Determination of the number of binding sites per mole of transketolase

Transketolase		Thiamine diphosphate		Moles thiamine diphosphate/mole transketolase	
mg/ml	nmoles/ml	dpm/ml	nmoles/ml		Corrected
0.029	0.21	4633	0.33	1.59	1.80

In a total volume of 200 µl, 0.17 mg apotransketolase in 0.1 *M* glycylglycine buffer, pH 7.3, were incubated with 5 m*M* $MgCl_2$ and 0.3 m*M* C^{14}-thiamine diphosphate (specific activity 6.3 µC/µmole). After a recombination time of 30 min at 25 °C, the incubation mixture was applied to a Sephadex G-25 column (1 × 55 cm), equilibrated with 20 m*M* sodium phosphate buffer, pH 5.7. The flow rate was 11.5 ml/h, fraction volume was 2.0 ml. The fractions were analyzed for transketolase activity and pooled (5.7 ml). Aliquots were taken for the determination of protein, thiamine diphosphate and transketolase activity. Protein was determined by the method of LOWRY, using a calibration curve which had been obtained with a transketolase solution standardized by the Biuret method[3]. Thiamine diphosphate was determined by radioactivity measurements. The counting efficiency was 82% when 0.5 ml of the transketolase pool were added to the scintillation liquid (4 g PBD/l ethanol/toluene, 1:1). The transketolase assay was carried out in the presence and absence of added thiamine diphosphate/Mg^{++}. A stimulation of 13% of enzymatic activity was found when the coenzyme was added, indicating a loss of bound coenzyme upon gel filtration. Assuming a linear relationship between enzymatic activity and thiamine diphosphate molecules bound to apotransketolase, we have corrected the value found for thiamine diphosphate by these 13%.

it can be seen that 1.8 moles of thiamine diphosphate were bound per mole of transketolase (MW 140 000). This result agrees well with the findings of KOCHETOV et al.[4], although these authors have used a pH of 7.6 for gel filtration, where we get a complete removal of thiamine diphosphate[2]. Furthermore, these authors determined protein according to LOWRY, but no calibration is mentioned. This is essential, since transketolase has a high tyrosine content[5].

Zusammenfassung. Nachweis, dass 2 Mole Thiamindiphosphat pro Mol Apotransketolase gebunden werden.

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Metabolic Evaluation of Sexual Dimorphism. IV. Metabolic Differences Related to the Oxidative Metabolism

It has been demonstrated that the metabolic differences of mice submaxillary glands are related to the different behaviour of some regulatory enzymes of the energy metabolism[1,2]. Moreover the glucose oxidation by slices of submaxillary glands, both males and females, is stimulated by epinephrine and dibutyryl-cyclic AMP[3]. Their effect is localized on the glycolytic pathway and mainly on the reaction catalized by phosphofructokinase[2]. This fact, however, does not eliminate the possibility that some metabolic differences observed in mice submaxillary glands[1] may be related to the oxidations of Krebs cycle and to the oxidative phosphorylation carried out by isolated mitochondria.

C3H, Balb/c and DBA/2 mice from AR/IRE colony, in continuation since 1956, were used. The animals were sacrificed by cervical dislocation and the submaxillary glands immediately removed and placed in either ice-cold 0.25 *M* sucrose or distilled water. The glands, blotted, weighed and minced, were homogenized using a precooled glass homogenizer with a teflon pestle[4].

The mitochondria were isolated according to JOHNSON and LARDY[5]. Oxygen consumption of homogenates and ADP: O ratio was measured polarographically with an Oxygraph (Mod. KM, G.M.E.) equipped with an oxygen electrode (Yellow Spring Instruments) polarized at −0.8 volts. The reaction mixture for the homogenates was composed of 20 µmoles KH_2PO_4 (pH 7.3 with NaOH), 8.0 µmoles of $MgCl_2$, 325 µmoles of sucrose and 220 µl of 10% homogenate in a final volume of 2.0 ml. Substrates were added in order to achieve a final concentration of 5×10^{-4} *M*. An aliquot of the homogenate was dried for 3 h at 120 °C for dry weight determination.

For the mitochondria, the reaction mixture was made up as follows: 0.25 *M* sucrose, 10 m*M* *Tris*-HCl pH 7.4, 10 m*M* KH_2PO_4, 5 m*M* $MgCl_2$, 25 m*M* KCl, 1×10^{-3}*M* EDTA, 1% BSA and 200 µl of mitochondrial suspension (~ 2.0 mg of protein) to a final volume of 2.0 ml. In both cases the experiments were performed at 30 °C and the concentration of dissolved oxygen was 240 umoles[6]. The determination of succinic-oxidase activity was carried out according to POTTER[7]. The protein concentration was

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determined by biuret method[8]. The data on the oxygen consumption by submaxillary glands homogenates in the presence and in the absence of substrates are reported in Table I.

The basal rate is higher for C3H and DBA/2 females, whereas a sex difference has not been observed for Balb/c. A substantial increase in oxygen consumption is produced in all strains by the addition of succinate. Moreover the respiration is significantly higher in the females of each strain.

Table I. Substrate oxidation by submaxillary gland homogenates of various strains of mice

	C3H		BALB/C		DBA/2	
	♀	♂	♀	♂	♀	♂
	0.210	0.198	0.053	0.056	0.088	0.051
Succinate	0.630	0.510	0.245	0.186	0.340	0.152
	0.210	0.154	0.060	0.050	0.080	0.046
α-Ketoglutarate	0.456	0.296	0.187	0.127	0.292	0.110
	0.236	0.154	0.060	0.051	0.088	0.050
Malate	0.350	0.200	0.120	0.080	0.174	0.080
	0.190	0.156	0.057	0.059	0.095	0.046
Pyruvate	0.210	0.164	0.104	0.093	0.180	0.085

The oxygen consumption is expressed as nmoles O_2/sec × mg dry wt. The substrates were added to achieve a final concentration of 5×10^{-4} *M*. The oxidation of succinate was carried out in the presence of Rotenone 1×10^{-6} *M*.

Table II. ADP: O and respiratory control ratio (RCR) values of mitochondria of submaxillary glands of male and female Balb/c mice

	Male		Female	
Substrates	ADP:O	RCR	ADP:O	RCR
Succinate[a]	1.84 ± 0.02	4.58	1.80 ± 0.05	4.60
α-Ketoglutarate	3.45 ± 0.07	3.80	3.10 ± 0.06	2.50
β-OH-butyrate	2.80 ± 0.01	3.80	2.42 ± 0.02	2.10
Malate	2.92 ± 0.04	3.70	3.00 ± 0.20	2.68

[a] In the presence of 1×10^{-6} *M* Rotenone.
The figures are averaged from 4 different determinations ± standard deviation.

Table III. Succinic-oxidase activity of submaxillary gland homogenates

Strain	µl O_2
C3H♂	46.0 ± 3.2
C3H ♀	63.0 ± 2.5
Balb/c♂	40.6 ± 1.3
Balb/c ♀	66.4 ± 1.2
DBA/2♂	38.0 ± 3.2
DBA/2 ♀	48.1 ± 1.8

The values are expressed as µl O_2/mg dry weight/h. The figures are averages from 4 different determinations ± standard deviation.

The addition of α-ketoglutarate increases the oxygen consumption with a greater increment in the females. The pyruvate is well oxidized by male and female submaxillary glands of Balb/c and DBA/2 but a lower rate of oxidation is found for C3H mice, both males and females. The malate raises the oxygen consumption less than succinate and α-ketoglutarate, even if the increase is greater for females.

The values of ADP: O ratio and respiratory control ratio are reported in Table II. The data clearly show that no statistically significant differences occur between mitochondria isolated from submaxillary gland of Balb/c mice.

The data reported above confirm the hypothesis that the metabolic differences of mice submaxillary glands do not depend on the cellular integrity, but are rather due to different activity of some enzymes which, in turn, act on the regulatory mechanisms of the energy metabolism[1,2]. The greater extent of the oxidation of the exogenous substrates reflect a higher activity of specific enzyme systems, as demonstrated for the succinic-oxidase (Table III).

Owing to the ADP: O ratio, the fact that the values differ from a whole number can be related to the occurence of some side reactions, which are due to the uncoupled reactions related to mechanical damage of mitochondria during isolation or to the contamination by some uncoupling agents which cannot be eliminated completly[9-11].

The values of respiratory control are not high compared with the data concerning other tissues[12-14]. Moreover without BSA in the isolation medium, the respiratory control (data not shown) became lower and the mitochondria lost their capacity to carry out the oxidative phosphorylation after a short period of time (1 h at 0 °C). Whether this is due to some subtle damage during isolation, or is a manifestation of the state of the mitochondria in the submaxillary gland cells, has yet to be determined.

Riassunto. Sono state studiate le ossidazioni del ciclo di Krebs e la fosforilazione ossidativa delle ghiandole sottomandibolari di topi di ambo i sessi. Le femmine hanno una capacità ossidativa più elevata, mentre non si sono riscontrate differenze relative al rapporto ADP: O.

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