Levels of Cytochromes in Heart, Liver, Kidney and Brain in the Developing Rat

Cytochromes b, c_1, c and aa_3 are the components in the terminal portion of the mitochondrial respiratory chain. The concentrations of these heme-proteins are individual for a given tissue[1] and vary according to the hormonal[2] or functional[3] status of the organism. It has been reported that in the liver and heart mitochondria of the rat, several components of the respiratory chain and the activities associated with oxidative phosphorylation increase postnatally[4].

In this study individual respiratory chain cytochromes were assayed in rats of various ages ranging from 20-day fetuses to adults. The contents of cytochromes increased shortly after birth. In brain and kidney, both organs that are immature at birth, the contents of cytochromes increased markedly even after the first postnatal week. The changes observed were due partly to an increase in the concentration of cytochromes in the mitochondria, partly to increase in mitochondrial protein in individual organs. The rise in the content of cytochrome c, which is synthesized outside the mitochondria, preceded that of the membrane-bound cytochromes, which are dependent on intramitochondrial protein synthesis.

Experimental. Rats of the Sprague-Dawley strain were used. Cytochromes in tissue homogenates were assayed within less than 2 h of decapitation. In exceptional cases the organs were stored at −80°C before the assays. The cytochrome spectra were measured with a laboratory-built dual wavelength spectrophotometer. The assay medium contained 50 m*M* *Tris*-Cl (pH 7.4), 85 m*M* KCl, 1 m*M* EDTA, 3 μ*M* rotenone, 0.1 m*M* malonate and 0.1% sodium deoxycholate. The reduction of cytochromes was accomplished by addition of 1 m*M* KCN (for cytochromes aa_3, c and c_1) or 2 μg/ml antimycin (A) (for cytochrome b). Additional details of the assay procedure are described elsewhere[5]. Heart, liver and kidney mitochondria were isolated as described earlier[5]. Brain mitochondria were obtained by the method of LØVTRUP and ZELANDER[6]. Protein was measured by a modification of the biuret method, with bovine serum albumin as standard[4]. Dry weight was calculated by incubating the homogenized samples at 80°C for 17 to 20 h. The dry weight of the assay medium was subtracted.

Results and discussion. The age of the rats ranged from 20-day fetuses to young adults, and the cytochrome contents of brain, heart, kidney and liver were assayed. As shown in the Figure, the concentrations and the developmental patterns of these proteins are specific for each tissue. However, in each case the concentrations of cytochromes increased shortly after birth. Furthermore, in brain the concentrations of cytochromes approximately doubled between the 8th and 30th postnatal days, while in kidney the cytochrome levels increased continuously by 3- to 4-fold during the developmental period studied.

As a check on whether the increase is due to a change either in mitochondrial protein or in the cytochrome contents of the mitochondria, the amounts of mitochondrial protein per g dry weight were calculated according to SCHOLLMEYER and KLINGENBERG[7]. This method is based on assays of the contents of the mitochondrial marker both in the total homogenate and in the isolated mitochondria. The purity of heart and liver mitochondria

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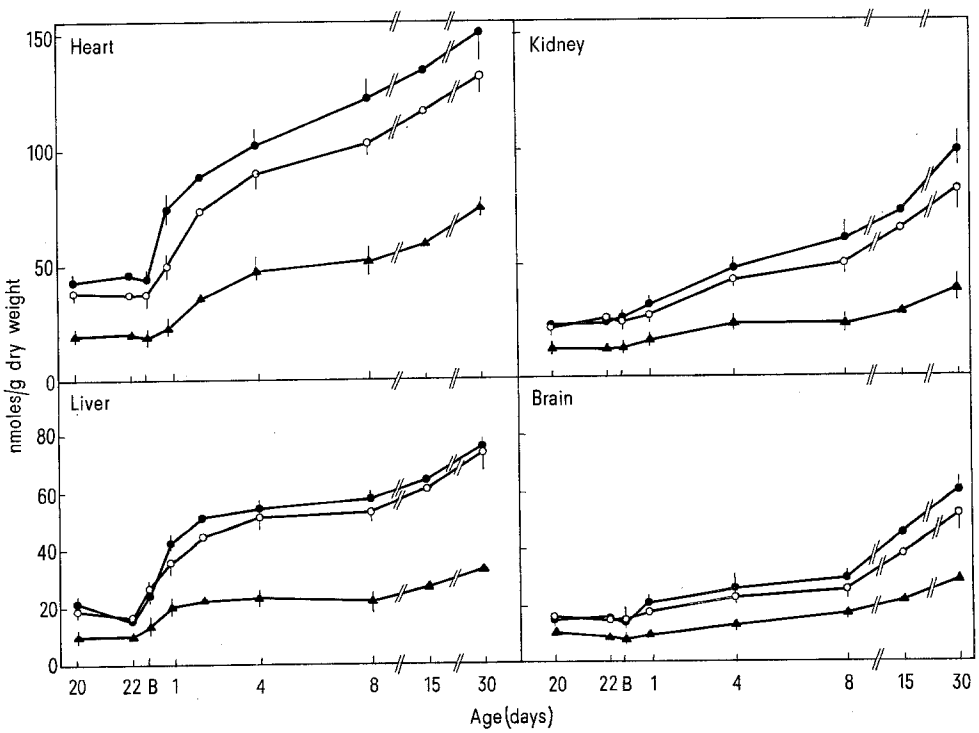

The contents of cytochromes $c+c_1$ (-●-), aa_3 (-○-), and b (-▲-) in the tissues of developing rat.

Table I. The amounts of mitochondrial protein and cytochrome aa_3 at different stages of development

	Heart	Kidney	Liver	Brain
Cytochrome aa_3 (nmoles/g dry wt.)				
A	36 (1.0)	26 (1.0)	17 (1.0)	14 (1.0)
B	89 (2.5)	42 (1.6)	52 (3.1)	23 (1.6)
C	135 (3.7)	99 (3.8)	80 (4.7)	53 (3.8)
Mitochondrial protein (mg/g dry wt.)				
A	159 (1.0)	128 (1.0)	130 (1.0)	145 (1.0)
B	231 (1.5)	—	212 (1.6)	—
C	356 (2.2)	296 (2.3)	305 (2.3)	178 (1.2)
Total protein (mg/g dry wt.)				
A	821 (1.0)	790 (1.0)	490 (1.0)	622 (1.0)
B	817 (1.0)	—	791 (1.6)	—
C	827 (1.0)	831 (1.1)	843 (1.7)	684 (1.1)

Mitochondrial protein was calculated as follows:

$$\frac{\text{cyt } aa_3 \text{ (nmoles/mg tissue prot.)}}{\text{cyt } aa_3 \text{ (nmoles/mg mitoch. prot.)}} \times \text{total protein (mg/g dry wt.)}$$

A, Fetus 22 days; B, Newborn 4 days; C, Adult 3–5 months.

Table II. Ratios of mitochondrial cytochromes c, aa_3, b and c_1 in rat heart during development

Age	c/aa_3	c/b	c/c_1
A, Fetus 22 days	0.96 ± 0.13 [a]	1.72 ± 0.09	1.45 ± 0.20
B, Newborn 20 h	1.35 ± 0.10	2.17 ± 0.19	1.96 ± 0.31
C, 30 days	0.85 ± 0.09 [b]	1.60 ± 0.12 [a]	1.48 ± 0.22

The results are the mean of 4 experiments $\pm$ S.E.M. [a] Significant difference ($p < 0.05$) as compared to B. [b] Significant difference ($p < 0.01$) as compared to B.

obtained from fetus and adult was tested by electron microscopy. These preparations contained well-preserved mitochondria and only small amounts of mitochondrial membranes. As shown in Table I, in each organ studied the concentration of mitochondrial protein in the tissue was greater in adults than in fetuses. However, in liver and brain the increase in the cytochrome contents of the tissue was largely due to a qualitative change in the mitochondria themselves.

There is evidence that the neonatal increase in ambient oxygen tension triggers the mechanism that leads to the increase in the components of the respiratory chain[4]. The assays shown here are in agreement with this suggestion. However, it is evident that oxygen tension is not the sole factor that regulates the contents of the respiratory chain during development. In brain and kidney the contents of cytochromes increased markedly even after the 8th postnatal day. During this period several enzymes that are needed for the functional maturation of the respective organs increase in activity (e.g. Na^+ and K^+-activated ATP phosphohydrolase in brain[8], carbonic anhydrase and glutaminase in kidney[9]).

The possibility that the increases in cytochromes after the 8th postnatal day are due to a change in the representation of different cell lines (e.g. neurons and glia in brain) cannot be excluded. On the other hand, there is considerable evidence that in the liver the rapid increase in the contents of functional respiratory chain components during the first neonatal day occurs on membranes existing before birth[10].

In Table II the ratios of cytochromes c, aa_3, b and c_1 in heart are presented. The ratios between cytochromes vary during development; in newborn rat the concentration of cytochrome c is high in respect to the other cytochromes. Moreover, the increases in the absolute amounts of cytochrome c in heart during the 22nd fetal day, 1st postnatal day and 30th postnatal day were 47, 122 and 1.5%, respectively. Therefore, it seems that during the rapid phase of increase of the respiratory chain components, cytochrome c increases in content somewhat earlier than the other cytochromes. The same tendency is also seen in other tissues of the rat.

Cytochrome c, which is associated with the inner membrane, is synthesized outside the mitochondria[11]. On

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the other hand, the cytochromes bound to the inner membrane need both intra- and extramitochondrial protein synthesis for their formation[12]. After birth intramitochondrial protein synthesis is rate-limiting for the formation of cytochrome aa_3[5]. The changes in the proportions of the respiratory chain cytochromes during development possibly reflect a difference in the factors that control the formation of these components (but see ref. 1). It is also possible that this phenomenon is due to differences in the assembly of various cytochromes. The latter alternative would support the view that mechanisms that regulate the rate of both intra- and extramitochondrial protein synthesis are somehow coupled to each other.

Zusammenfassung. Der Gehalt an Cytochromen ($c+c_1$, aa_3 und b) in Herz, Leber, Gehirn und Niere der Ratte wurde bestimmt. Ihre Vermehrung nach der Geburt erwies sich in den Geweben als relativ individuell. Die Vermehrung des ausserhalb der Mitochondrien synthetisierten Cytochromen c ging den übrigen, von der intramitochondrialen Proteinsynthese unabhängigen Cytochromen voraus.

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Absence of Naturally Occurring Coronary Atherosclerosis in Squirrel Monkeys (*Saimiri sciurea*) Treated with Chondroitin Sulfate A

Previous studies indicate that administration of chondroitin sulfates, which are widely distributed in mammalian, fish and fowl connective tissues, have an inhibitory effect on the development of experimentally induced atherosclerosis. KURITA[1] in 1955 reported that i.v. injections of 5 mg/kg of body weight daily of chondroitin sulfate C inhibited atherosclerosis in cholesterol-fed rabbits. OHDOI[2] found that sodium chondroitin sulfate inhibited the formation of atheromatous aortic lesions in cholesterol-fed cockerels when administered orally at a level of 20 mg/kg of body weight per day. MURATA[3] noted that daily i.v. injections of 5 mg/kg of body weight of a chondroitin polysulfate prepared by sulfation of chondroitin from shark cartilage reduced the severity of cholesterol-induced atherosclerosis in rabbits. MORRISON et al.[4] observed that when chondroitin sulfate A (CSA) was administered for 9 months at a level of 10 mg daily by s.c. injection to squirrel monkeys (*Saimiri sciurea*) fed a diet consisting of 1.5% cholesterol, 20% butter and 78.5% ground Purina Monkey Chow, the severity of atheromatous aortic lesions was substantially less than that of animals fed a similar diet which did not receive the chondroitin sulfate A treatment. More recently, MORRISON et al.[5] found that orally administered chondroitin sulfate A was highly effective in reducing the incidence and severity of coronary atherosclerosis in x-irradiated cholesterol-fed rats and in preventing the occurrence of aortic and coronary athero-arteriosclerotic lesions in rats fed a hypervitaminosis D atherogenic diet[6].

Available data indicate that the adult squirrel monkey as a sub-human primate is unique among a number of animal species studied for its development of a high incidence of naturally occurring aortic and coronary atherosclerotic lesions similar in many respects to those in adult man[7]. These lesions although macroscopically rare in younger animals, occur with increasing frequency and severity with advancing age as in humans. They are especially pronounced and most marked in severity and number in the coronary arteries of the basal section of the heart as has been found in human subjects at necropsy[8,9]. In the present experiment it was observed that coronary atherosclerotic lesions were completely absent both microscopically and macroscopically in the basal section of the heart of $7^1/_2$- to 8-year-old squirrel monkeys administered daily i.m. injections of CSA for 90 days preceding necropsy in contrast to their presence in squirrel monkeys of similar age administered physiologic saline solution.

Eight squirrel monkeys of approximately $7^1/_2$ to 8 years of age were employed in the present experiment. The males averaged 727 g (range 649 to 818 g) and the females 586 g (range 534 to 675 g) in body weight. The animals were obtained at approximately $2^1/_2$ to 3 years of age as judged by their weight, sexual development and dentition and were flown from a monkey compound in Leticia, Columbia, to our laboratory where they were fed a diet of Purina Monkey Chow and water ad libitum. Twice weekly they were fed fresh fruit such as grapes, apples, oranges and bananas. For 5 years they were kept on the above regime during which they maintained a good state of health with minimal changes in body weight. The animals were weighed once monthly during this period.

At the outset of the experiment the squirrel monkeys were divided into 2 groups: Group I controls consisted of 3 females and 1 male; Group II consisted of 2 males and 2 females. Weights were comparable. Group I was administered daily i.m. injections (6 days per week) of sterile physiologic saline solution; Group II was administered daily i.m. injections (6 days per week) of a sterile solution of 20 mg chondroitin sulfate A (CSA) dissolved in physiologic saline solution and with the pH adjusted to 7.0. Each injection consisted of 0.5 ml of solution, i.e., either physiologic saline solution or CSA solution. The identical dietary regime was followed as in the past 5 years.

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