METABOLISM OF THE MOUSE PLACENTA

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Most of the studies of placental metabolism (Wang & Hellman, 1943; Page, 1948; Hellman, Harris & Andrews, 1950; Villee, 1953; MacKay, 1958; Friedman & Sachtleben, 1960; Baird & McDonald, 1964) have involved tissue slice preparations using Warburg's apparatus. In an isolated study by Villee & Hagerman (1953), homogenates of human placentae were prepared and their O₂ utilization was tested using glucose, pyruvate, acetate, citrate, α -ketoglutarate, succinate, malate and oxalacetate as substrates.

In the present study, an attempt was made to isolate mitochondria from mouse placentae at different stages of gestation and to study their metabolic activity by a polarographic method.

The experiment involved the use of 195 albino mice, 12 to 19 days pregnant. Animals at the same stage of pregnancy were killed by cervical dislocation, the placentae were removed and the mitochondria were prepared by a method based on the one described by Lardy & Wellman (1952). The placentae were placed in ice-cold isolation medium containing 0.25 M-sucrose and 0.002 M-EDTA, pH 7.4. They were rinsed twice in ice-cold isolation medium and homogenized in a Potter-Elvehjem homogenizer tube fitted with a Teflon pestle. The homogenate was made up to 20 ml with isolation medium. The homogenization procedures were carried out in a cold room at 2°C. The homogenate was then divided equally into two plastic centrifuge tubes and centrifugations were carried out in an M.S.E. Model high-speed refrigerated centrifuge. The nuclear fraction was sedimented by centrifugation of the homogenate for 10 min at 600 *g*. The supernatant which contained the mitochondria was carefully separated into two centrifuge tubes cooled in crushed ice. The recovery of mitochondria was improved by washing the sedimented nuclear fraction as described by Johnson & Lardy (1967). The combined supernatant fluids were centrifuged for 10 min at 8500 *g* to sediment the mitochondrial fraction. The pellets were then washed twice in cold isolation medium, centrifuged, pooled and finally suspended in about 2 ml of final preparation. Two 0.1-ml samples of the mitochondrial preparation were taken for protein estimation.

The protein content of the mitochondrial preparations was estimated by a method based on that of Gornall, Bardawill & David (1949), and the respiration rates of the preparations were recorded by the polarographic technique with a Clark oxygen electrode (Chapell, 1964) in a reaction vessel described by Estabrook (1967). The substrates used included succinate, α -ketoglutarate, β -hydroxybutyrate, malate and glutamate. The response of placental mito-

chondria to ADP was also tested. All measurements were carried out at 25°C, using a reaction medium of isotonic buffer containing 0.006 M-MgCl₂, 0.01 M-KH₂PO₄ and 0.107 M-KCl at pH 7.4. A reaction volume of 3.15 ml was used throughout the experiment which consisted, in almost all determinations, of 2.35 ml reaction medium, 0.4 ml mitochondrial preparation and 0.4 ml substrate. When α -ketoglutarate, β -hydroxybutyrate, glutamate and malate

TEXT-FIG. 1. The difference spectrum of the reduced minus the oxidized pigments in suspensions of mouse placental mitochondria.

Table 1. Concentration of pigments of mouse placental mitochondria

Pigment	Measuring wavelength (nm)	ϵ m M	Optical density change	Concentration of pigments	
				nmol/2.5 ml diluted suspension	mol $\times 10^{-10}$ /mg
Cytochrome a	605 to 630	16.0	0.0140	2.2	3.7
Cytochrome b	562 to 575	20.0	0.0245	3.1	5.3
Cytochrome c + c ₁	551 to 540	19.1	0.0280	3.7	6.3
Cytochrome a ₃	445 to 455	90.0	0.1025	4.8	8.1
Flavoprotein	465 to 500	11.5	0.0220	2.9	4.9

were used as substrates, 20 μ l of 10 mM-nicotinamide adenine dinucleotide (NAD) were sometimes added to the reaction medium. The activity of the preparation was expressed as QO_2 (μ l O₂/mg protein/hr).

A Phoenix dual-wavelength scanning spectrophotometer was used to detect the components of respiratory chain. A suspension (1 ml) of mitochondria (11.8 mg protein/ml) was diluted to 5 ml with the reaction medium and divided into two cuvettes; 0.02 ml of 0.1 M-solution of ADP was then added to each

cuvette and the baseline of equal light absorption was recorded. Potassium succinate (0.025 ml of a 0.5 M-solution) was then added to one cuvette and, after utilization of the O₂, the difference in light absorption of the reduced minus the oxidized pigments was recorded (Text-fig. 1).

Applying the method used by Estabrook & Holowinsky (1961), the concentrations of the cytochrome pigments a, a₃, b, c and c₁, and flavoprotein

Table 2. Utilization of different substrates by mouse placental mitochondria on Days 18 and 19 of pregnancy

Substrate*	No. of determinations	QO ₂ (μl O ₂ /mg protein/hr)	
		Without exogenous NAD	With exogenous NAD
Succinate (0.5 M)	37	13.47 ± 0.18	
α-Ketoglutarate (0.135 M)	2	9.64	9.87
Malate (0.2 M)	2	5.63	5.72
Glutamate (0.25 M)	2	3.24	3.35
β-Hydroxybutyrate (0.2 M)	2	3.58	3.74

* The value for succinate represents the mean estimation ± S.E.M. for Days 12 to 19 of pregnancy

Table 3. Effect of ADP on mouse placental respiration, using 0.5 M-succinate as substrate

Stage of pregnancy	QO ₂ (μl O ₂ /mg protein/hr)	
	Resting	Stimulated (20 μl of 0.1 M-ADP)
12 (2)	12.25	21.60
17 (2)	11.68	24.65
19 (3)	11.12	24.63

Figures in parentheses represent the number of preparations on which the tests were made.

were determined (Table 1). The absorption spectrum showed a pattern essentially similar to the one reported by Chance (1957) and Estabrook & Holowinsky (1961) for mitochondria of other organs, but the absorption band of C₁ with b₁ at 420 nm was absent in mouse placental mitochondria. A similar pattern at this wavelength has been recorded for rat heart muscle sarcosomes (Chance, 1957). The concentrations of cytochrome pigments reported here were higher than those of rat liver mitochondria (Estabrook & Holowinsky, 1961) though the flavoprotein showed similar values. No significance has yet been attached to the organ or species variations in the concentration of these cytochrome pigments.

Table 2 illustrates that the mouse mitochondrial preparation is capable of utilizing all the substrates tested and must have contained all the enzymes of the tricarboxylic acid cycle. The values for O₂ consumption reported by Wang & Hellman (1943), Page (1948), Hellman, Harris & Andrews (1950), Villee

(1953), MacKay (1958) and Friedman & Sachtleben (1960) in the human placenta, and by Baird & McDonald (1964) in the bovine placenta, ranged from 0.46 to 7.5 $\mu\text{l O}_2/\text{mg dry weight/hr}$, decreasing with increasing gestational age. Since these studies, with the exception of that of Villee (1953), were carried out on tissue slices, no comparison with the present values could be made. The Q_{O_2} value of 17.56 for succinate (Villee, 1953) represented $\mu\text{l O}_2/\text{mg N/hr}$ which would represent a much lower value when expressed in terms of mg protein. The addition of NAD made little difference to the rate of O_2 utilization. Stimulation of respiration with ADP resulted in increased O_2 utilization (Table 3).

These preparations were thus capable of carrying out oxidative phosphorylation and must have contained intact mitochondria. Enhancement of respiratory rate by ADP is a sensitive test of mitochondrial integrity (Johnson & Lardy, 1967). The presence of mitochondrial-bound NAD in such preparations (Jacobson & Kaplan, 1957) made the addition of exogenous NAD of little effect in increasing the rate of respiration.

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