The Initial Synthesis of Proteins during Development PHOSPHOENOLPYRUVATE CARBOXYLASE IN RAT LIVER AT BIRTH

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1. A specific antibody, prepared by immunizing rabbits with phosphoenolpyruvate carboxylase (EC 4.1.1.32) purified from adult rat liver, was used to study the appearance of this enzyme in livers from developing rats. 2. Although some inactive precursor of the enzyme may be present in foetal liver, the amount is not sufficient to account for the enzyme appearance at birth. 3. The rate of phosphoenolpyruvate carboxylase synthesis relative to other cytosol proteins increases 20-fold from the foetus to the 1-day-old rat. The high rate of synthesis was maintained at least until 3 days after birth. 4. There was no measurable degradation of phosphoenolpyruvate carboxylase during the first day after birth. During this period the hepatic enzyme content increased 12-fold. 5. When phosphoenolpyruvate carboxylase attained a constant activity in the liver of rats 2 days after birth the half-time of degradation was approx. 13 h. 6. We suggest that the pattern of changes occurring during appearance of phosphoenolpyruvate carboxylase is similar to substrate-induced enzyme induction in bacteria.

When enzyme activities increase in tissues from mature mammals, the alterations are from activities that are already substantial (Schimke & Doyle, 1970). The appearance of an enzyme during development, however, is a change from a negligible to a high activity, a situation analogous with enzyme induction in bacteria. It has been established that induction in bacteria is produced by a stimulation in the rate of protein synthesis (Cohn, 1957), and no enzyme is degraded, at least in the growing culture (Mandelstam, 1963).

We wished to examine the similarities and differences between mammalian and bacterial growth and have chosen to study the development of phosphoenolpyruvate carboxylase in rat liver. As the increase in phosphoenolpyruvate carboxylase activity at birth is prevented by actinomycin D or puromycin, Yeung & Oliver (1968) have inferred that new enzyme protein is formed at this time. Nevertheless, the appearance of the enzyme could be produced not only by an increase in phosphoenolpyruvate carboxylase synthesis, but also by a relative decrease in the specific degradation rate of the enzyme.

In the present paper we report the use of immunochemical techniques to measure the rates of synthesis and degradation of phosphoenolpyruvate carboxylase. Our results show that enzyme appearance is ac-

companied by a large increase in the rate of synthesis, with no phosphoenolpyruvate carboxylase degradation during the accumulation phase.

Materials and Methods

Chemicals

L-[U-¹⁴C]Leucine (specific radioactivity 340 mCi/mmol) and NaH¹⁴CO₃ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Complete Freund's adjuvant was from Difco Laboratories, Detroit, Mich., U.S.A., and hydroxyapatite (Bio-Gel HPT) was a product of Bio-Rad Laboratories, Richmond, Calif., U.S.A. Malate dehydrogenase, phosphoenolpyruvate, NADH and IDP required for the assay of phosphoenolpyruvate carboxykinase were from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Animals

Adult Wistar rats were fed on a high-carbohydrate diet containing 5% fat. The age of foetuses used was assessed according to the time of mating or from age-to-weight relationships determined on animals within the colony. Young rats used at 2 h or more

after birth were left with their mother until they were killed. For injection of leucine into foetal rats the mothers were anaesthetized with ether and a small incision was made in the abdominal wall. The uterus was partially removed and injection into the foetuses was done through the uterine wall. The uterus was put back in the animal and the incision closed (Philippidis & Ballard, 1970).

Purification of phosphoenolpyruvate carboxylase

This enzyme was purified from livers of starved rats as described by Ballard & Hanson (1969), with the exception that the final electrofocusing step was replaced by chromatography on hydroxyapatite columns at 2°C. After elution from DEAE-cellulose, the enzyme was precipitated with $(\text{NH}_4)_2\text{SO}_4$ and extracted from the precipitate with decreasing concentrations of $(\text{NH}_4)_2\text{SO}_4$ (Ballard & Hanson, 1969). Those fractions with highest specific activity, which was usually 4–6 μmol of HCO_3^- fixed at 37°C/min per mg of protein, were passed through a Sephadex G-25 column equilibrated with 0.01 M-potassium phosphate buffer, pH 7.0. The combined fractions containing 30 to 50 mg of protein were pumped on to a hydroxyapatite column (1.5 cm² × 20 cm) and eluted with a linear gradient of potassium phosphate buffer, pH 7.0, from 0.01 M to 0.25 M, the enzyme eluting at about 0.1 M-potassium phosphate (Fig. 1). Usually 70% of the enzyme applied was recovered in fractions of specific activity 14–16 units/mg of protein, with constant specific activity across the enzyme peak. The eluate volume was decreased and the enzyme precipitated by dialysis against saturated $(\text{NH}_4)_2\text{SO}_4$ containing 1 mM-dithiothreitol and 1 mM-EDTA at pH 7.0. Phosphoenolpyruvate carboxylase was collected by centrifugation, dissolved in 0.15 M-NaCl, then dialysed overnight against 0.15 M-NaCl and stored frozen.

This modification to the purification procedure was preferable to the isoelectric focusing described by Ballard & Hanson (1969) as it was more reproducible and took approx. 3 h instead of 2 days.

Immunochemical techniques

Phosphoenolpyruvate carboxylase was dissolved in 0.15 M-NaCl and mixed thoroughly with an equal volume of complete Freund's adjuvant, then injected subcutaneously into rabbits. Approx. 2 mg of enzyme was injected/2 kg body wt. and the injections were repeated every 2 weeks. A high titre of antibody was usually attained 6 weeks after the first injection. After this approx. 40 ml of blood was obtained from an ear vein at monthly intervals. Blood was left to clot overnight and the serum was collected by centrifugation. The γ -globulin fraction was precipitated by addition

Fig. 1. *Chromatography of phosphoenolpyruvate carboxylase on a hydroxyapatite column*

The gradient from 0.01 M- to 0.25 M-potassium phosphate, pH 7.0, was begun after 100 ml had been collected. Other details are given in the Materials and Methods section. ●, Phosphoenolpyruvate carboxylase activity; ○, protein.

Fig. 2. *Effects of different amounts of antibody on phosphoenolpyruvate carboxylase activity*

Phosphoenolpyruvate carboxylase, either purified (●) or from a cytosol fraction of rat liver (○) was mixed with increasing volumes of antibody prepared in rabbits against the purified enzyme, and incubated at 37°C for 15 min and at 0°C for 24 h. In each tube the volume was 0.3 ml. After centrifugation, the enzyme activity was measured in the supernatant.

of $(\text{NH}_4)_2\text{SO}_4$ to 50% saturation and the precipitate was washed twice by suspension in 50% $(\text{NH}_4)_2\text{SO}_4$. The washed antibody preparation was dissolved in 0.15M-NaCl at half the original serum volume and was then dialysed against 0.15M-NaCl overnight and stored frozen at -20°C .

The potency of the antibody preparation was determined by titration either against purified enzyme or a 100000g supernatant of rat liver. These titrations (Fig. 2) showed that a constant amount of enzyme was inactivated by a fixed volume of antibody. A linear relationship of this type has been reported for the inactivation of tyrosine aminotransferase by antibody (Granner *et al.*, 1968). The linear relationship between phosphoenolpyruvate carboxylase activity and antibody permits the measurement of antibody titre. One unit of antibody is the volume of γ -globulin preparation that will inactivate 1 unit of phosphoenolpyruvate carboxylase under the conditions described in Fig. 2. One unit of phosphoenolpyruvate carboxylase activity is defined as that amount of enzyme which catalyses the fixation of $1\mu\text{mol}$ of $\text{HCO}_3^-/\text{min}$ at 37°C (Chang & Lane, 1966). Normally, the dialysed γ -globulin had an antibody titre of 2–6 units/ml. Ouchterlony double diffusion (Ouchterlony, 1966) of antibody against antigen of different specific activities showed a single precipitin line when γ -globulin of high specific activity was used. With antibody preparations of activity below 5 units/ml other precipitin lines were apparent in the Ouchterlony test, indicating a lack of specificity of the antigen-antibody reaction. Specificity was obtained by treatment of antibody with a 100000g supernatant prepared from a 1:2 (w/v) homogenate of foetal rat liver in 0.25M-sucrose. One part of liver supernatant was mixed with 3 parts of antibody and incubated for 15 min at 37°C and then 24 h at 0°C . After centrifugation to remove any precipitate caused by non-specific combination of γ -globulin with cytosol proteins, the treated antibody was dialysed against 0.15M-NaCl and stored at -20°C . This treatment did not cause any significant loss of antibody activity and produced an antibody preparation that gave a single continuous precipitin line on Ouchterlony double-diffusion analysis.

Incorporation of radioactive isotope

$[^{14}\text{C}]\text{Leucine}$ ($3\mu\text{Ci}$, $50\mu\text{l}$) was injected intraperitoneally into foetal and newborn rats. For pulse studies the animals were killed 60 min later, then the liver was removed and homogenized in 3 vol. of cold 0.25M-sucrose. This homogenate was centrifuged at 100000g for 40 min and the supernatant was removed, care being taken to avoid any turbid material. Portions of the supernatant were taken for the measurement of radioactivity in proteins and

amino acids (Mans & Novelli, 1961) and for measurement of immunoprecipitation and total radioactivity.

To obtain complete precipitation by antibody, a slight excess of antibody over antigen is required. The amount of phosphoenolpyruvate carboxylase antigen in each liver supernatant was determined by enzyme assay. We have described some slight modifications (Ballard & Hanson, 1967) to the assay method of Chang & Lane (1966), but since this method involves the determination of $\text{NaH}^{14}\text{CO}_3$ fixation, particular care must be taken to minimize any radioactivity introduced as $[^{14}\text{C}]\text{leucine}$. To accomplish this, reactions were stopped by the addition of trichloroacetic acid to give a final concentration of 5% and the reaction tubes were cooled in ice to facilitate the complete precipitation of radioactive protein. The tubes were then centrifuged at 2000g for 10 min and the supernatant was removed and gassed with CO_2 for 3 min. The radioactivity measured in these samples included any ^{14}C -labelled amino acids in the liver preparation and usually represented a blank of less than 200 c.p.m. This blank was subtracted from test measurements.

Immunoprecipitation was done in duplicate with 0.18 unit of enzyme and 0.25 unit of antibody. These amounts were chosen to obtain a satisfactory precipitate. The precipitate contained approx. $130\mu\text{g}$ of protein as measured by the Lowry *et al.* (1951) procedure, which was similar to the amount present at equivalence. If 0.25 unit of phosphoenolpyruvate carboxylase was not available in the liver supernatant, additional non-radioactive enzyme of specific activity 1–2 units/mg was added. The volume was usually 0.3 ml and was standardized for any series of experiments. The solutions were incubated at 37°C for 15 min and then overnight at 0°C . The tubes were then centrifuged and the supernatants were transferred to similar tubes. A second immunoprecipitation was then done by adding 0.18 unit of non-radioactive phosphoenolpyruvate carboxylase and 0.25 unit of antibody. Both first and second precipitates were washed by suspension on 0.15M-NaCl at 0°C . The tubes were centrifuged and the supernatants discarded. This washing was repeated twice more, after which the precipitates were dissolved in 0.5 ml of 0.5M-NaOH and transferred to scintillation vials containing 1 ml of water and 10 ml of a solution containing 4 g of 2,5-diphenyloxazole, 100 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 1 litre of toluene and 500 ml of Triton X-100. The additional water was required to prevent phase separation. The radioactivity in these samples was measured in a Packard liquid-scintillation counter (model 3375) with 72% efficiency. Specific radioactivity in phosphoenolpyruvate carboxylase was taken as being equal to the radioactivity in the first precipitate minus the non-specific radioactivity in the second precipitate (Schimke *et al.*, 1965).

In some experiments the supernatant from the first precipitation was analysed by Ouchterlony double-diffusion against antibody. In no case was a precipitin line visible, indicating complete precipitation of phosphoenolpyruvate carboxylase. An additional control precipitation was attempted by using serum from rabbits immunized with Freund's adjuvant which had been treated with a cytosol fraction of foetal liver as described for the specific antibody, but without phosphoenolpyruvate carboxylase. No precipitate was obtained when this serum was mixed with liver supernatants and incubated at 37°C for 15 min and then overnight at 0°C.

Measurement of leucine in liver

Livers were homogenized in 2 vol. of 4% (w/v) sulphosalicylic acid, and centrifuged at 15000g for 10 min; the supernatant and precipitate were then separated. The precipitate was resuspended in 1 vol. of the sulphosalicylic acid and centrifuged as before. The supernatants were combined and freeze-dried, then the amino acids were separated on a Beckman model 120B amino acid analyser (Moore *et al.*, 1958).

Results

The rapid increase in hepatic phosphoenolpyruvate carboxylase activity that occurs immediately after birth (Ballard & Hanson, 1967) could possibly be caused by activation of a pre-existing apoenzyme. Should such an apoenzyme be present in foetal liver, it would probably combine with antibody prepared against the native enzyme. To test this possibility we titrated increasing amounts of phosphoenolpyruvate carboxylase from a cytosol fraction of adult liver against a fixed volume of antibody, in both the presence and absence of foetal liver extracts. The equivalence point with adult cytosol enzyme was found to correspond to 22.5 milliunits of phosphoenolpyruvate carboxylase (Fig. 3). When 0.2 ml of a cytosol fraction from a 33% (w/v) homogenate of foetal liver was added, the equivalence point was decreased to 19.5 milliunits. This would indicate that 3 milliunits of non-enzymically active antigen was present in the foetal extract added, or 45 milliunits/g of liver. In experiments on four preparations of foetal liver the amount of non-enzymically active antigen present was 30 ± 8 (S.E.M.) milliunits/g of liver. This value is significantly greater than zero ($P < 0.05$). The determination is, however, subject to considerable experimental error and would give erroneously high values if any mitochondrial phosphoenolpyruvate carboxylase was present in the supernatant prepared from foetal liver.

An alternative method of measuring the amount of enzymically inactive antigen in a preparation is to titrate it with an antibody of known titre (see Fig. 2).

Fig. 3. Measurement of non-enzymically active antigen in foetal liver cytosol

Antibody (10 μ l) prepared against phosphoenolpyruvate carboxylase was mixed with increasing amounts of enzyme from a cytosol fraction of adult rat liver and incubated as described in the Materials and Methods section (●). A cytosol fraction of foetal liver (0.2 ml) was present in some tubes (○). The total volume was 0.4 ml. After centrifugation, the activity of phosphoenolpyruvate carboxylase was measured. The equivalence points in the presence and absence of foetal cytosol are 19.5 and 22.5 milliunits of enzyme.

In this way the volume of antibody required to inactivate completely a fixed amount of enzyme may be obtained by extrapolation of the titration line. However, the accuracy of this procedure is not great when the solution to be titrated contains a very low activity of enzyme, such as the cytosol fraction of foetal liver. To overcome this difficulty, different volumes of enzymically active antigen were added to constant volumes of foetal rat liver supernatant and the resultant mixtures titrated as shown in Fig. 2. After several titrations we have plotted the amount (μ l) of antibody required for complete inactivation against milliunits of antigen added and extrapolated to zero addition of enzyme (Fig. 4). This gives the amount of antibody required to inactivate all the antigen, both enzymically active and inactive, in the foetal liver cytosol. In curve *a* (Fig. 4) a value of 3.6 μ l of antibody was obtained. The antibody preparation used had a titre of 2.0 units/ml, so that 7.2 milliunits of antigen were present. Phosphoenolpyruvate carboxylase assays indicated that 5.7 milliunits of enzyme were present in the cytosol fraction used, indicating 1.5 milliunits of antigen without enzymic activity. This was present in 0.4 ml of a supernatant fraction prepared from a 20% (w/v) homogenate of liver, and represents 19 milliunits of enzymically inactive antigen/g of liver. Similar calculations from another liver preparation (Fig. 4, curve *b*) gave 24 milliunits/g of liver.

Fig. 4. Titration curves for two experiments in which different volumes of a partially purified preparation of phosphoenolpyruvate carboxylase were added to foetal liver cytosol

The volume of antibody required to inactivate completely any phosphoenolpyruvate carboxylase antigen was determined by extrapolation of plots such as those illustrated in Fig. 2. In experiment (a) 3.6 μ l of antibody (7.2 milliunits) was required to inactivate all the antigen present in 0.4 ml of foetal liver cytosol. In experiment (b) the extrapolated value was 2.3 μ l (4.6 milliunits). In these two experiments there were 5.7 and 2.7 milliunits respectively of active enzyme in the foetal extracts.

Rates of phosphoenolpyruvate carboxylase synthesis

Although the same amount of radioactive leucine was injected into foetal and newborn rats, the incorporation of this label into cytosol proteins was much lower in the foetus (Table 1). This may reflect a proportionally greater incorporation in tissues other than liver, or alternatively the label may have leaked from the foetus. For these reasons, and because the hepatic leucine pool size changes markedly during the period studied (Table 2), we have expressed phosphoenolpyruvate carboxylase synthesis as a fraction of the incorporation of label into the cytosol proteins of liver. There is a large increase in phosphoenolpyruvate carboxylase synthesis from the foetal liver to the liver of 2-day-old animals. As shown in Fig. 5 there is a 20-fold increase in the relative rate of hepatic phosphoenolpyruvate carboxylase synthesis from the foetus to the 1-day-old animal, with much of this change occurring in the first 2 h after birth. The high rate of synthesis observed in livers of 1-day-old animals is maintained at least until an age of 3 days.

Rate of phosphoenolpyruvate carboxylase degradation

Measurements of enzyme degradation may be made by labelling the enzyme with [14 C]leucine and

Table 1. Incorporation of [14 C]leucine in liver

Leucine (3 μ Ci) was injected into foetal rats and 2-day-old animals. After 60 min the livers were removed and the radioactivity was measured in protein and amino acid fractions (Mans & Novelli, 1961) of the cell cytosol. Incorporation into phosphoenolpyruvate carboxylase is represented by the difference between precipitate 1 (test) and precipitate 2 (blank). Values are the means $\pm$ S.E.M. for measurements on 12 livers.

Age ...	[14 C]Leucine (d.p.m./100 μ l of liver cytosol)	
	21½-22 day foetus	2-day-old infant rat
Protein	6340 $\pm$ 400	14700 $\pm$ 1900
Amino acids	2010 $\pm$ 85	2560 $\pm$ 360
Precipitate 1	61.6 $\pm$ 5.0	400 $\pm$ 39.5
Precipitate 2	50.8 $\pm$ 4.2	125 $\pm$ 15.5
Phosphoenolpyruvate carboxylase	10.8 $\pm$ 1.5	275 $\pm$ 26.0

Table 2. Leucine concentrations in livers of young rats

Leucine was measured as described in the Materials and Methods section.

Litter	Time after delivery (h)	Leucine (μ mol/g of liver)
1	0	0.261
	5	0.106
2	0	0.257
	5	0.156
	7	0.093
	12	0.093
3	5	0.117
	12	0.109

determining the rate of removal of radioisotope from the enzyme pool. In foetal rat liver the rate of phosphoenolpyruvate carboxylase synthesis was too small to permit accurate measurements of decay. However, the amount of phosphoenolpyruvate carboxylase in liver (Fig. 6) gradually increases during the last 2 days of gestation, whereas the rate of synthesis is not significantly altered. This would suggest that if degradation of phosphoenolpyruvate carboxylase is occurring at this time, the rate must be low.

For the postnatal animal the rate of enzyme degradation can be conveniently measured. At 1 h after injection of [14 C]leucine some of the animals were killed to give a zero-time value of labelling, and 6 μ mol of non-radioactive leucine was given to the other

Fig. 5. Incorporation of [^{14}C]leucine into phosphoenolpyruvate carboxylase in the livers of rats of various ages

Results are expressed as a percentage of radioactivity found in the cytosol proteins of liver. Values are the means $\pm$ S.E.M. of 7–12 determinations.

animals to dilute out the radioactive leucine pool. In this way the synthesis of new enzyme from radioactive leucine is minimized. Other experiments showed that this leucine injection did not cause any change in phosphoenolpyruvate carboxylase activity.

As with the studies of enzyme synthesis, we have expressed rates of enzyme degradation relative to the disappearance of label from total cytosol proteins. It is important, for this method of measuring phosphoenolpyruvate carboxylase degradation, that degradation of total cytosol proteins is relatively slow compared to this enzyme. An indication of the degradation rate of cytosol protein was obtained from experiments in which 32 rats between 1 and 3 days old were injected with [^{14}C]leucine. The radioactivity in cytosol proteins of 16 animals killed after 1 h was 37100 ± 1390 (S.E.M.) d.p.m./liver. The remaining animals were injected with $6\mu\text{mol}$ of unlabelled leucine 1 h after the [^{14}C]leucine injection and were killed 13 h later. The radioactivity in liver cytosol proteins of these animals was 33100 ± 1050 d.p.m./liver. The approximate half-life of cytosol proteins is therefore 4 days, in agreement with measurements on adult animals (Schimke *et al.*, 1968). This long half-life will not markedly affect calculations of the half-life of phosphoenolpyruvate carboxylase.

The development of phosphoenolpyruvate carboxylase after birth shows a period of rapid enzyme accumulation from birth to 24 h, and a slower rate of increase in enzyme between 2 days and 3 days after birth (Fig. 6). A steady state is therefore indicated

Fig. 6. Appearance of phosphoenolpyruvate carboxylase activity in rat liver

Values are calculated from the activity in units/g of tissue and the liver weights, and are plotted as the means $\pm$ S.E.M. for 6–12 determinations.

Fig. 7. Rates of degradation of hepatic phosphoenolpyruvate carboxylase at different ages after birth

Animals were injected with [^{14}C]leucine and were either killed after 60 min or given a 'chase' of $6\mu\text{mol}$ of non-radioactive leucine. The remaining animals were killed several hours later. Degradation is expressed as the percentage of radioactivity in phosphoenolpyruvate carboxylase relative to total liver cytosol proteins. In this experiment 53 animals from seven different litters were grouped into the ten time-points indicated by the means $\pm$ S.E.M. Half-times of degradation are given in parentheses.

during this last period. From 3 to 15 days after birth the activity of phosphoenolpyruvate carboxylase remains between 5 and 7 units/g of liver (F. J. Ballard, unpublished work), but the liver begins to increase in weight from 5 days after birth.

During the period of rapid enzyme accumulation, there is no degradation of phosphoenolpyruvate carboxylase (Fig. 7). In the period from 1 to 2 days after birth the half-time of degradation shown in Fig. 7 is 14h. Other experiments gave values between 12 and 20h, the variation being due presumably to differences between the rate of enzyme appearance in individual litters. By 2 to 3 days after birth the rate of degradation shows a half-life of 10–15h, with a mean of 13h (Fig. 7). Since this last period corresponds to the time when the amount of enzyme is constant, the rate of phosphoenolpyruvate carboxylase synthesis will equal the rate of degradation.

Discussion

Foetal liver

The immunochemical titration experiments in Figs. 3 and 4 suggest that foetal liver contains some substance that reacts with antibody prepared against phosphoenolpyruvate carboxylase but is not enzymically active. This antigen is present at approx. 0.02 unit/g of liver, compared with 6 units/g in the liver of rats 2 days after birth. It therefore appears that this foetal antigen is quantitatively unimportant and its presence cannot account for the rapid postnatal appearance of enzyme. This conclusion would not hold if the material in foetal liver has an antigenic activity much less than that of the native enzyme, a possibility that is difficult to test. Nevertheless, the postnatal appearance of phosphoenolpyruvate carboxylase is prevented by actinomycin D (Yeung & Oliver, 1968), suggesting that RNA-dependent protein synthesis is involved. Further, the rate of enzyme development initiated by delivery is the same in prematurely delivered rats as at the normal time of birth (Yeung & Oliver, 1967). If an enzymically inactive precursor was accumulating during gestation, we would expect that the activation of precursor would produce a more rapid increase in phosphoenolpyruvate carboxylase activity at birth as compared with the prematurely delivered rat.

Although the rate of phosphoenolpyruvate carboxylase synthesis is extremely low in foetal liver, there is a gradual accumulation of enzyme towards the end of gestation. We interpret this as an indication that the rate of enzyme degradation is lower than the rate of synthesis. If glucagon is injected into rat fetuses, several enzymes increase in activity and then return to low values (Greengard & Dewey, 1967, 1968). Phosphoenolpyruvate carboxylase is also induced by glucagon in foetal liver (Philippidis &

Ballard, 1969) and we have shown in preliminary studies that the activity returns to control values within 16h after administration. As these increased activities do not occur in the presence of actinomycin D (Greengard, 1969), it is likely that protein synthesis is involved. The subsequent fall in activity therefore implies that an appreciable rate of phosphoenolpyruvate carboxylase degradation can occur in foetal rat liver.

Postnatal changes

After birth there is a rapid increase in the rate of synthesis of phosphoenolpyruvate carboxylase relative to other cytosol proteins. There is no degradation of enzyme, but by 2–3 days after birth the half-life is 13h. This rate of degradation is slower than the half-time of about 5h reported for insulin-induced degradation of phosphoenolpyruvate carboxylase in livers (Shrago *et al.*, 1963) or adipose tissue (Reshef *et al.*, 1970) of adult, diabetic rats. Immunochemical techniques were not used in these experiments and the insulin caused a net loss of enzyme activity. Consequently those studies are not strictly comparable with the degradation experiments reported in this communication, and the apparent differences in rates of degradation of the enzyme may be differences between a steady state and a condition of net enzyme loss.

The change in rate of phosphoenolpyruvate carboxylase synthesis at birth is probably related to the hypoglycaemia that occurs soon after birth. This hypoglycaemia could result in an increase of glucagon and a decrease in insulin concentrations, both of which would favour phosphoenolpyruvate carboxylase synthesis. No explanation can be offered for the change in enzyme degradation. Protein degradation appears to be an energy-dependent process (Kenney, 1967; Hershko & Tomkins, 1971), which may involve the lysosome. Since proteins differ in their turnover rates, and phosphoenolpyruvate carboxylase and other proteins have rates of degradation that may be altered, it would seem that any system of protein catabolism must be adaptable. Whatever this adaptability may involve, it can occur in the liver of 2-day-old rats.

General considerations

Experiments in adult rats have shown that both synthesis and degradation rates of enzymes can be altered by dietary or hormonal changes. For example, changes in the activities of arginase (Schimke, 1964) and acetyl-CoA carboxylase (Majerus & Kilburn, 1969) involve alterations in both synthesis and degradation rates. The most striking differences between phosphoenolpyruvate carboxylase appearance after

birth and enzyme adaptation in adults are the complete lack of enzyme degradation during the accumulation phase in newborn animals and the very large increase in the rate of enzyme synthesis at this time. This pattern is very similar to substrate-induced induction in bacteria, in which enzyme degradation does not occur, at least during the period of rapid growth (Mandelstam, 1963). Perhaps the initial accumulation of enzymes in mammalian tissues follows this procedure. The onset of degradation would reflect a maturation of cells, which would then have the more complex regulatory controls on enzymes that are characteristic of higher organisms. There are no other comparable studies on enzyme appearance in mammals, but Silpananta & Goodridge (1971) have measured rates of synthesis and degradation of NADP malate dehydrogenase (EC 1.1.1.40) in liver from developing chicks. Their findings are very similar to those reported here for phosphoenolpyruvate carboxylase, with accumulation accompanied by a 50-fold increase in enzyme synthesis whereas degradation was rapid only after high enzyme activities were reached.

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