Hormone-Initiated Maturation of Rat Liver Mitochondria after Birth

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1. The injection of adrenaline, glucagon or cyclic AMP into foetal rats *in utero* initiates the maturation of energy transduction in rat liver mitochondria before birth. 2. The injection of the β -blocker, propranolol, prevents this maturation process. 3. The maturation of mitochondrial energy transduction is measured in terms of the increase in the respiratory control index and mitochondrial adenine nucleotide concentration. 4. It is postulated that the actions of the hormones, acting through cyclic AMP, affect glycogenolysis and glycolysis to give rise to transient localized high concentrations of ATP. 5. It is the ATP that acts as the molecular trigger, effecting mitochondrial maturation.

After birth, many physiological and biochemical changes take place in a newborn animal, enabling it to adapt to its new environment. Many of the regulatory functions previously carried out by the placenta and other maternal organs must be quickly assumed by the lungs, liver, kidneys and other organs of the neonate (Dawkins, 1966). In the liver the activity of several enzymes first becomes measurable on the first day after birth (the neonatal cluster of enzymes) (Greengard, 1971). In several instances the postnatal appearance of an enzyme has been shown to be dependent on the increase in the plasma concentrations of glucagon and/or adrenaline, two hormones that stimulate cyclic AMP formation in the liver by the interaction of their receptor complex with adenylate cyclase (Robison *et al.*, 1971). These hormones have been shown to act through cyclic AMP at different levels of control in the neonatal rat. In some instances they act at the transcriptional level (Yeung & Oliver, 1968), resulting in true enzyme induction, and at least one example is known where cyclic AMP acts at the translational level, controlling the termination step of protein synthesis in the neonatal rat liver (Oliver, 1973). Cyclic AMP is also known to stimulate glycogenolysis by causing the activation of a phosphorylase kinase, which in turn activates glycogen phosphorylase (Fisher *et al.*, 1970). Before birth, glycogen phosphorylase is almost completely in the inactive *b* form, whereas shortly after birth more than 80% of the glycogen phosphorylase is in the activated *a* form (Devos & Hers, 1974).

It has been proposed that adrenaline and glucagon are involved in the initiation of yet another process

at birth, namely the maturation of energy transduction in rat liver mitochondria (Pollak, 1977). The activation of glycogenolysis and glycolysis as a result of the cyclic AMP-mediated actions of these two hormones results in an increase in the cytoplasmic ATP concentration, which causes the hypercontraction of the mitochondrial inner membrane, leading to mitochondrial maturation (Pollak, 1977).

Mitochondria isolated from foetal rat liver possess a low respiratory control index (Hallman, 1971; Nakazawa *et al.*, 1973; Pollak, 1975), and their adenine nucleotide concentration is also significantly lower than at later developmental ages (Nakazawa *et al.*, 1973; Sutton & Pollak, 1978). After premature delivery by caesarean section 1 day before natural birth, maturation *in vivo* is already apparent after 30 min and is even more marked after 60 min, when the respiratory control index increases concomitantly with the increased concentrations of all three adenine nucleotides (Sutton & Pollak, 1978). This maturation is not due to protein synthesis *de novo* (Sandor & Pollak, 1976). After the rats have been in a humidicrib for 2 h, the mitochondrial adenine nucleotide concentration increases by more than 2-fold and keeps increasing until 5 days after birth (Sutton & Pollak, 1978).

An alternative mechanism for the triggering of mitochondrial maturation can also be proposed. It was suggested that the low energy-transducing activity of foetal rat liver mitochondria is due to hypoxia in foetal blood (Nakazawa & Nunokawa, 1977). This hypoxia is thought to cause the depletion of mitochondrial adenine nucleotides. At birth the increased oxygen tension to which the neonatal

liver is exposed causes mitochondrial maturation by increasing the mitochondrial adenine nucleotide concentrations and also their respiratory control indices (Nakazawa *et al.*, 1973).

The present series of experiments was devised to test these two hypotheses.

Experimental

Details of animals and incubation procedures in vivo

Random-bred Wistar rats from the University Animal House were used throughout the investigation. For experiments involving adult rat liver mitochondria, female Wistar rats weighing between 150 and 200 g were used. Foetal rats of known age were obtained from overnight (16 h) matings. In maturation studies, fetuses were delivered by caesarean section 1 day before normal birth and kept in a humidicrib at 35–37°C for up to 2 h.

The humidicrib was designed so that it could be provided with a stream of prewarmed air or N₂, as required. A flap-valve ensured that the gas pressure inside the humidicrib remained at atmospheric pressure.

Isolation of mitochondria

Mitochondria were isolated as described previously (Pollak, 1975) by a method especially devised to isolate mitochondria from foetal and neonatal rat liver. The isolation medium contained 250 mM-sucrose, 1 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.4, 1 mM-EDTA adjusted to pH 7.4 with NaOH and 0.2% bovine serum albumin.

Measurement of mitochondrial respiration and respiratory control index

State 3 and State 4 respiration and respiratory control index were measured by using a Rank (Bottisham, Cambs., U.K.) oxygen electrode in a volume of 2 ml. The incubation mixture was main-

tained at 30°C and contained 25 mM-Hepes buffer, pH 7.4, 5 mM-sodium phosphate buffer, pH 7.4, 5 mM-MgCl₂, 50 mM-KCl and 0.4 ml of isolation medium (see above), including the volume of the isolation medium in which the mitochondria were suspended. Sodium succinate (5 mM) was used as substrate.

Analytical methods

Adenine nucleotides were determined by the very sensitive luciferase assay of ATP, with the use of conversion enzymes for AMP and ADP as described by Sutton & Pollak (1978).

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (fraction V) [Sigma (St. Louis) Chemical Co.] as standard.

Results and Discussion

Correlation between adenine nucleotide concentration and respiratory control index

The low respiratory control index of foetal compared with adult rat liver mitochondria (Table 1) may suggest that these mitochondria are loosely coupled and/or contaminated with other cellular organelles. It must therefore be emphasized that the cell-fractionation procedure used for the isolation of perinatal rat liver mitochondria has been especially devised to avoid these problems (Pollak, 1975). The results presented in Table 1 show clearly that the low respiratory control index is due to the low rate of State 3 respiration and not to an increased State 4 respiration, as would be the case in uncoupled or loosely coupled or 'leaky' mitochondria. Furthermore the additions of uncouplers such as 2,4-dinitrophenol or carbonyl cyanide *m*-chlorophenylhydrazine causes increases in the respiratory rate comparable with those observed in well-coupled adult rat liver mitochondria (J. K. Pollak, unpublished work).

The contamination of mitochondrial fractions

Table 1. *Comparison of the respiration of foetal and adult rat liver mitochondria*

Mitochondria were isolated from the livers of fetuses taken less than 1 day before normal birth, or from female adult rats, and the respiratory control indices determined, as described in the Experimental section. Mean values $\pm$ S.E.M. are given for the numbers of experiments indicated. Incubations with or without ATP were compared by a dependent *t* test: ns, not significant.

	Presence of ATP	Respiration rates (ng-atoms of oxygen/min per mg of protein)		Respiratory control index
		State 3	State 4	
Foetal (<i>n</i> = 28)	—	98 $\pm$ 4	54 $\pm$ 2	1.9 $\pm$ 0.1
	+	154 $\pm$ 7 <i>P</i> < 0.001	60 $\pm$ 2 <i>P</i> < 0.001	2.6 $\pm$ 0.1 <i>P</i> < 0.001
Adult (<i>n</i> = 5)	—	297 $\pm$ 12	55 $\pm$ 4	5.5 $\pm$ 0.4
	+	351 $\pm$ 33 ns	67 $\pm$ 4 ns	5.3 $\pm$ 0.4 ns

isolated from foetal rat liver with microsomal or lysosomal fractions is minimal; this has been demonstrated by the use of marker enzymes (Pollak & Munn, 1970; Pollak, 1975).

The respiratory control index of foetal rat liver mitochondria increases when ATP is added to suspensions of mitochondria *in vitro* (Hallman, 1971; Nakazawa *et al.*, 1973; Pollak, 1975; Sandor & Pollak, 1976; Sutton & Pollak, 1978). The results shown in Table 1 indicate that the action of ATP is on State 3 respiration, State 4 respiration being only marginally affected. ATP has no effect on the respiratory control index of adult rat liver mitochondria (Table 1). The effect of ATP on foetal rat liver mitochondria has been shown not to depend on the provision of energy by its hydrolysis, as the addition of ATP plus oligomycin or the addition of adenyllyl imidodiphosphate (an analogue of ATP which cannot be hydrolysed by ATPase) causes the same maturation effect (Pollak, 1975).

In this respect foetal rat liver mitochondria are very different from brown-fat mitochondria; the latter have been shown to be loosely coupled (Hohurst & Rafael, 1968). The addition of ATP and particularly GTP or GDP to brown-fat mitochondria produces tighter coupling, but this is an energy-dependent reaction (Flatmark & Pederson, 1975), unlike the situation in foetal rat liver mitochondria (Pollak, 1975). GTP has no effect on the respiratory control index of foetal rat liver mitochondria (Pollak, 1975), in spite of the fact that it can be hydrolysed by these mitochondria (L. Olson & J. K. Pollak, unpublished observation).

In a detailed study that examined the changes in the adenine nucleotide concentration of rat liver and rat liver mitochondria it was shown that a positive correlation exists between the mitochondrial adenine nucleotide concentration and the respiratory control index (Sutton & Pollak, 1978). In the present

investigation it is shown that the manipulation of perinatal rats, obtained by caesarean section 1 day before natural birth, yields results that again demonstrate the correlation between the mitochondrial adenine nucleotide concentration and respiratory control index (Table 2). Neonatal rats are capable of surviving for limited periods in an atmosphere of N₂; thus if neonatal rats are placed for 30 min into a humidicrib in an atmosphere of N₂, the mitochondrial adenine nucleotide content and the respiratory control index increase only marginally (Table 2). On the other hand, in air, normal maturation in terms of increases in the adenine nucleotide concentration and respiratory control index takes place. If the exposure to anaerobic conditions is followed with a period during which air is admitted to the humidicrib, then the mitochondrial adenine nucleotide concentration and the respiratory control index increase (Table 2). Reversing this treatment by placing the pups for 30 min in an atmosphere of air followed by 30 min in an atmosphere of N₂ decreases both parameters (Table 2). Therefore it may be concluded that at this developmental age the maturation process is freely reversible.

Under different experimental conditions when the mitochondrial total adenine nucleotide concentrations change significantly, the proportions of the three individual adenine nucleotides remain fairly constant (Table 2). The proportions of the three adenine nucleotides in isolated mitochondria should not be regarded as having any metabolic significance, since 60–90 min will have elapsed between the death of the rats and the isolation of the purified mitochondrial fractions.

At this stage it should be pointed out that one of the reasons for regarding the changing mitochondrial concentration as a maturation step is the fact that there is a small but steady increase in mitochondrial adenine nucleotides during the

Table 2. *Effect of exposing premature delivered rats to 100% N₂ on mitochondrial adenine nucleotides and respiratory control indices*

These data represent mean values $\pm$ S.E.M. for three experiments in each of which two litters of rats were delivered less than 1 day prematurely and kept in a humidicrib for 30 or 60 min in air or in 100% N₂ as indicated. Mitochondria were prepared from the livers of rats of each treatment, and the respiratory control indices measured and the adenine nucleotides assayed as described in the Experimental section. The adenine nucleotide concentrations are expressed as nmol/mg of mitochondrial protein. The correlation coefficient between mitochondrial ATP + ADP + AMP and respiratory-control-index values is 0.97 ($P < 0.01$).

	Respiratory control index	ATP	ADP	AMP	ATP + ADP + AMP
0 min	1.5 $\pm$ 0.1	0.75 $\pm$ 0.08	0.54 $\pm$ 0.10	0.51 $\pm$ 0.08	1.80 $\pm$ 0.20
30 min air	2.2 $\pm$ 0.2	0.85 $\pm$ 0.17	0.62 $\pm$ 0.18	0.84 $\pm$ 0.25	2.31 $\pm$ 0.59
30 min N ₂	1.5 $\pm$ 0.2	0.76 $\pm$ 0.20	0.68 $\pm$ 0.08	0.58 $\pm$ 0.14	2.02 $\pm$ 0.36
60 min air	2.5 $\pm$ 0.1	0.90 $\pm$ 0.11	0.70 $\pm$ 0.11	1.08 $\pm$ 0.15	2.68 $\pm$ 0.36
30 min N ₂	2.0 $\pm$ 0.2	0.87 $\pm$ 0.12	0.52 $\pm$ 0.14	0.73 $\pm$ 0.14	2.13 $\pm$ 0.38
+ 30 min air					
30 min air	1.7 $\pm$ 0.1	0.85 $\pm$ 0.18	0.62 $\pm$ 0.15	0.50 $\pm$ 0.14	1.97 $\pm$ 0.47
+ 30 min N ₂					

last 5 days of gestation, followed by a doubling of the adenine nucleotide concentration during the first 2 h after birth. During the period of 5 days before birth to 5 days after birth the mitochondrial adenine nucleotide content increases by 7-fold ($1.49\text{--}11.01\ \mu\text{mol/g}$ of mitochondria protein) (Sutton & Pollak, 1978). In contrast, the adenine nucleotide content of the liver during that period remains virtually constant ($2.58\text{--}2.93\ \mu\text{mol/g}$ of liver) (Sutton & Pollak, 1978). The protein concentration per g of liver increases by more than 4-fold during this period (R. Sutton & J. K. Pollak, unpublished observation).

Involvement of adrenaline and glucagon in mitochondrial maturation

If adrenaline tartrate is injected intraperitoneally into foetal rats *in utero* and 60 min is allowed to elapse before the pups are delivered by caesarean section, the mitochondria isolated from the livers of these pups have a significantly higher concentration of total adenine nucleotides and also exhibit a higher respiratory control index than the mitochondria isolated from non-injected littermates or littermates

that had been injected with 0.9% NaCl (Table 3). The same effect is also observed if the foetal rats are injected *in utero* with either glucagon or dibutyryl cyclic AMP (Table 3).

The non-injected littermates or the ones that had been injected with 0.9% NaCl give results that are statistically indistinguishable from each other; consequently for statistical analysis the results of the two types of controls (non-injected or NaCl-injected) are combined and compared with the hormone- or dibutyryl cyclic AMP-injected pups in independent *t* tests (Table 3). In addition, since the effects of all three treatments (adrenaline, glucagon and dibutyryl cyclic AMP) are really dependent on the cellular effects of cyclic AMP, it was considered justifiable to pool the adrenaline, glucagon and dibutyryl cyclic AMP data and compare these with the pooled non-injected and NaCl-injected controls (Table 3). The results presented in Table 3 indicate that the cyclic AMP-mediated actions cause the maturation of rat liver mitochondria *in utero*, as demonstrated by the increased mitochondrial total adenine nucleotide concentrations and raised respiratory control indices.

Table 3. *Effects of adrenaline, glucagon and dibutyryl cyclic AMP injection in utero on the mitochondria of foetal liver* Adrenaline tartrate (Parke Davis and Co.) ($10\ \mu\text{g}$), glucagon (Eli Lilly and Co.) ($10\ \mu\text{g}$) or dibutyryl cyclic AMP (Sigma Chemical Co.) ($50\ \mu\text{g}$) was injected in a $5\ \mu\text{l}$ volume of 0.9% NaCl intraperitoneally into four foetuses *in utero*. Four other foetuses were not injected, and another four foetuses were injected with $5\ \mu\text{l}$ of 0.9% NaCl. If less than 12 pups were present in the two horns of the uterus, either non-injected or the NaCl-injected pups served as controls. The pregnant rats (1 day before term) were anaesthetized with ether throughout the operation, during which the uterus was lifted from the body cavity and placed on a warm tray at 37°C . After the injections, the uterus was replaced into the peritoneal cavity, the abdominal wall sewn up with nylon thread and the skin clamped together with Michelle clips. After the operation the rat was placed into a warm cage and was usually quite active during the 60 min allowed to elapse before it was killed by dislocation of the neck. After caesarean section the pups were all checked for viability and possible haematomas due to the injection, and any damaged or non-viable foetuses were discarded (this occurred only rarely). Mitochondria were isolated, and adenine nucleotides and respiratory control indices determined as described in the Experimental section. Results are presented as mean values $\pm$ S.E.M. for the numbers of experiments given in parentheses. Total adenine nucleotides are expressed as nmol/mg of protein, and State 3 and State 4 rates of respiration are in ng-atoms of oxygen/min per mg of protein. In each case the hormone- or dibutyryl cyclic AMP-treated pups were compared with both control groups by using an independent *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Treatment	ATP + ADP + AMP	Respiratory control index	Rates of respiration	
			State 3	State 4
Glucagon (3)	$3.23 \pm 0.1^{**}$	$2.0 \pm 0.1^*$	98 ± 11	48 ± 2
Non-injected control (2)	2.43	1.6	83	52
0.9% NaCl (3)	2.57 ± 0.1	1.7 ± 0.2	91 ± 6	53 ± 2
Adrenaline (3)	$3.16 \pm 0.02^*$	$2.1 \pm 0.1^{**}$	117 ± 17	55 ± 6
Non-injected control (3)	2.56 ± 0.05	1.6 ± 0.1	82 ± 13	52 ± 5
0.9% NaCl (1)	2.66	1.7	94	55
Dibutyryl cyclic AMP (5)	$2.83 \pm 0.2^*$	$2.1 \pm 0.1^*$	118 ± 13	57 ± 4
Non-injected control (4)	2.46 ± 0.1	1.8 ± 0.01	104 ± 11	59 ± 6
0.9% NaCl (1)	2.09	1.5	95	63
Dibutyryl cyclic AMP + glucagon + adrenaline (11)	$3.03 \pm 0.1^{***}$	$2.1 \pm 0.2^{***}$	$112 \pm 8^*$	52 ± 2
Non-injected control (9)	2.49 ± 0.04	1.7 ± 0.04	92 ± 7	54 ± 3
0.9% NaCl (5)	2.43 ± 0.1	1.7 ± 0.1	93 ± 3	55 ± 2

The participation of adrenaline in the maturation of foetal rat liver mitochondria was confirmed further by an additional series of experiments, in which the β -blocker propranolol was injected intraperitoneally into a pregnant rat 2h before the removal of the pups by caesarean section. Propranolol has been shown to pass through the bovine placental barrier (Van Petten & Willes, 1970), and it was predicted that its presence in the foetal circulation would prevent the normal mitochondrial maturation 60min after birth if adrenaline is responsible for triggering off this process. This experiment requires that the litters of two pregnant rats are compared, one pregnant rat being injected with propranolol, the litter of the other non-injected rat being processed in parallel and acting as a control (non-injection was considered to be the more stringent control, avoiding any stresses other than the ones occurring at parturition). Mitochondria were isolated from some pups of both litters at 0min, and the remaining pups were maintained in the humidicrib for 60min. The results are presented in Table 4 and demonstrate that mitochondria isolated from pups obtained from the propranolol-injected rats, which were kept in the humidicrib for 60min, have not matured, as evidenced by their low adenine nucleotide content and low respiratory control index, which are statistically indistinguishable from the 0min controls of either the propranolol-injected or the non-injected control litters (Table 4). On the other hand, the mitochondria isolated from pups of the control rats that were maintained for 60min in the humidicrib show an increase in both the mitochondrial adenine nucleotide content and their respiratory control index (Table 4). The results presented in Tables 3 and 4 are therefore compatible with the hypothesis that cyclic AMP triggers off mitochondrial maturation by switching glycogen

synthesis off and turning glycogenolysis on, with the result that transient localized high concentrations of ATP cause a hyperconcentration through an interaction with -SH groups on the inner mitochondrial membrane (Pollak, 1975; Sandor & Pollak, 1976; Pollak & Baum, 1977). The matured mitochondria in turn will contribute to the maintenance of a raised ATP concentration within the hepatocyte, ensuring that the mitochondria remain in a state in which they carry out effective oxidative phosphorylation.

At first sight the experiments presented in Table 2 also appear to provide evidence for the hypothesis that an increased oxygen tension experienced by the neonate is the key to the maturation of rat liver mitochondria. No actual measurements exist of the oxygen tension of foetal rat liver, although it may be considered 'functionally' anoxic, since the [lactate]/[pyruvate] ratio before birth is 223, whereas in the well-oxygenated 1-day-old rat this ratio decreases to 13.5 (Phillippidis & Ballard, 1969). The failure of foetal rat liver mitochondria to oxidize pyruvate at a substantial rate could be due to a lack of oxygen, a low activity of pyruvate dehydrogenase or an inherent limitation of the rate of electron transport.

There are no compelling reasons to assume that the injection of adrenaline, glucagon or dibutyryl cyclic AMP increases the oxygen tension in the liver *in utero*, nor conversely are there cogent reasons to assume that propranolol lowers the oxygen tension in the neonatal rat. Hence the present series of experiments indicates that oxygen itself is not the trigger for the maturation of the energy-transducing activity of foetal mitochondria, but rather it is required to maintain mitochondrial ATP production through oxidative phosphorylation once mitochondrial maturation has been hormonally triggered.

Table 4. *Inhibition of mitochondrial maturation by propranolol*

Pregnant rats were injected intraperitoneally, 1 day before giving birth, with 10mg of propranolol hydrochloride (ICI)/kg in 0.9% NaCl (10mg/ml). After 2h the pups were delivered by caesarean section. Pups obtained from non-injected rats at the same stage of pregnancy served as controls. Mitochondria were isolated either from the livers of newly born pups (0min) or from pups maintained in a humidicrib for 60min in an atmosphere of air. The adenine nucleotide and protein concentrations and respiratory control indices were determined as described in the Experimental section. Results are mean values $\pm$ S.E.M. from three experiments. The units are as for Table 3. The respiratory control index of the mitochondria from the control neonates kept in a humidicrib for 60min differs significantly ($P < 0.05$), as assessed by independent *t* tests, from the respiratory control index of the mitochondria from both 0min control fetuses and the 60min propranolol-treated neonates. The liver mitochondria from propranolol-treated neonates kept in a humidicrib for 60min do not differ significantly from those of their littermates taken at 0min.

Mitochondria	ATP + ADP + AMP	Respiratory control index	Rate of respiration	
			State 3	State 4
Control, 0min	2.41 $\pm$ 0.14	1.6 $\pm$ 0.1	85 $\pm$ 7	52 $\pm$ 3
Propranolol, 0min	2.76 $\pm$ 0.22	1.8 $\pm$ 0.1	96 $\pm$ 9	55 $\pm$ 3
Control, 60min	3.54 $\pm$ 0.13	2.1 $\pm$ 0.1	106 $\pm$ 7	50 $\pm$ 4
Propranolol, 60min	2.38 $\pm$ 0.21	1.7 $\pm$ 0.2	86 $\pm$ 4	49 $\pm$ 1

It may be argued that cyclic AMP causes lung maturation by enhancing surfactant production in alveolar type II cells (Hallman, 1977), and that the more matured lungs contribute to tissue oxygenation, which affects mitochondrial maturation *in utero*. Such an argument can be discounted, as the fluid-filled lungs do not become functional until after parturition, in spite of any increase in surfactant material that may be present. Therefore the maturation of mitochondria *in utero* cannot be explained in terms of greater oxygenation brought about by lung maturation.

It is therefore proposed that the chain of events leading to the maturation of rat liver mitochondria after parturition commences with the stress experienced during parturition as the foetus passes down the birth-canal (or the stress set up by caesarean section), stimulating catecholamine secretion (DiMarco *et al.*, 1976), which in turn enhances glucagon secretion (Girard *et al.*, 1974). The catecholamine and glucagon secretion leads to an increased rate of glycogenolysis in the liver due to the activation of glycogen phosphorylase by cyclic AMP. The released catecholamines also cause a decrease in the insulin release by the pancreas (Robison *et al.*, 1968). This change in the hormone balance causes glycogen synthase to become phosphorylated and hence inactivated (Rubin & Rosen, 1975). As a result glycogen synthesis ceases and glycogenolysis is activated. This represents a marked change in the direction of carbohydrate metabolism, as up to that point in development the foetal liver is almost entirely concerned with the accumulation of glycogen (Devos & Hers, 1974).

Before birth the [ATP]/[AMP] ratio in the liver is significantly lower than 60 min after birth [5 as opposed to 33 (Sutton & Pollak, 1978); 2.8 as opposed to 15.7 (Ballard, 1971)]; this ratio controls phosphofructokinase, the main controlling step of the glycolytic sequence, which is activated by the low [ATP]/[AMP] ratio (Atkinson, 1966) existing in foetal rat liver. Thus glycogenolysis would be followed in the short term by rapid glycolytic activity, which could cause transient localized increases in the ATP concentration and mitochondrial hypercontraction (Pollak, 1975). Mitochondrial maturation therefore may be the result of an increased hepatic ATP concentration which has been shown to rise by 51% within 30 min after birth (Sutton & Pollak, 1978).

This study does not rule out other possible means of action of adrenaline and glucagon on the liver mitochondria. However, earlier attempts to substitute cyclic AMP and cyclic GMP for ATP to effect the maturation of isolated mitochondria *in vitro* were unsuccessful (J. K. Pollak, unpublished results). Glucagon, when injected, has been shown to increase mitochondrial adenine nucleotide concen-

trations and proticity in adult rat liver (Prpic *et al.*, 1978; Halestrap, 1978), and also enhances pyruvate entry into mitochondria (Halestrap, 1978; Tith-eradge & Coore, 1976). Halestrap (1978) has suggested that in the adult rat glucagon exerts its effects on mitochondria via cyclic AMP to cause phosphorylation of a protein by means of a protein kinase. The fact that in isolated foetal rat liver mitochondria ATP or adenylyl imidophosphate will cause an increase in the respiratory control index (Pollak, 1975) suggests that the cyclic AMP action in foetal rat liver is different from the one described by Halestrap (1978) in adult liver.

Both adrenaline and glucagon have been shown to cause a rapid enhancement of cyclic AMP concentrations in the liver, when injected intraperitoneally into 2-day-old rats (DiMarco & Oliver, 1978). This rapid increase in the concentration of liver cyclic AMP, most of which is dissipated 5 min after injection, tends to support the concept that mitochondrial maturation in the perinatal rat requires only to be triggered.

The evidence presented in this paper is compatible with the hypothesis (Pollak, 1977), that adrenaline and glucagon, through cyclic AMP, trigger an increase in cytoplasmic ATP (Sutton & Pollak, 1978), which in turn effects the hypercontraction of mitochondria (Pollak, 1975; Sandor & Pollak, 1976), an increased mitochondrial adenine nucleotide content (Sutton & Pollak, 1978) and a raised respiratory control index (Pollak, 1975; Sutton & Pollak, 1978) and therefore mitochondrial maturation. These matured mitochondria would in turn further increase the cytoplasmic [ATP]/[ADP] ratio, bringing about the maturation of other mitochondria.

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