Mitochondrial Development in Liver of Foetal and Newborn Rats

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The development of the inner mitochondrial membrane in foetal and neonatal rat liver was studied by following three parameters: (1) the activity of several respiratory enzymes in homogenates and purified mitochondria, (2) the spectrophotometric determination of cytochrome content in the mitochondria and (3) the cardiolipin content in both homogenates and purified mitochondria. Respiratory-enzyme activities of homogenates of foetal liver were one-quarter to one-twentieth of those of homogenates of adult liver, and the enzyme specific activities in purified mitochondria from foetal liver were one-half to one-eighth of those in mitochondria from adult liver. The cardiolipin content of liver homogenates increased approximately twofold during the development period, but there was no significant change in the cardiolipin content of purified mitochondria. It is concluded that cell mitochondrial content approximately doubles in the immediate postnatal period. There was no evidence for an increase in the relative amount of cristae protein in mitochondria during this period to account for increases in mitochondrial enzyme specific activity, since cardiolipin and cytochrome concentrations remained unchanged and electron micrographs revealed no differences. The cause of the lower respiratory-enzyme specific activity in foetal liver mitochondria is unclear. Qualitative differences in respiratory units in foetal and mature animals are suggested.

In the last few years considerable information about the synthesis and assembly of mitochondria has accumulated. Although our understanding is far from complete, it definitely appears that mitochondria have a degree of autonomy in the cell. They have their own DNA, ribosomes, tRNA and mechanism of protein synthesis (Roodyn & Wilkie, 1968; Rabinowitz & Swift, 1970). However, little is known about how mitochondrial components, synthesized either inside or outside mitochondria, are assembled into a fully formed organelle. It is still not established whether the mitochondrion or a major unit of it is formed by the synchronous assembly of all or many of its elements or by the ordered sequential addition of its major components to pre-existing structures. The purpose of the present paper is to provide information on this problem by examining the development of different mitochondrial components in the foetal and postnatal period of the rat.

Several markers of mitochondrial structure and function have been followed. Activities of four respiratory enzymes of the inner mitochondrial membrane that belong to different parts of the electron-transport system have been evaluated during embryogenesis and postnatally. The concentration of the phospholipid cardiolipin, which

has been shown to be localized exclusively in the mitochondria of rat and sheep liver (Fleischer, Fleischer, Rouser, Casu & Kritchevsky, 1967; Getz, Bartley, Lurie & Notton, 1968) and particularly in the inner mitochondrial membrane of guinea-pig and rat liver (Parsons, Williams, Thompson, Wilson & Chance, 1967; McMurray & Dawson, 1969), was also determined. The findings indicate that, in addition to a greater amount of inner mitochondrial membrane in adult liver, mitochondria from adult rats have an enhanced specific activity indicative of a qualitative difference between mitochondria from foetal and mature animals.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats were used in all experiments. Total age was estimated from the time of conception and was accurate to within 12 h. Each animal was killed by decapitation. The liver was excised immediately and rinsed with ice-cold 0.3 M-sucrose containing 1 mM-EDTA, pH 7.4. All homogenates except for adult controls were prepared from pooled livers to obtain sufficient amounts of material for analysis.

Isolation of cell fractions. Except for the isolation of the purified nuclear fraction, which was carried out by the method of Lawford, Sadowski & Schlachter (1967), 5% (w/v) liver homogenates were prepared in 0.3 M-sucrose

containing 1 mM-EDTA, pH 7.4, in a glass Potter-Elvehjem homogenizer with a loosely fitting Teflon pestle at maximal speed with one up-and-down movement of the pestle for foetal liver and five for adult tissue. The homogenate was filtered through a layer of silk bolting cloth (110 mesh; Blaw-Knox Co., Chicago, Ill., U.S.A.) and centrifuged at 1100g for 10 min. The pellet was discarded and the supernatant was centrifuged at 7700g for 10 min. The mitochondrial pellet was washed by suspension in 0.3 M-sucrose containing 1 mM-EDTA, pH 7.4, and centrifugation at 5900g for 10 min. The washings were repeated five times in the experiment designed to evaluate mitochondrial localization of cardiolipin in foetal liver. In all other experiments mitochondria were washed three times. Microsomes were prepared by centrifuging the supernatant at 78500g in the Spinco ultracentrifuge for 1 h; the pellet was suspended in 0.3 M-sucrose containing 1 mM-EDTA, pH 7.4, and centrifuged at 12100g for 30 min. The pellet was discarded and the supernatant was then centrifuged at 78500g for 1 h to yield the microsomal pellet. Samples were kept at 0°C throughout preparation.

Enzyme assays. Each sample was assayed in duplicate for the different enzyme activities and for protein content (Lowry, Rosebrough, Farr & Randall, 1951). Succinate-cytochrome *c* reductase activity was determined spectrophotometrically at 25°C by measuring the change in E_{550} in the presence of succinate (Rabinowitz & DeBernard, 1957). Antimycin-sensitive NADH-cytochrome *c* reductase activity was assayed after treating the mitochondria by a swelling and shrinking procedure described by Sottocasa, Kuylenshierna, Ernster & Bergstrand (1967). The reduction of cytochrome *c* by NADH in the presence and absence of 1 µg of antimycin A per ml was measured in the same way as for the succinate-cytochrome *c* reductase and was calculated by subtracting the values obtained in the presence of antimycin from those obtained in its absence. Cytochrome *c* oxidase activity was assayed by measuring the rate of oxidation of cytochrome *c* reduced with dithionite in the Gilford automatic spectrophotometer by the modified method of Cooperstein & Lazarow (1951) described by Rabinowitz, Getz, Casey & Swift (1969). Succinate dehydrogenase activity was measured with and without phenazine methosulphate as an intermediate electron carrier between reduced succinate dehydrogenase and tetrazolium. Formazans formed on reduction of tetrazolium salts were extracted with ethyl acetate and the E_{490} value was measured (Pennington, 1961). Units of activity are expressed as nmol of cytochrome *c* reduced/min per mg of protein for succinate-cytochrome *c* reductase and NADH-cytochrome *c* reductase, as mg of formazan produced/h per mg of protein for succinate dehydrogenase and as the first-order rate constant k /min per ml of reaction mixture per mg of protein for cytochrome *c* oxidase.

Phospholipid analysis. Phospholipids were extracted from each sample by the method of Letters (1966). Lipid extracts containing approx. 20 µg of P were analysed by two-dimensional t.l.c. as described by Getz, Jakovcic, Heywood, Frank & Rabinowitz (1970). Phosphorus analysis was performed by the method of Barlett (1959).

Cytochrome determinations. Difference spectra (oxidized-reduced) of mitochondrial preparations solubilized with deoxycholate were obtained by the method of Williams (1964) with a Cary model 14 recording spectro-

photometer. Cytochrome concentrations were calculated from these spectra by using the method of Williams (1964).

Electron microscopy. Small blocks of liver tissue were fixed for 1 h in 1% OsO₄ in 0.1 M-phosphate buffer, pH 7.4. Small pellets of isolated mitochondria were fixed for 2-4 h in 4% glutaraldehyde in 0.1 M-phosphate buffer, pH 7.4, and 1.5% sorbitol, and post-fixed in OsO₄ in phosphate buffer. Material was embedded in Epon 812; thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Results represent mean values for two to four separate experiments.

Enzyme patterns during the development of rat liver. Cytochrome oxidase, succinate-cytochrome *c* reductase, antimycin-sensitive NADH-cytochrome *c* reductase and succinate dehydrogenase activities in liver of foetal (15-21 days of gestation), postnatal (1-30 days *post partum*) and adult (3-month-old) rats were measured. Homogenate enzyme activities are presented in Fig. 1. The mean specific

Fig. 1. Specific activities of respiratory enzymes of liver homogenates of foetal and neonatal rats. Activities are expressed as percentages of adult values: $\blacktriangle$, cytochrome oxidase; $\times$, succinate dehydrogenase; $\bullet$, succinate-cytochrome *c* reductase; $\square$, antimycin A-sensitive NADH-cytochrome *c* reductase. NB, Newborn. The mean $\pm$ 1 s.d. absolute specific activities for adult rat liver homogenates were 48 ± 11 units for cytochrome oxidase, 99 ± 18 units for succinate-cytochrome *c* reductase, 80 ± 17 units for antimycin-sensitive NADH-cytochrome *c* reductase, and 21 ± 5 units for succinate dehydrogenase.

Fig. 2. Specific activities of respiratory enzymes of purified liver mitochondria from foetal and neonatal rats. Activities are expressed as percentages of adult values: ▲, cytochrome oxidase; ×, succinate dehydrogenase; ●, succinate-cytochrome *c* reductase; □, antimycin A-sensitive NADH-cytochrome *c* reductase. NB, Newborn. The mean absolute specific activities for adult rat liver mitochondria were 144 ± 17 units for cytochrome oxidase, 296 ± 17 units for succinate-cytochrome *c* reductase, 253 ± 18 units for antimycin-sensitive NADH-cytochrome *c* reductase and 46 ± 14 units for succinate dehydrogenase.

activities $\pm$ 1 S.D. in adult rats were 48 ± 11 units for cytochrome *c* oxidase, 99 ± 18 units for succinate-cytochrome *c* reductase, 80 ± 17 units for antimycin-sensitive NADH-cytochrome *c* reductase and 21 ± 5 units for succinate dehydrogenase. Enzyme activities from liver of rats at 15–17 days of gestation were one-third to one-twentieth of those in adult liver. Except for cytochrome *c* oxidase, the activity of which is low and decreases further until birth, the activities of the other three respiratory enzymes start to rise during the last 3–4 days before delivery and continue to increase postnatally.

The specific activities of the respiratory enzymes of purified mitochondria (Fig. 2) followed the same development pattern as that of the homogenate, but the increase was less marked. The values obtained 15–17 days prenatally were one-half to one-eighth of adult values. Mean adult specific activities $\pm$ 1 S.D. were 144 ± 17 units for cytochrome *c* oxidase, 296 ± 17 units for succinate-cytochrome *c* reductase, 253 ± 18 units for antimycin-sensitive NADH-cytochrome *c* reductase and 46 ± 14 units for succinate dehydrogenase. These results suggest that the increase in the specific activities of all respiratory enzymes is due both to the increased number of mitochondria postnatally and to

Fig. 3. Effects of addition of Lubrol (0.4mg/mg of protein) on cytochrome *c* oxidase activities in liver mitochondria and homogenates of foetal and neonatal rats. ●—●, Mitochondria; ●---●, mitochondria + Lubrol; ○—○, homogenates; ○---○, homogenates + Lubrol. NB, Newborn.

the increased functional activity of individual mitochondrial units.

It also appears from Figs. 1 and 2 that the rates of increase of respiratory-enzyme activities are not identical for the four enzymes studied. Succinate-cytochrome *c* reductase activity appears to increase more rapidly than do the activities of antimycin-sensitive NADH-cytochrome reductase and cytochrome *c* oxidase. Succinate dehydrogenase is the last to reach its maximal activity, at about 4 weeks after birth. A rather striking example of asynchrony is the decline of homogenate and mitochondrial cytochrome oxidase activities between days 18 and 21 of gestation, a period when the other three enzymes measured were showing marked increase in activity.

To evaluate the possibility that the difference in activity of foetal and adult cytochrome *c* oxidase is due to differences in enzyme activation during preparation and assay *in vitro*, enzyme activities were measured in the presence of the detergent Lubrol (0.4mg/mg of protein). It has been claimed that this pretreatment with Lubrol produces maximal activation of cytochrome *c* oxidase (Schnaitman & Greenawalt, 1968). Assays were carried out on liver homogenates and mitochondrial fractions in the foetal and postnatal rats (Fig. 3). There was an increase in the activity by as much as 100% in the homogenate and 80% in the mitochondria in both foetal and adult samples. Irrespective of the degree

Table 1. Concentrations of cytochromes in mitochondria from foetal and adult rats

Concentrations of cytochromes are given in nmol/mg of protein. F, Foetal; A, adult.

Cytochrome <i>a</i>		Cytochrome <i>b</i>		Cytochrome <i>c</i> ₁		Cytochrome <i>c</i>	
F	A	F	A	F	A	F	A
0.30	0.27	0.31	0.26	0.13	0.19	0.24	0.15

Table 2. Succinate-cytochrome *c* reductase, cytochrome *c* oxidase and cardiolipin content in fractions of foetal and adult rat liver

F, Foetal; A, adult.

	Homogenate		Nuclear	Mitochondrial		Microsomal	
	F	A		F	A	F	A
Succinate-cytochrome <i>c</i> reductase (nmol/min per mg of protein)	—	98.7	Trace	—	296.1	—	10.7
Cytochrome <i>c</i> oxidase (k/min per mg of protein)	8.1	48.5	0.49	41.9	144.9	5.5	5.7
Cardiolipin (μg/mg of protein)	0.1	0.21	0.0025	0.59	0.65	0.1	0.08
Cardiolipin (% of total phospholipids)	6.3	5.4	0.38	13.5	15.3	0.67	0.65

of activation *in vitro*, the foetal activities remained one-third of adult values in the homogenate and one-half of adult values in the mitochondria.

Cytochrome content. The lower specific activities of mitochondrial enzymes in foetal liver may be due to a smaller content of respiratory carriers or to their presence in less active form. To distinguish between these possibilities spectral analysis of purified mitochondria from foetal and adult rats was carried out. The mitochondrial fraction of 21-day-old foetal liver was compared with that of an adult control. No significant difference in the concentration of cytochrome *a*/mg of mitochondrial protein was found between the prenatal and adult liver mitochondria (Table 1).

Cardiolipin concentration of the foetal and adult liver. Cardiolipin was selected as a marker of the inner mitochondrial membrane. It was first necessary to establish that the mitochondrial membrane is the major, if not exclusive, site of cardiolipin in both foetal and adult hepatocytes. Cardiolipin concentrations and the activities of mitochondrial markers, cytochrome *c* oxidase and succinate-cytochrome *c* reductase, were measured in the homogenate and in nuclear, mitochondrial and microsomal fractions. Results are presented in Table 2. The phospholipid composition of the adult liver cell fractions is similar to that previously reported with the use of different methods of phospholipid fractionation (Getz *et al.* 1968). Only a very small amount of cardiolipin was present in the nuclear and microsomal fractions. When corrections are made for mitochondrial contamination on the basis of marker enzyme activities, the calculated cardiolipin content in the nuclear fraction of adult rats is 0.31% of nuclear phospholipids. Since pure nuclei contain a very small proportion of the total

rat liver phospholipids this low content of cardiolipin is a negligible proportion of total hepatocyte cardiolipin.

Assuming that about 50% of the homogenate protein is microsomal protein, as derived from the data of Dallner, Siekevitz & Palade (1966), then the microsomes of foetal and adult livers do not contain much more than 10% of the total homogenate cardiolipin (11.2 and 11.9% respectively). It is also apparent from the results in Table 2 that cardiolipin is enriched in the mitochondria relative to the homogenate to about the same extent as are enzymes cytochrome *c* oxidase and succinate-cytochrome *c* reductase. Thus, if the percentage of adult homogenate protein found in the mitochondria is calculated on the basis of the cytochrome *c* oxidase, succinate-cytochrome *c* reductase and cardiolipin contents, one arrives at the values 33.6, 33.4 and 35.4% respectively. Similar computations for the percentage of foetal liver homogenate protein found in the mitochondria on the basis of cytochrome *c* oxidase and cardiolipin contents yield results of 19.3 and 16.9% respectively. All of these computations, employing conservative assumptions, suggest that 88–100% of the cardiolipin in both foetal and adult rat liver is located in the mitochondria. Cardiolipin can therefore be used as phospholipid marker of the mitochondrial membrane in both foetal and adult rat liver. It is of note that the overall phospholipid compositions of foetal and adult liver mitochondria are similar.

The change in cardiolipin concentration during the development of rat liver was used as another parameter to elucidate the biogenesis of mitochondria. Fig. 4 shows that the cardiolipin concentration/mg of liver homogenate protein in foetal animals (18–21 days of gestation) was about

Table 3. *Phospholipid composition in fractions of foetal and adult liver*

F, Foetal; A, adult

The values marked * are means of five separate determinations; all other values are the results of single experiments. In the case of the foetal determinations, these were made on livers of 21-day foetuses. n.d., Not detected.

	Phospholipids (μg of P/mg of protein)										Phospholipids (% of total)			
	Homogenate					Nuclear					Microsomal		Mitochondrial	
	F	A*	F	A*	F	A	F	A*	F	A*	F	A	F	A
Diphasphatidyl glycerol (cardiolipin)	0.10	0.21	0.088	0.13	0.0025	0.08	0.10	0.08	2.72	1.53	0.38	0.7	0.7	0.7
Phosphatidylethanolamine	0.38	0.77	0.203	0.38	1.88	1.93	1.43	1.53	3.83	3.83	23.8	23.0	21.9	23.0
Phosphatidylcholine	n.d.	n.d.	n.d.	n.d.	0.07	0.04	0.07	0.04	47.9	50.2	42.7	33.6	36.1	52.1
Phosphatidylglycerol	n.d.	n.d.	0.05	n.d.	n.d.	n.d.	0.01	n.d.	n.d.	n.d.	n.d.	1.6	n.d.	0.4
Phosphatidic acid	0.085	0.19	0.085	Trace	Trace	0.34	0.17	0.34	0.01	1.3	Trace	0.1	n.d.	0.1
Phosphatidylserine	n.d.	n.d.	0.015	0.015	n.d.	0.08	0.15	0.08	5.3	4.8	Trace	1.4	2.5	3.0
Lysophosphatidylethanolamine	0.11	0.22	0.015	0.015	Trace	0.48	0.11	0.48	6.8	5.6	Trace	0.6	2.2	0.7
Sphingomyelin	0.12	0.37	0.056	Trace	Trace	1.35	0.28	1.35	7.4	9.1	Trace	2.4	7.0	5.7
Phosphatidylinositol	0.04	0.08	0.051	Trace	Trace	0.25	0.08	0.25	2.5	1.9	Trace	6.6	9.2	11.5
Lysophosphatidylcholine	n.d.	n.d.	n.d.	0.005	n.d.	0.10	n.d.	0.10	2.5	1.9	Trace	1.3	1.2	2.2
Unidentified	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.8

Fig. 4. Cardiolipin content of mitochondria and homogenates of foetal and neonatal rats. The broken horizontal lines represent ± 1 s.d. from the mean of the adult values. NB, Newborn.

twice that in adults. In contrast similar cardiolipin concentrations were found in liver mitochondria from adult and foetal rats. A relatively elevated cardiolipin content in the 16–18 days of gestation was observed in both mitochondria and homogenates. From the ratios of homogenate to mitochondrial cardiolipin content (Fig. 4) the mitochondria of foetal liver constitute about half the proportion of total homogenate protein compared with the situation in adult liver.

If one analyses the ratio of respiratory-enzyme specific activities to cardiolipin concentration, the ratio is lower in foetal liver and markedly increases during development. The same pattern of increased ratio during development is observed in the homogenate and the mitochondria. The changes in specific activities of respiratory enzymes expressed on the basis of cardiolipin are quite similar to those expressed per mg of mitochondrial protein. This confirms that the respiratory-enzyme specific activities increase much more significantly during liver maturation than does the cardiolipin concentration.

Fig. 5 displays the results of changes in the concentration of mitochondrial phosphatidylethanolamine and phosphatidylcholine (lecithin) during the development of neonatal rat liver. Liver mitochondria from foetal and neonatal rats contain

Fig. 5. Mitochondrial phosphatidylcholine and phosphatidylethanolamine concentrations during development. ●, Phosphatidylcholine; ○, phosphatidylethanolamine.

approximately the same amounts of phosphatidylethanolamine and more phosphatidylcholine than do liver mitochondria from adults.

Morphology of mitochondria of foetal, neonatal and adult liver. Electron micrographs of sections of rat liver (Plate 1) and of purified mitochondrial pellets (Plate 2) were obtained from 20-day-old embryos and from 4-day-old, 15-day-old and adult rats. Mitochondria of the embryo and newborn were larger in width than those of the 15-day and adult livers, but no consistent changes in quantity or development of cristae were observed. Mitochondrial pellets also showed no evidence for greater development of cristae in livers of mature rats. Contamination in the pellets was slight, consisting of occasional glycogen-particle microbodies, lysosomes and platelets. Mitochondria are almost exclusively of the large type ascribed to hepatocytes.

DISCUSSION

In this study we have measured three parameters to follow the development of the inner mitochondrial membrane of the neonatal rat liver, namely the activities of several respiratory enzymes in the homogenate and purified mitochondria, the spectrophotometrically determined contents of the cytochromes in the mitochondria, and the cardiolipin content in both the homogenate and mitochondria of the developing liver. The data confirm previous reports that the total activity of rat liver respiratory enzymes increases markedly after birth (Dawkins, 1959; Lang, 1965). Adult values are reached 2–4 weeks *post partum*. The increase in respiratory-enzyme activity begins 2–3 days before birth for all the enzymes studied, except for cytochrome oxidase, in which activity declines until the last day of gestation and begins to rise only after birth.

The increase in the respiratory-enzyme activity

of the homogenate could be the result of one or all of several changes. These include an increase in the total mass of mitochondria within the liver, an increase in the amount of respiratory element per mitochondrion, and/or an increase in the activity of each of the mitochondrial electron-transfer units.

Evidence in favour of an increase in the mass of mitochondria present within the developing hepatocyte derives from two types of data. First, the cardiolipin content of the homogenate increases approximately twofold during this developmental period. These studies as well as those conducted previously (Fleischer *et al.* 1967; Parsons *et al.* 1967; Getz *et al.* 1968; McMurray & Dawson, 1969), have indicated that cardiolipin is a useful marker of mitochondrial mass, since most, if not all, of the hepatic cardiolipin is located within the mitochondrial membrane. Second, the ratio of homogenate to mitochondrial specific activities of respiratory enzymes suggests that during the course of development the proportion of hepatic protein made up of mitochondrial protein increases twofold. However, a twofold increase in mitochondrial mass is insufficient to account for the total increment in mitochondrial enzyme activity. The increasing specific activity of respiratory enzymes in mitochondria isolated from developing rat liver suggests qualitative changes in mitochondria at various stages of hepatic development. All four mitochondrial respiratory enzymes measured show lower specific activities in the purified foetal than in the purified adult mitochondria. Thus the relatively simple flavoprotein, succinate dehydrogenase and the more complex respiratory complexes, succinate-cytochrome *c* reductase, antimycin-sensitive NADH-cytochrome *c* reductase and cytochrome oxidase all have lower activities in foetal mitochondria.

It is noteworthy that several investigators (Dawkins, 1959; Mintz *et al.* 1967) have suggested the existence of qualitative differences between the mitochondria of foetal and adult rat liver. This conclusion is based on the assumption that during the course of preparation of mitochondria from foetal and adult rat liver, there is no differential activation of respiratory enzymes occurring as a preparative artifact. The similar activation of respiratory-enzyme activity in both foetal and adult rat liver mitochondria and homogenates lends support to this assumption.

If the observed changes in the respiratory-enzyme specific activities of isolated rat liver mitochondria do indeed represent true changes in enzyme activity, they could be the result of variations in the amount of inner mitochondrial membrane as a proportion of total mitochondrial protein. That this is not the explanation for the changes in specific activity of respiratory enzymes is suggested by two factors.

EXPLANATION OF PLATE I

Sections of intact rat liver, showing regions of hepatocytes containing mitochondria. (a) 20-day embryo; (b) 4 days old; (c) 15 days old; (d) adult. Magnification $\times 16000$.

EXPLANATION OF PLATE 2

Pellets of isolated rat liver mitochondria. (a) 20-day embryo; (b) 4 days old; (c) 15 days old; (d) adult. Magnification $\times 16000$.

First, there is no apparent difference in the content of the cytochromes, determined spectrophotometrically, per mg of mitochondrial protein. Second, the specific activities of the respiratory enzymes as expressed on the basis of cardiolipin, a presumed component of the inner mitochondrial membrane, reflects changes quite similar to those seen when these activities are expressed per mg of mitochondrial protein. Further, the electron microscopic studies show no evidence of a change in the proportion of cristae between foetal and adult rat liver mitochondria. This is in contrast with the situation in developing rat brain (Gregson & Williams, 1969) and in liver and skeletal muscle of hypothyroid animals treated with thyroid hormone (Gustafsson, Tata, Lindberg & Ernster, 1965; Gross, 1969; N. J. Gross & H. Swift, unpublished work).

Apart from a definite increase in respiratory-enzyme activities in the postnatal period, there is a suggestion of an asynchronous pattern of elevation of the activities of each of these respiratory enzymes. The asynchronous development of mitochondrial components could conceivably reflect the changing cell population in the neonatal liver during development, if one assumes that a different type of mitochondrion is present in hemopoietic cells. However, it is extremely unlikely that this is the sole explanation for the asynchrony of respiratory-enzyme development, since hemopoietic cells, though constituting a large proportion of foetal hepatic mass, nevertheless contain relatively few mitochondria compared even with foetal hepatocytes. Thus, the respiratory activity and cardiolipin content probably reflect mainly the hepatocyte composition.

The asynchronous enzyme development observed during development does not necessarily conflict with studies measuring the turnover of various mitochondrial components that have led to the hypothesis that at least one major subunit of the inner mitochondrial membrane turns over as a unit (Fletcher & Sanadi, 1961; Gross, Getz & Rabinowitz, 1969). Simultaneous assembly and degradation of such a unit is assumed. The inner membrane cytochromes, *c*, *b* and *a₃*, all turn over with the same half-life in rat heart and liver (Druyan, DeBernard & Rabinowitz, 1969; Aschenbrenner, Druyan & Rabinowitz, 1970). These studies were carried out under steady-state conditions and are valid only in the framework of the relatively long turnover times measured. They do not exclude the possibility of asynchronous assembly during such non-steady state conditions as are present in the rapidly growing foetal and newborn liver.

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