DISTRIBUTION OF AMMONIA AND METHYLAMINE BETWEEN MITOCHONDRIA AND SUSPENSION MEDIUM

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1. Introduction

Liver mitochondria accumulate a number of metabolically important anions as if a Donnan situation prevailed [1, 2]; other anions are relatively impermeable under some conditions so if generated internally their concentration rises [3]. This means that the concentrations of reactants applied to the enzymes of the mitochondrial interior and those in the cytoplasm are displaced from the value obtained if the total content in the tissue of a metabolite is divided by the water content. To understand the process of metabolic regulation it is necessary to know how reactants are partitioned. This requirement extends to the ammonium ion because it is involved in the glutamate dehydrogenase reaction as well as in urea synthesis. The partition of ammonia and similar bases between the mitochondrial interior and the medium may also provide indications of the internal charge.

2. Methods

Rat liver mitochondria were prepared by the methods described before [1, 2]. Ammonia† was determined by a Nessler reaction carried out in the cuvette of a dual wavelength spectrophotometer using the wavelengths 380–620 nm. The advantage of this was that the system is sensitive to changes of specific absorbance without being so sensitive to changes of turbidity. The reagent was prepared by adding 25 ml saturated mercuric chloride to 3.5 g potassium iodide in 20 ml water to obtain a permanent precipitate, then 12 g KOH in 55 ml water was added. Aliquots of 0.1 ml of the reagent were used with 1.5 ml 3 M NaOH in the cuvette. The base line was recorded and the samples of either medium or acid extract of mitochondria were injected into the cuvette with a microsyringe.

The injection of the mitochondrial extract led to an immediate deflection which was succeeded by a slow drift lasting for a few minutes. Since the latter could be mimicked by adding glutamine, while prior addition of ammonia increased the initial deflection, only the latter was used in the evaluation of ammonia. Internal standards gave deflections equal to those obtained with equal additions of pure ammonia.

The separation of the mitochondria from the medium was carried out by centrifugation through a layer of silicone into a lower pool of 20% w/v sulphosalicylic acid. Apart from the change in the lower fluid the method is as described by Harris and van Dam [4]. The sulphosalicylic acid was preferred as extractant because it led to a lower blank ammonia than perchloric acid.

The determinations were run in triplicate or duplicate, the values agreed within 5%.

3. Results

The analysis of the content of endogenous ammonia† in the mitochondria showed that there was always an appreciable quantity present, though the amount increased with the age of the preparation. The lowest content measured was 1.3 nmole/mg protein but most values were 4 or more nmole/mg at 30–60 min after

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† Or substance reacting like ammonia to the Nessler reagent.

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Table 1
Ammonia† contents of mitochondria separated from media to which ammonium chloride had been added.

	Concentration of ammonia in medium (mM)	Content in mitochondria (nmole/mg)
(1)	0.04 (endogenous)	5.5
	0.76	5.7
	1.34	4.8
	2.12	4.1
	2.70	4.3
(2)	0.15	4.5
	1.60	4.0
	Medium with 1 mM hydroxybutyrate added	

Contents are corrected for the ammonia carried with the medium measured by the sucrose accessible volume.

Table 2
Ammonia† contents of mitochondria and medium during glutamate oxidation.

	Incubation time (min)	Concentration of ammonia in medium (mM)	Content in mitochondria (nmole/mg)
(1)	1	0.06 (endogenous)	8.4
	15	0.15	7.8
	45	0.60	7.7
(2)	1	0.06	9.0
	13	0.40	9.0
	33	1.00	9.0
	Medium with 50 μM DNP and 5 mM glutamate		

the last wash of the preparation. After 3 hr storage at 0° the increase of ammonia content in a particular example was 6 nmole/mg.

If ammonium chloride was added to the medium and the mitochondria were separated, their ammonia† content, after correction for the ammonia in the volume of medium carried through the silicone (usually about 3.5 ml/g protein), was imperceptibly increased (table 1).

If a reaction was carried out which generates ammonia internally, namely the oxidation of glutamate, the ammonia formed was found to accumulate in the medium and there was little or no change in the mitochondrial content (table 2). An attempt was made to remove the internal ammonia by carrying out a

Table 3
Ammonia† contents of mitochondria during oxidation of either (1) oxoglutarate or (2) isocitrate.

	Time of incubation (min)	Conc. of ammonia in medium (mM)	Content in mitochondria (nmole/mg)
Basic medium plus	½	0.06	9.0
(1) 5 mM oxo-	11	0.06	9.0
glutarate	30	0.07	9.0
Basic medium plus (2) 10 mM isocitrate (threo-d), 2 mM malate, 0.5 mM arsenite, 50 μM ADP, 2 unit hexokinase/ml, 5 mM glucose, 1 mM $MgCl_2$.	½	not determined	6.9
	10	not determined	6.7

Table 4
Distribution of methylamine between rat liver mitochondria and medium.

	Conc. in medium (mM)	Corr. mitochondrial content (nmole/mg)
(1) with 1 mM	0.44	0.20
hydroxybutyrate	1.07	0.60
	2.16	1.73
(2) with DNP 50 μM	0.49	0.26
	1.13	0.38
	1.99	0.74

reaction which consumes ammonia, namely reductive amination of oxoglutarate. Since the latter is not a good penetrant [3] and reducing equivalents are required, the reaction was tried both with oxoglutarate and with isocitrate. Table 3 shows that long incubation with these substrates caused no change in the ammonia measured.

Since it was clear that the mitochondria carried some form of combined ammonia† which was not responding to addition nor possible removal, some experiments were made to find the distribution of ^{14}C-methylamine, added as hydrochloride. The results

obtained (table 4) show that the corrected content in nmole/mg of this cationic substance is numerically lower than the applied concentration and it may even be relatively excluded from the interior.

4. Discussion

Two points emerge from these results; first, there is some source of ammonia† in the isolated mitochondria which does not respond to treatments lasting 10–30 min which might be expected to alter the content and second, methylamine is not concentrated, pointing to an internal positivity. It seems that there is present some unidentified compound which reacts like ammonia with Nessler's reagent.

Acknowledgements

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References

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