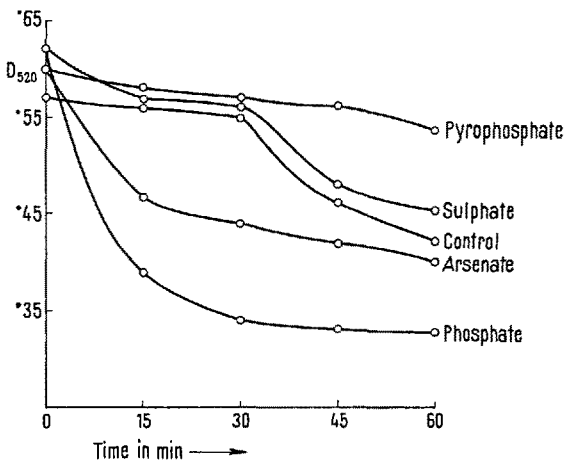
The Correlation between Anion-Induced Mitochondrial Swelling and Glutaminase I Activity of Guinea Pig Liver

In a previous communication[1], we reported the conditions necessary for phosphate activation of glutaminase I of guinea pig liver mitochondria and suggested the possibility of a correlation between anion-induced swelling and the observed glutaminase I activity of mitochondria. Evidence is presented here in favour of such a possible correlation.

Methods. Mitochondria was prepared in 0·44 *M* sucrose according to the method of SCHNEIDER[2] and washed thrice with ice-cold sucrose. The basic medium of incubation was 0·44 *M* sucrose containing 0·05 *M* Veronal buffer pH 7·2. Enzyme activity was determined by measuring the ammonia formed as reported previously[1], while the corresponding swelling was followed by reading the changes in absorbancy at 520 mμ. Mitochondria equivalent to about 75 mg of wet tissues were taken in all cases and added last to the systems within 15 min of preparation. All additions were brought beforehand to pH 7·2. Ageing of mitochondria was made by preincubation for 20 min at 40°C.

Results. Among the anions tested (0·2 *M*), only phosphate and arsenate produced marked swelling, whereas sulphate was without any effect; pyrophosphate, on the other hand, inhibited even the spontaneous swelling of mitochondria (Fig.). The corresponding glutaminase activity indicated that phosphate and arsenate activated the enzyme, whereas sulphate produced a very slight increase in activity. On the contrary, pyrophosphate, known as one of the strongest activator of glutaminase I[3], completely failed to activate the enzyme even with higher concentration (0·4 *M*). But when aged and preswollen mitochondria were employed, both pyrophosphate and sulphate produced significant activation of glutaminase I at 37°C (Table I).

Effect of temperature variation on phosphate-induced swelling and glutaminase I activation also showed close correlation between these two functions of the anion. At 37°C, 0·2 *M* phosphate induced both maximum swelling and glutaminase I activation. At 27°C similar results were found with higher phosphate concentrations (0·3 to 0·4 *M*), but at 15°C neither swelling of mitochondria nor glutaminase I activation was possible with any concentration of phosphate.



Effect of various anions (0·2 *M*) on swelling of guineapig liver mitochondria suspended in 0·44 *M* sucrose – 0·05 *M* Veronal buffer, pH 7·2 at 37°C.

Tab. I. Anion activation of glutaminase I of guineapig liver mitochondria

Anions added (0·2 *M*)	μg Ammonia formed/h	
	Fresh mitochondria	Aged mitochondria
1. None	7·2	2·0
2. Phosphate	24·0	20·5
3. Arsenate	16·2	18·5
4. Sulphate	9·6	12·0
5. Pyrophosphate	6·8	16·5

All incubations were carried out with L-glutamine (5×10^{-3} *M*), at pH 7·2 and temperature 37°C.

Tab. II. Inhibition of phosphate (0·2 *M*) induced swelling and glutaminase I activation of guineapig liver mitochondria

Inhibitors added	Fresh mitochondria						Aged mitochondria	
	Changes in O.D./h			% inhibition of swelling	μg Ammonia formed/h	Glutaminase activity (relative)	μg Ammonia formed/h	Glutaminase I Activity (relative)
	Initial	Final	Difference					
1. None	0·72	0·385	0·335	Nil	18·5	100	13·0	100·0
2. KCN (0·1 *M*)	0·72	0·60	0·12	64·2	8·0	43·2	13·5	103·8
3. NaN_3 (0·2 *M*)	0·68	0·54	0·14	58·3	2·0	10·8	1·5	11·5
4. Na-Amytal (0·01 *M*)	0·72	0·57	0·15	56·3	11·0	59·4	13·0	100·0
5. Antimycin A (5 μg)	0·72	0·55	0·17	49·3	12·5	67·5	13·0	100·0
6. Sucrose (0·88 *M*)	0·67	0·55	0·12	64·2	7·5	40·0	13·0	100·0
7. Sucrose (0·88 *M*) + 0·4 *M* PO_4	0·67	0·39	0·28	16·5	16·0	86·4	—	—

All incubations were carried out with L-glutamine (5×10^{-3} *M*) at pH 7·2 and temperature 27°C.

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Known antiswelling agents of mitochondria like KCN, Na-azide, amytal, antimycin A, and sucrose (0·88 *M*) were tested to observe their effects on both glutaminase I and the corresponding swelling. It was observed that both glutaminase I and swelling of fresh mitochondria were inhibited by these substances. However, with aged and preswollen mitochondria, all these inhibitors, except Na-azide, failed to inhibit the glutaminase I activity (Table II), indicating that the inhibition of enzymic activity by the above substances was primarily due to their antiswelling action on fresh mitochondria. Na-azide, however, was inhibitory to both swelling and glutaminase I. Hypertonic sucrose (0·88 *M*) in fresh mitochondrial preparation prevented swelling as well as glutaminase I activity with the usual 0·2 *M* phosphate concentration, but on further addition of phosphate to a final concentration of 0·4 *M*, both these activities reappeared.

That swelling of mitochondria releases some hydrolytic enzymes has been reported previously[4]; but no work has been done with regard to glutaminase I. Data presented above strongly suggest that swelling of mitochondria caused either by the added anions themselves, like phosphate and arsenate, or due to induced ageing or preswollen condition, as with pyrophosphate and sulphate, is a prerequisite condition for the demonstration of anion activation of glutaminase I of guineapig liver mitochondria.

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Zusammenfassung

Die dargelegten Versuche beweisen, dass die durch Anionen (Phosphat und Arsenat) herbeigeführte Anschwellung von Mitochondrien der Meerschweinchenleber eine Vorbedingung ist für die Glutaminase I-Aktivierung. Pyrophosphat, das die Schwellung hemmt, und Sulfat, das sie nicht beeinflusst, aktiviert das Enzym erst nach vorheriger Anschwellung der Mitochondrien.

Distributions of Cholesterol Amongst Liver Subcellular Fractions

Assays for the cholesterol content of various subcellular fractions derived from rat liver homogenates have been reported by a number of investigators[1–5] and are presented in Table I. In each instance mitochondria were isolated under conditions of centrifugation (18 000–24 000 × *g* for 10 min) sufficient to sediment microsomes as well.

Mitochondria obtained from rat and mouse liver homogenates by centrifugation at 8500 × *g* for 10 min, then washed by resuspension and recentrifugation, have been employed in studies of the cholesterol oxidase system[6–9]. Addition of the microsomal fraction to these '8500 × *g*' mitochondria, omission of the washing procedure, or isolation of mitochondria under higher centrifugal forces for the same time interval, in each case severely depressed oxidation of added cholesterol —C^{14} by the cholesterol oxidase present in the mitochondria. This has been traced in part to the relatively high endogenous content of free cholesterol in the liver microsomal fraction which effectively dilutes any added radioactive cholesterol.

Table II records the relative distribution of liver cholesterol amongst various subcellular fractions of a number of animals. The fractions were isolated according to the scheme presented in the Figure. Liver specimens were washed three times with 10% w/v sucrose before homogenization in a Potter-Elvehjem homogenizer. The subcellular fractions were digested in warm 30% aq. KOH and the cholesterol extracted with petroleum ether. After the petroleum ether extracts were dried over anhydrous Na_2SO_4 aliquots were analyzed for cholesterol using the method of TRINDER[10].

The consistency of the liver mitochondrial cholesterol content in several animal species is of interest. SCHOTZ, RICE, and ALFIN-SLATER[3] found that their preparations of rat liver mitochondria contained 0·20 mg cholesterol. Our preparations, derived from the same volume of liver homogenate (4·0 ml), contained on the average only about 0·13 mg.

Tab. I. Percentage Recovery of Total Cholesterol in Mouse and Rat Liver Fractions

Fraction	Ref. 1[a]	Ref. 2[b]	Ref. 3[a]	Ref. 4[a]	Ref. 5[a]
Nuclei	—	—	6·0	0·7	—
Mitochondria	1·3	3·5	13·7	1·2	2·2
Microsomes	—	5·1	—	1·9	—
Supernatant	—	2·8	—	0·3	—

[a] Rat. [b] Mouse.

Tab. II. Average Percentage of Total Liver Cholesterol Recovered (No. of experiments in parentheses)

Fraction	Species					
	Mouse[a] (2)	Rat (3)	Chicken (3)	Rabbit (2)	Monkey (1)	Human (1)
Debris and nuclei	39·0	53·7	60·1	58·0	58·7	56·4
Mitochondria	7·8	6·3	7·6	8·1	6·5	6·9
Mitochond. wash	14·9	7·0	7·0	10·9	6·5	6·9
Microsomes	33·5	25·0	15·3	15·1	9·5	10·5
Supernatant	4·8	8·0	10·0	7·9	18·6	19·5

[a] Pooled livers of 4 mice used.

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