Rowell *et al.*, 1969b; Snell, 1971a), whereas no significant changes were observed in L-alanine-2-oxoglutarate aminotransferase activity up to 24 h after injection (Rowell *et al.*, 1969b). The elevation of the glyoxylate aminotransferase activity after injection of glucagon or cyclic AMP is confined

Fig. 3. Activity of L-alanine-2-oxoglutarate aminotransferase in whole liver homogenates as a function of age

Points are the means $\pm$ S.E.M. of six to ten determinations ($\circ$) or a single determination on the pooled liver from two littermates ($\bullet$). Each determination before birth was on the pooled liver from two animals. Activity was determined as described in the text and is expressed as $\mu\text{mol/min per g of liver}$. B, birth; A, adult.

to the mitochondrial isoenzyme, and no significant change is observed for the cytosol isoenzyme activity (Table 1). The effect of glucagon and cyclic AMP together on whole-homogenate aminotransferase activity is no greater than that due to either agent alone and this suggests that the glucagon effect may be mediated via cyclic AMP, the liver concentration of which is increased after glucagon treatment (Butcher *et al.*, 1968).

Fig. 4. Activity of L-alanine-2-oxoglutarate aminotransferase in the cytosol ($\blacktriangle$) and particulate ($\bullet$) fractions of liver homogenates as a function of age

Each point is the mean $\pm$ S.E.M. of six to ten determinations. Each determination was on the liver from a single animal (postnatal) or on the pooled liver from two animals (foetal). Activities were determined as described in the text and are expressed as $\mu\text{mol/min per g of liver}$. B, birth.

Table 1. Effects of glucagon and cyclic AMP on the mitochondrial and cytosol forms of L-alanine-glyoxylate aminotransferase in adult rat liver

Animals were injected intraperitoneally with glucagon (0.5 mg/100 g body wt.), cyclic AMP (5.0 mg/100 g body wt.), or dibutyryl cyclic AMP (5.0 mg/100 g body wt.). Control animals were injected intraperitoneally with an appropriate volume of 0.9% NaCl. The animals were killed 24 h after injection and hepatic L-alanine-glyoxylate aminotransferase activity was assayed in whole homogenate and in the supernatant and in the resuspended sediment, prepared as described in the text. Activities are the means $\pm$ S.E.M. with the numbers of observations in parentheses. —, Not determined. Significance of results: *, $P < 0.001$.

Treatment	L-Alanine-glyoxylate aminotransferase activity (μmol of pyruvate formed/min per g of liver)		
	Whole homogenate	Mitochondrial	Cytosol
Controls (0.9% NaCl)	3.48 ± 0.02 (12)	2.67 ± 0.13 (4)	0.98 ± 0.03 (4)
Glucagon	5.40 ± 0.20 (4)*	4.60 ± 0.17 (4)*	0.83 ± 0.05 (4)
Cyclic AMP	5.15 ± 0.13 (4)*	4.13 ± 0.15 (3)*	1.03 ± 0.04 (3)
Dibutyryl cyclic AMP	6.75 ± 0.38 (3)*	—	—
Glucagon + cyclic AMP	5.33 ± 0.17 (3)*	—	—

Table 2. *Stimulation of aminotransferase activities in foetal rat liver 2 days before term*

Foetuses were injected intraperitoneally *in utero* with the indicated hormonal agents as described in the text. Littermates injected with 0.9% NaCl served as controls. Animals were killed 5 h after injection and the pooled liver from two or three foetuses was used for assay as described in the text. Enzyme activities are given as the means $\pm$ S.E.M. with the numbers of observations in parentheses. Significance of results: ***, $P < 0.05$; **, $P < 0.01$; *, $P < 0.001$.

Treatment	Activity (μ mol of pyruvate formed/min per g of liver)			
	L-Alanine-glyoxylate aminotransferase		L-Alanine-2-oxoglutarate aminotransferase	
	Mitochondrial	Cytosol	Mitochondrial	Cytosol
Controls (0.9% NaCl)	1.10 $\pm$ 0.07 (12)	0.22 $\pm$ 0.02 (12)	1.12 $\pm$ 0.08 (6)	0.28 $\pm$ 0.06 (6)
Glucagon	1.67 $\pm$ 0.11 (6)*	0.20 $\pm$ 0.04 (6)	0.97 $\pm$ 0.12 (6)	0.95 $\pm$ 0.11 (6)*
Cortisol	1.08 $\pm$ 0.09 (4)	0.42 $\pm$ 0.03 (4)*	1.36 $\pm$ 0.07 (4)***	0.31 $\pm$ 0.09 (4)
Thyroxine	1.47 $\pm$ 0.10 (6)*	0.33 $\pm$ 0.03 (6)**	1.58 $\pm$ 0.09 (4)**	1.08 $\pm$ 0.16 (4)*

Hormonal regulation of aminotransferase isoenzymes in foetal rat liver

A number of enzymes have been shown to respond to hormones injected *in utero* into foetal rats, either by an increase in the rate of their natural development or by the appearance of activity that was previously absent (Greengard, 1970, 1971). Some hormonal agents were tested for an effect on the isoenzyme activities of liver L-alanine-2-oxoglutarate aminotransferase and L-alanine-glyoxylate aminotransferase. Table 2 shows that 5 h after an injection of glucagon into foetal rats 2 days before birth, mitochondrial L-alanine-glyoxylate aminotransferase activity was about 50% greater than in 0.9% NaCl-injected controls and cytosol L-alanine-2-oxoglutarate aminotransferase activity was threefold greater than control values. In contrast the cytosol glyoxylate aminotransferase and the mitochondrial 2-oxoglutarate aminotransferase activities did not respond significantly to glucagon treatment. The activity of the latter isoenzymes did respond, however, to injection of cortisol acetate; cytosol L-alanine-glyoxylate aminotransferase activity was doubled and there was an increase of about 20% in the mitochondrial L-alanine-2-oxoglutarate aminotransferase activity compared with 0.9% NaCl-injected controls. No effect of cortisol was observed on the mitochondrial glyoxylate aminotransferase or the cytosol 2-oxoglutarate aminotransferase. Thyroxine injection had a significant stimulatory effect on all the measured aminotransferase isoenzyme activities (Table 2).

Table 3 compares the effect of various hormonal agents on mitochondrial L-alanine-glyoxylate aminotransferase activity in rats at different stages of development. Activity was increased significantly by glucagon and dibutyryl cyclic AMP at all ages before birth, and at 3 ($P < 0.01$) and 4 ($P < 0.001$) days

before birth the effect of dibutyryl cyclic AMP was significantly greater than that of glucagon. At 2 days after birth, when the normal mitochondrial glyoxylate aminotransferase activity exceeds the adult value, glucagon was without effect. Cortisol acetate was without effect during foetal development, but thyroxine significantly increased the isoenzyme activity before birth at all the ages tested.

Glucagon or dibutyryl cyclic AMP were without effect on the cytosol L-alanine-glyoxylate aminotransferase activity during foetal development (Table 4). Both cortisol and thyroxine significantly increased cytosol isoenzyme activity at all ages before birth, but the stimulation by cortisol at 4 days before birth was significantly greater than that by thyroxine ($P < 0.02$). The stimulation by cortisol was still present at 5 days after birth.

Regulation of aminotransferase isoenzyme activities in postnatal rat liver

The further changes in the isoenzyme activities of L-alanine-glyoxylate aminotransferase and L-alanine-2-oxoglutarate aminotransferase that occur during postnatal development appear to be initiated around the 15th postnatal day (see Figs. 2a, 2b and 4). Although rats in our colony are normally completely weaned on to a solid diet at about 30 days, it has been observed that animals as young as 13 days will nibble solid food intended for the mother and the gradual process of weaning can be said to begin at about that age. The natural development of certain enzymes can be accelerated if rats are weaned prematurely on to a completely solid diet (Walker & Eaton, 1967; Vernon & Walker, 1968b; Lockwood *et al.*, 1970).

The fall in mitochondrial glyoxylate aminotransferase activity that occurs naturally at about 15 days

Table 3. *Effect of developmental age on the response of mitochondrial L-alanine-glyoxylate aminotransferase to hormonal agents*

The doses employed and the method of injections *in utero* are given in the text. Animals were killed 24 h (postnatal rats) or 5 h (foetuses) after a single intraperitoneal injection of the indicated agent. Littermates injected with 0.9% NaCl served as controls. Mitochondrial L-alanine-glyoxylate aminotransferase was assayed as described in the text. Activities are the means $\pm$ S.E.M. with the numbers of observations in parentheses. —, Not determined. Significance of results: **, $P < 0.01$; *, $P < 0.001$.

Time before (–) or after (+) birth (days) ... Treatment	L-Alanine-glyoxylate aminotransferase activity (μ mol of pyruvate formed/min per g of liver)				
	–4	–3	–2	–1	+2
Controls (0.9% NaCl)	0.30 $\pm$ 0.04 (12)	0.63 $\pm$ 0.05 (10)	1.10 $\pm$ 0.07 (12)	1.72 $\pm$ 0.10 (12)	3.77 $\pm$ 0.17 (3)
Glucagon	0.58 $\pm$ 0.04 (4)*	1.02 $\pm$ 0.08 (5)**	1.67 $\pm$ 0.11 (6)*	2.54 $\pm$ 0.12 (5)*	3.80 $\pm$ 0.14 (3)
Dibutyryl cyclic AMP	1.35 $\pm$ 0.10 (3)*	1.67 $\pm$ 0.03 (3)*	—	2.69 $\pm$ 0.11 (6)*	—
Cortisol	0.28 $\pm$ 0.08 (4)	—	1.08 $\pm$ 0.09 (4)	1.73 $\pm$ 0.14 (6)	—
Thyroxine	0.60 $\pm$ 0.07 (6)*	0.93 $\pm$ 0.05 (4)**	1.47 $\pm$ 0.10 (6)*	2.48 $\pm$ 0.13 (4)*	—

Table 4. *Effect of developmental age on the response of cytosol L-alanine-glyoxylate aminotransferase to hormonal agents*

Details of treatments were as in Table 3. Cytosol L-alanine-glyoxylate aminotransferase was assayed as described in the text. Activities are the means $\pm$ S.E.M. with the numbers of observations in parentheses. —, Not determined. Significance of results: ***, $P < 0.05$; **, $P < 0.01$; *, $P < 0.001$.

Time before (–) or after (+) birth (days) ... Treatment	L-Alanine-glyoxylate aminotransferase activity (μ mol of pyruvate formed/min per g of liver)				
	–4	–3	–2	–1	+5
Controls (0.9% NaCl)	0.12 $\pm$ 0.01 (12)	0.17 $\pm$ 0.01 (10)	0.22 $\pm$ 0.02 (12)	0.28 $\pm$ 0.04 (12)	0.43 $\pm$ 0.02 (9)
Glucagon	0.13 $\pm$ 0.02 (4)	0.18 $\pm$ 0.03 (5)	0.20 $\pm$ 0.04 (6)	0.27 $\pm$ 0.05 (5)	—
Dibutyryl cyclic AMP	0.12 $\pm$ 0.02 (3)	0.18 $\pm$ 0.04 (3)	—	0.26 $\pm$ 0.04 (6)	—
Cortisol	0.32 $\pm$ 0.04 (4)*	—	0.42 $\pm$ 0.03 (4)*	0.47 $\pm$ 0.06 (6)***	0.66 $\pm$ 0.02 (9)*
Thyroxine	0.18 $\pm$ 0.02 (6)***	0.23 $\pm$ 0.02 (4)***	0.33 $\pm$ 0.03 (6)**	0.50 $\pm$ 0.04 (6)**	—

(Fig. 2a) is greater in animals weaned prematurely at that age compared with normally developing rats (Table 5). The activities of cytosol L-alanine-glyoxylate aminotransferase and the cytosol and mitochondrial forms of L-alanine-2-oxoglutarate aminotransferase were all markedly increased in the prematurely weaned rats.

Cortisol and thyroxine are two hormonal agents that have been implicated in the increases in activity of certain enzymes that occur naturally at the time of weaning (Greengard, 1970; Lockwood *et al.*, 1970). Cortisol was found to stimulate cytosol L-alanine-glyoxylate aminotransferase at 5 days *post partum*, but was ineffective at 11 days (Table 6). Cytosol and mitochondrial L-alanine-2-oxoglutarate aminotransferases, however, could be stimulated by cortisol

at both 5 and 11 days *post partum*. Table 6 shows that thyroxine significantly decreased cytosol glyoxylate aminotransferase and both cytosol and mitochondrial 2-oxoglutarate aminotransferase activities at 11 days *post partum*. A decrease after thyroxine injection has also been observed in the adult rat for whole-homogenate L-alanine-2-oxoglutarate aminotransferase activity, but not for L-alanine-glyoxylate aminotransferase activity (Snell, 1971b).

The effect of increasing the protein intake of neonatal rats by intragastric administration of casein at 12 days *post partum* is shown in Table 7. Cytosol L-alanine-glyoxylate aminotransferase activity was not significantly affected by intubation of casein for 3 days, whereas there was some stimulation of cytosol and mitochondrial L-alanine-2-oxoglutarate amino-

transferase activities ($P<0.01$). If animals were injected with cortisol acetate 24h before being killed, i.e. on the third day of intubation with casein, however, there was a significant increase in cytosol L-alanine-glyoxylate aminotransferase activity. Further, the increases observed in cytosol and mitochondrial L-alanine-2-oxoglutarate aminotransferase activities after cortisol injection were significantly enhanced ($P<0.05$) if the animals were also intubated with casein.

Discussion

Developmental patterns and enzyme function

It is sometimes possible to correlate patterns of enzymic development with variations in metabolic processes (see reviews by Walker, 1968, 1971;

Räihä, 1971; Greengard, 1971). The developmental pattern observed for mitochondrial L-alanine-glyoxylate aminotransferase (Fig. 2a) is similar to that exhibited by a number of enzymes known to be involved in gluconeogenesis (Vernon & Walker, 1968a; Yeung & Oliver, 1967; Burch *et al.*, 1963), and this suggests that the mitochondrial glyoxylate aminotransferase may be involved in the gluconeogenic process, the capacity of which is enhanced similarly in the developing rat (Vernon & Walker, 1972a,b; Vernon *et al.*, 1968; Ballard & Oliver, 1963, 1965; Yeung & Oliver, 1967). This implication is supported by the observation that mitochondrial glyoxylate aminotransferase activity in adult liver is stimulated by glucagon (Table 1), because glucagon is known to increase the capacity for hepatic gluconeogenesis as well as increasing the activities of gluconeogenic enzymes (Exton *et al.*, 1970; Exton & Park,

Table 5. Effect of premature weaning on to a normal diet on aminotransferase isoenzyme activities in rat liver

Suckling neonatal rats were completely weaned on to a normal diet when 15 days old and were killed 4 days later, when 19 days old. Some littermates were left with their mother to develop normally and a few littermates were killed when 15 days old and they served as pre-weaned controls. Further details are described in the text, and the results are expressed as the means $\pm$ S.E.M. with the numbers of observations in parentheses. Significance of results (weaned compared with unweaned): **, $P<0.01$; *, $P<0.001$.

Dietary status	Activity (μ mol of pyruvate formed/min per g of liver)			
	L-Alanine-glyoxylate aminotransferase		L-Alanine-2-oxoglutarate aminotransferase	
	Cytosol	Mitochondrial	Cytosol	Mitochondrial
Pre-weaned (15 days)	0.45 $\pm$ 0.03 (4)	6.10 $\pm$ 0.38 (4)	1.52 $\pm$ 0.28 (4)	1.18 $\pm$ 0.16 (4)
Normal, unweaned	0.55 $\pm$ 0.04 (3)	4.53 $\pm$ 0.22 (3)	2.10 $\pm$ 0.36 (3)	1.38 $\pm$ 0.18 (3)
Weaned on to normal diet	0.96 $\pm$ 0.02 (4)*	2.87 $\pm$ 0.15 (4)**	7.15 $\pm$ 0.64 (4)*	2.72 $\pm$ 0.32 (4)**

Table 6. Effect of postnatal age on the response of rat liver aminotransferase isoenzymes to hormonal agents

Neonatal rats were killed at the ages indicated 24h after a single intraperitoneal injection of cortisol acetate (125 μ g for 5-day-old rats; 250 μ g for 11-day-old rats), thyroxine (10 μ g) or 0.9% NaCl (controls). Enzyme activities were determined as described in the text and results are expressed as the means $\pm$ S.E.M. with the numbers of observations in parentheses. —, Not determined. Significance of results: ***, $P<0.05$; **, $P<0.01$; *, $P<0.001$.

Postnatal age (days) ...	Activity (μ mol of pyruvate formed/min per g of liver)					
	L-Alanine-glyoxylate aminotransferase in cytosol		L-Alanine-2-oxoglutarate aminotransferase			
	5	11	Cytosol 5	Mitochondrial 5	Cytosol 11	Mitochondrial 11
Treatment						
Control (0.9% NaCl)	0.43 $\pm$ 0.02 (9)	0.38 $\pm$ 0.03 (6)	1.45 $\pm$ 0.22 (9)	1.92 $\pm$ 0.23 (9)	1.10 $\pm$ 0.09 (6)	0.93 $\pm$ 0.11 (6)
Cortisol	0.66 $\pm$ 0.02 (9)*	0.41 $\pm$ 0.04 (6)	2.42 $\pm$ 0.36 (9)***	3.65 $\pm$ 0.48 (9)**	8.30 $\pm$ 0.41 (6)*	4.43 $\pm$ 0.15 (6)*
Thyroxine	—	0.20 $\pm$ 0.02 (4)*	—	—	0.33 $\pm$ 0.14 (4)*	0.50 $\pm$ 0.02 (4)*

Table 7. *Effects of casein incubation and cortisol injection on aminotransferase isoenzyme activities in postnatal rat liver*

Neonatal rats were intubated intragastrically with 15% (w/v) casein in water from 12 days old as described in the text. After each intubation the rats were returned to the nursing mother. Intubation was continued for 3 days; some rats received a single intraperitoneal injection of cortisol acetate (50 µg) on the third day of intubation when 14 days old. Some littermates that had been fed normally received a single injection of cortisol acetate (50 µg) only when 14 days old and other littermates that had been fed normally served as controls. All animals were killed when 15 days old. Enzyme activities were assayed as described in the text and results are expressed as the means $\pm$ s.e.m. with the numbers of observations in parentheses. Significance of results: **, $P < 0.01$; *, $P < 0.001$.

Treatment	Activity (μ mol of pyruvate formed/min per g of liver)		
	L-Alanine-glyoxylate aminotransferase in cytosol	L-Alanine-2-oxoglutarate aminotransferase	
		Cytosol	Mitochondrial
Control (0.9% NaCl)	0.35 $\pm$ 0.02 (6)	1.31 $\pm$ 0.14 (6)	1.24 $\pm$ 0.18 (6)
Casein intubation	0.40 $\pm$ 0.03 (6)	1.93 $\pm$ 0.15 (6)**	2.23 $\pm$ 0.22 (6)**
Cortisol injection	0.38 $\pm$ 0.04 (6)	9.44 $\pm$ 0.63 (6)*	3.98 $\pm$ 0.16 (6)*
Casein intubation + cortisol injection	1.05 $\pm$ 0.04 (6)*	12.33 $\pm$ 0.93 (6)*	4.60 $\pm$ 0.19 (6)*

1968; Ashmore & Weber, 1968). That it is the mitochondrial isoenzyme of L-alanine-glyoxylate aminotransferase which increases in activity in gluconeogenic situations is consistent with the route proposed by Rowsell *et al.* (1972) for the conversion of glyoxylate into glucose. The enzymes involved in this pathway, via glycine, serine, hydroxypyruvate, glyceralate and 2-phosphoglycerate, are all localized in the mitochondria.

The developmental pattern observed for cytosol L-alanine-glyoxylate aminotransferase (Fig. 2b) differs markedly from that observed for the mitochondrial form, but is very similar to the patterns observed for the cytosol and mitochondrial isoenzymes of L-alanine-2-oxoglutarate aminotransferase (Fig. 4). Several other enzymes are known to develop in this biphasic fashion, with increases in activity from the foetal stage up to about 5 days *post partum* and then a further increase to adult values beginning between 10 and 15 days *post partum*, i.e. the urea-cycle enzymes (Illnerová, 1966; Rähä & Suihkonen, 1968; Miller & Chu, 1970), ornithine-2-oxoglutarate aminotransferase (Rähä & Kekomäki, 1968; Herzfeld & Greengard, 1969), asparaginase (McGee *et al.*, 1971), glutaminase (Linder-Horowitz, 1969) and glutamine synthetase (Knox *et al.*, 1971).

L-Alanine-2-oxoglutarate aminotransferase may serve as a link between pyruvate transamination and glutamate dehydrogenase to promote the general oxidative deamination of amino acids (Rowsell, 1956). The participation of cytosol L-alanine-glyoxylate aminotransferase (catalysing glycine

transamination with pyruvate) in this scheme seems unlikely, as the transamination reaction is virtually irreversible (Rowsell *et al.*, 1969b; Snell, 1971b; Thompson & Richardson, 1967).

The relatively low activity of L-alanine-2-oxoglutarate aminotransferase during suckling, when there is enhanced gluconeogenesis, introduces the question of the role of alanine as a gluconeogenic substrate at this time. A high proportion of the amino acids derived from milk proteins is used in protein synthesis, leaving very little available for gluconeogenic purposes (Hahn *et al.*, 1961). The low rate of urea excretion and low ureogenic capacity of liver slices (Illnerová, 1966; Rähä & Kretschmer, 1965) also suggest a low rate of amino acid catabolism during the neonatal period. A low rate of net carbohydrate synthesis in liver slices from neonatal rats (compared with adults) with alanine as substrate was reported by Vernon *et al.* (1968). There was, however, a discrepancy between those results and the rate of incorporation of [14 C]alanine into carbohydrate. The apparent increase in rate of incorporation of [14 C]alanine into carbohydrate at about 15 days may be a consequence of the elevated mitochondrial L-alanine-glyoxylate aminotransferase activity acting to promote formation of [14 C]pyruvate and hence [14 C]glucose, but without net glucose synthesis. Re-formation of alanine from the pyruvate produced in this transamination reaction would be necessary to obtain a continued net synthesis of glycine (and glucose) from glyoxylate, and in this situation pyruvate itself would not contribute to net glucose synthesis.

Hormonal regulation of enzyme development before birth

The cytosol and mitochondrial isoenzymes of both L-alanine-glyoxylate and L-alanine-2-oxoglutarate aminotransferases begin to develop in late-foetal liver, at a time when the pituitary, the adrenal cortex and the thyroid glands are all functional (Jost & Picon, 1970). An increase in whole-homogenate L-alanine-2-oxoglutarate aminotransferase in foetal rat liver after cortisol injection has been noted (Harding *et al.*, 1961).

The mitochondrial and cytosol forms of both aminotransferases are increased by different hormones (glucagon and cortisol), and thyroxine appears to stimulate all the isoenzymes (Table 2). Aspartate aminotransferase behaves in a similar way: the cytosol form, but not the mitochondrial form, is stimulated by cortisol before birth, whereas thyroxine stimulates both isoenzyme forms (Herzfeld & Greengard, 1971). Glucose 6-phosphatase, another enzyme that begins to develop naturally before birth, is also stimulated in foetal liver by both thyroxine and glucagon (Greengard, 1969a). The apparent non-specificity of thyroxine suggests that it may be acting simply to increase the general rate of protein synthesis at this time. Two enzymes that are absent before birth, tyrosine aminotransferase and serine dehydratase (Greengard, 1969b; Greengard & Dewey, 1967), are not stimulated prenatally by thyroxine but can be stimulated by glucagon. Herzfeld & Greengard (1971) found that thyroxine was effective in increasing cytosol aspartate aminotransferase at an earlier gestational day than was cortisol, and they suggested a sequential action on enzyme development in which thyroxine operates at an early developmental stage and serves perhaps as a prerequisite for the further responsiveness to other hormones. For cytosol L-alanine-glyoxylate aminotransferase (Table 4), however, cortisol was more effective than thyroxine in stimulating activity at the earlier gestational ages. Further results are clearly required before generalizations on the role of thyroxine in the sequential action of hormones at this time are justified.

Greengard (1969b) has also proposed a sequential development of competence in regulating the activities of enzymes that respond to glucagon and cyclic AMP before birth. At 3 and 4 days before birth dibutyryl cyclic AMP is more effective in increasing mitochondrial glyoxylate aminotransferase activity than is glucagon, but by the last day of gestation both agents are equally effective (Table 3). Yeung & Oliver (1971) have also reported an increased responsiveness of serine dehydratase to glucagon with increasing gestational age. As the effects of glucagon on enzyme stimulation are mediated via cyclic AMP (Jost *et al.*, 1969; Wicks *et al.*, 1969; Snell, 1971a), this pattern of responsiveness

suggests that the ability of glucagon to raise the concentration of cyclic AMP develops between 3 days and 1 day before birth. However, this cannot be the complete explanation, as even at 3 and 4 days before birth glucagon is able to elicit some increase in mitochondrial glyoxylate aminotransferase activity, albeit a smaller increase than that obtained with dibutyryl cyclic AMP (Table 3). The hormonal activation of adenylate cyclase (which catalyses cyclic AMP formation) by glucagon occurs to the same extent on each of the last 4 days of gestation and is similar to that found in the adult liver (Hommes & Beere, 1971; Bär & Hahn, 1971). The presence of an adenylate cyclase that is less hormone-sensitive at the earlier gestational ages is not therefore likely to explain the diminished enzyme response to glucagon; the latter may be related to the low basal activity of adenylate cyclase found at this time, which increases markedly between the fourth prenatal day and birth (Hommes & Beere, 1971).

Immediately after birth glucagon is no longer able to increase mitochondrial L-alanine-glyoxylate aminotransferase activity (Table 3). Activity at this time exceeds that of the adult rat and this natural stimulation is probably effected by endogenous glucagon released in response to postnatal hypoglycaemia. When activity has fallen to the adult value, exogenous glucagon is again able to elicit an increase (Table 1).

Enzyme regulation in postnatal rat liver

The capacity for stimulation by cortisol of the cytosol L-alanine-glyoxylate aminotransferase observed before birth continues up to 5 days after birth (Table 6). Activity at this time is below the adult value and the absence of any further increase is probably due to low concentrations of endogenous glucocorticoid between the fifth and tenth postnatal days (Haltmeyer *et al.*, 1966). This probably accounts also for the similar pattern observed for the L-alanine-2-oxoglutarate aminotransferase isoenzymes, as these are also stimulated at 5 days *post partum* by exogenous cortisol (Table 6). The response of the cytosol 2-oxoglutarate aminotransferase to cortisol after birth is noteworthy, as cortisol had no effect on the isoenzyme before birth, although glucagon was effective at that time (Table 2); a similar effect has been observed for tyrosine aminotransferase (Greengard, 1969b).

The further increase in activity of the cytosol glyoxylate aminotransferase and the cytosol and mitochondrial isoenzymes of L-alanine-2-oxoglutarate aminotransferase that occurs at about 10–15 days *post partum* is probably the consequence of physiological changes associated with weaning, because premature weaning accelerates the development of these isoenzymes (Table 5). These physiological changes include the re-establishment of

pituitary-adrenocortical function (Levine & Mullins, 1966; Halmeyer *et al.*, 1966), an increase in thyroid secretion rate and plasma thyroid-hormone concentration (Beltz & Reineke, 1968; Hommes *et al.*, 1969), and changes in specific dietary components associated with the change from rat milk to solid food. An increase in thyroid activity is unlikely to be a factor responsible for the increase in isoenzyme activities, as thyroxine administered to rats at 11 days *post partum* resulted in lower activities (Table 6). However, cortisol injection at 11 days increased the activities of both the cytosol and mitochondrial forms of L-alanine-2-oxoglutarate aminotransferase. The re-establishment of the pituitary-adrenal axis at about the 12th postnatal day means that factors such as stress can lead to corticosterone release. The process of weaning at this time may itself be sufficient stress to trigger this release and account for the observed increase in activity of the L-alanine-2-oxoglutarate aminotransferase isoenzymes. The activity of ornithine aminotransferase, which shows a similar natural pattern of development, can also be precociously elevated by cortisol administration (Herzfeld & Greengard, 1969; Rih & Kekomki, 1968). Cortisol alone, however, is not sufficient stimulus at 11 days to promote the further development of the cytosol L-alanine-glyoxylate aminotransferase activity (Table 6).

There is an increase in the protein intake at weaning as the animal transfers from milk (9.6%, w/v, protein) to solid food (21.5%, w/w, protein) (Miller, 1970). Although accurate results on the milk consumption of individual rats are not available, it can be calculated from those of Hahn & Koldovsky (1966) that an 18-day-old rat receiving milk only would have a protein intake of about 0.34 g/day, but that if it were completely weaned on to a solid diet the value would be about 1.2 g of protein/day. Many of the enzymes of amino acid metabolism increase in activity with increasing dietary protein in the adult animal (Schimke, 1962; Knox & Greengard, 1965; Harper, 1968). Rat pancreatic proteolytic enzymes show a similar relationship, and it is noteworthy that their activity in the small intestine begins to increase from low values at about 15 days *post partum* (Robberecht *et al.*, 1971).

Increasing the protein intake of 12-day-old rats by administration of casein intragastrically for 3 days had no effect on the cytosol glyoxylate aminotransferase activity at the end of that time, but it did slightly increase cytosol and mitochondrial L-alanine-2-oxoglutarate aminotransferase activities (Table 7). An effect of increasing amounts of dietary protein on L-alanine-2-oxoglutarate aminotransferase activity has been observed in adult rat liver (Rosen *et al.*, 1959). However, an increase in cytosol L-alanine-glyoxylate aminotransferase activity was observed if rats intubated with casein were given an

injection of cortisol on the third day of intubation, 24 h before assay (Table 7). Casein intubation also enhanced synergistically the response of the L-alanine-2-oxoglutarate aminotransferase isoenzymes to cortisol (Table 7). The synergism of protein and cortisol has been noted in adult rat liver for L-alanine-2-oxoglutarate aminotransferase and other amino acid-metabolizing enzymes (Hurvitz & Freedland, 1968; Freedland *et al.*, 1968; Rosen *et al.*, 1959; Schirmer & Harper, 1970). An apparent dependence on both protein and cortisol has been demonstrated for an increase in serine dehydratase activity in adult rat liver (Peraino, 1967). A similar interaction between diet and cortisol has been shown to account for the development of glucokinase and tryptophan pyrrolase at the time of weaning (Jamdar & Greengard, 1970; Greengard & Jamdar 1971; Greengard & Dewey, 1971), and, here again, a dietary-hormonal interaction on glucokinase activity had been noted in adult rat liver (Peraino, 1967).

In summary, the present results suggest that before birth, when the foetus is in a nutritionally constant environment, hormonal agents are important in controlling enzyme development; after birth the changing patterns of nutrition introduce additional influences and further development is promoted by the joint operation of hormonal and dietary factors.

We are grateful to Mrs. A. G. Phillips for skilled and conscientious technical assistance and to the Wellcome Trust for generous financial support.

References

- Ashmore, P. G. & Weber, G. (1968) in *Carbohydrate Metabolism and its Disorders* (Dickens, F., Randle, P. J. & Whelan, W. J., eds.), vol. 1, pp. 335-374, Academic Press London and New York
- Augenfeld, J. & Fritz, I. B. (1970) *Can. J. Biochem.* **48**, 288-294
- Ballard, F. J. & Oliver, I. T. (1963) *Biochim. Biophys. Acta* **71**, 578-588
- Ballard, F. J. & Oliver, I. T. (1965) *Biochem. J.* **95**, 191-200
- Br, H.-P. & Hahn, P. (1971) *Can. J. Biochem.* **49**, 85-89
- Beltz, A. D. & Reineke, E. P. (1968) *Gen. Comp. Endocrinol.* **10**, 103-108
- Burch, H. B., Lowry, O. H., Kuhlman, A. M., Skerjance, J., Diamant, F. J., Lowry, S. R. & Von Dippe, P. (1963) *J. Biol. Chem.* **238**, 2267-2273
- Butcher, R. W., Robinson, G. A., Hardman, J. G. & Sutherland, E. W. (1968) *Advan. Enzyme Regul.* **6**, 357-389
- Exton, J. H. & Park, C. R. (1968) *Advan. Enzyme Regul.* **6**, 391-407
- Exton, J. H., Mallette, L. E., Jefferson, L. S., Wong, E. H. A., Friedmann, N. & Park, C. R. (1970) *Amer. J. Clin. Nutr.* **23**, 993-1003
- Freedland, R. A., Murad, S. & Hurvitz, A. I. (1968) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 1217-1222
- Greengard, O. (1969a) *Science* **163**, 891-895
- Greengard, O. (1969b) *Biochem. J.* **115**, 19-24

- Greengard, O. (1970) in *Biochemical Actions of Hormones* (Litwack, G., ed.), vol. 1, pp. 53–87, Academic Press, New York
- Greengard, O. (1971) *Essays Biochem.* **7**, 159–205
- Greengard, O. & Dewey, H. K. (1967) *J. Biol. Chem.* **242**, 2986–2991
- Greengard, O. & Dewey, H. K. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 1698–1701
- Greengard, O. & Jamdar, S. C. (1971) *Biochim. Biophys. Acta* **237**, 476–483
- Hahn, P. & Koldovský, O. (1966) in *Utilization of Nutrients during Postnatal Development*, p. X, Pergamon Press, Oxford
- Hahn, P., Koldovský, O., Křeček, J., Martinek, J. & Vacek, Z. (1961) *Somatic Stabil. Newly Born, Ciba Found. Symp.*, 131–138
- Haltmeyer, G. C., Dennenberg, V. H., Thatcher, J. & Zarrow, M. X. (1966) *Nature (London)* **212**, 1371–1373
- Harding, H. R., Rosen, F. & Nichol, C. A. (1961) *Amer. J. Physiol.* **201**, 271–275
- Harper, A. E. (1968) *Amer. J. Clin. Nutr.* **21**, 358–366
- Herzfeld, A. & Greengard, O. (1969) *J. Biol. Chem.* **244**, 4892–4898
- Herzfeld, A. & Greengard, O. (1971) *Biochem. Biophys. Acta* **237**, 88–98
- Hommes, F. A. & Beere, A. (1971) *Biochim. Biophys. Acta* **237**, 296–300
- Hommes, F. A., Wilmink, C. W. & Richters, A. (1969) *Biol. Neonatorum* **14**, 69–73
- Hurvitz, A. I. & Freedland, R. A. (1968) *Arch. Biochem. Biophys.* **127**, 548–555
- Illnerová, H. (1966) *Biol. Neonatorum* **9**, 197–202
- Jamdar, S. C. & Greengard, O. (1970) *J. Biol. Chem.* **245**, 2779–2783
- Jost, A. & Picon, L. (1970) *Advan. Metab. Disord.* **4**, 123–184
- Jost, J.-P., Hsie, A. W. & Rickenberg, H. V. (1969) *Biochem. Biophys. Res. Commun.* **34**, 748–754
- Katunuma, N., Mikumo, K., Matsuda, M. & Okada, M. (1962) *J. Vitaminol. (Kyoto)* **8**, 68–73
- Knox, W. E. & Greengard, O. (1965) *Advan. Enzyme Regul.* **3**, 247–313
- Knox, W. E., Kupchik, H. Z. & Liu, L. P. (1971) *Enzyme* **12**, 88–98
- Levine, S. & Mullins, R. F. (1966) *Science* **152**, 1585–1592
- Linder-Horowitz, M. (1969) *Biochem. J.* **114**, 65–70
- Lockwood, E. A., Bailey, E. & Taylor, C. B. (1970) *Biochem. J.* **118**, 155–162
- McGee, M., Greengard, O. & Knox, W. E. (1971) *Enzyme* **12**, 1–12
- Miller, A. L. & Chu, P. (1970) *Enzymol. Biol. Clin.* **11**, 497–503
- Miller, S. A. (1970) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **29**, 1497–1502
- Peraino, C. (1967) *J. Biol. Chem.* **242**, 3860–3867
- Räihä, N. C. R. (1971) in *The Biochemistry of Development* (Benson, P. F., ed.), pp. 141–160, Spastics International Medical Publications, London
- Räihä, N. C. R. & Kekomäki, M. P. (1968) *Biochem. J.* **108**, 521–525
- Räihä, N. C. R. & Kretchmer, N. (1965) *J. Pediatr.* **67**, 950
- Räihä, N. C. R. & Suihkonen, J. (1968) *Biochem. J.* **107**, 793–797
- Robberecht, P., Deschodt-Lanckman, M., Camus, J., Bruylants, J. & Christophe, J. (1971) *Amer. J. Physiol.* **221**, 376–381
- Rosen, F., Roberts, N. R. & Nichol, C. A. (1959) *J. Biol. Chem.* **234**, 476–480
- Rowell, E. V. (1956) *Biochem. J.* **65**, 246–252
- Rowell, E. V., Carnie, J. A. & Snell, K. (1969a) *Biochem. J.* **112**, 7p
- Rowell, E. V., Snell, K., Carnie, J. A. & Al-Tai, A. H. (1969b) *Biochem. J.* **115**, 1071–1073
- Rowell, E. V., Snell, K., Carnie, J. A. & Rowell, K. V. (1972) *Biochem. J.* **127**, 155–165
- Schimke, R. T. (1962) *J. Biol. Chem.* **237**, 459–468
- Schirmer, M. D. & Harper, A. E. (1970) *J. Biol. Chem.* **245**, 1204–1212
- Snell, K. (1971a) *Biochem. J.* **123**, 657–659
- Snell, K. (1971b) Ph.D. Thesis, University of Manchester
- Snell, K. & Walker, D. G. (1971a) *Biochem. J.* **125**, 68p
- Snell, K. & Walker, D. G. (1971b) *Biochem. J.* **125**, 68p–69p
- Swick, R. W., Barnstein, P. L. & Stange, J. L. (1965) *J. Biol. Chem.* **240**, 3334–3340
- Thompson, J. S. & Richardson, K. E. (1967) *J. Biol. Chem.* **242**, 3614–3619
- Vernon, R. G. & Walker, D. G. (1968a) *Biochem. J.* **106**, 321–330
- Vernon, R. G. & Walker, D. G. (1968b) *Biochem. J.* **106**, 331–338
- Vernon, R. G. & Walker, D. G. (1972a) *Biochem. J.* **127**, 521–529
- Vernon, R. G. & Walker, D. G. (1972b) *Biochem. J.* **127**, 531–537
- Vernon, R. G., Eaton, S. W. & Walker, D. G. (1968) *Biochem. J.* **110**, 725–731
- Walker, D. G. (1968) in *Carbohydrate Metabolism and its Disorders* (Dickens, F., Randle, P. J. & Whelan, W. J., eds.), vol. 1, pp. 465–496, Academic Press London and New York
- Walker, D. G. (1971) in *The Biochemistry of Development* (Benson, P. F., ed.), pp. 77–95, Spastics International Medical Publications, London
- Walker, D. G. & Eaton, S. W. (1967) *Biochem. J.* **105**, 771–777
- Wicks, W. D., Kenney, F. T. & Lee, K.-L. (1969) *J. Biol. Chem.* **244**, 6008–6013
- Yeung, D. & Oliver, I. T. (1967) *Biochem. J.* **103**, 744–748
- Yeung, D. & Oliver, I. T. (1971) *Comp. Biochem. Physiol.* **40A**, 135–144