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Effect of Arsenate on Oxidative Phosphorylation and Related Reactions

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Crane and Lipmann [1] have shown in 1953 that arsenate causes uncoupling of respiration from phosphorylation in washed particles from rat liver, accompanied by a depression of the respiration in the presence of various substrates. The uncoupling occurred in a gradual manner, increasing in extent with the duration of the incubation. We have now been able to confirm these findings using intact rat liver mitochondria and a variety of substrates including glutamate, succinate and β-hydroxybutyrate. We have found, furthermore, that arsenate also induces an adenosine triphosphatase activity in the mitochondria, as well as a release of endogenous inorganic and high-energy phosphates, and that these effects take place prior to the uncoupling of the electron transport coupled phosphorylation. These findings are illustrated in Tables 1 and 2.

Table 1 shows that the arsenate-induced ATP-ase (which has recently also been observed by Azzone *et al.*[2] and by Wadkins [3]) reached a maximal activity at 3 mM arsenate, and that this activity was about 8 times higher than that found in the absence of arsenate; this maximum was about 2-3-fold lower than the ATPase activity induced by 10^{-4} M 2,4-dinitrophenol. Addition of succinate (or other substrates) resulted in an apparent depression of the ATPase activity, due to the phosphate uptake accompanying the oxidation of these substrates which, as could be ascertained in parallel tests, was not seriously impaired by low concentrations of arsenate in these short-term experiments. As expected, this effect of added substrates could be abolished by inhibiting the respiration with antimycin A (and, in the case of DPN-linked substrates, also with amytal).

The depleting effect of arsenate on mitochondrial endogenous phosphates is illustrated in Table 2. For these studies, so-called "^{32}P-labelled mitochondria" were prepared according to Beyer *et al.*[4], *i. e.*, preparations of mitochondria obtained from liver to which a suitable quantity of ^{32}P-orthophosphate was added during the homogenization procedure. These mitochondria, as demonstrated by Beyer *et al.*[4], contain most of the bound ^{32}P in the form of orthophosphate and high-energy phosphates, the ratio of the former to the latter varying between 7:3 and 5:5. When such mitochondria were suspended in a sucrose-KCl medium and incubated at 30°C in the presence of 1—3 mM arsenate, a rapid release of the bound ^{32}P took place, reaching nearly completeness within 5 min. 2 mM amytal, which caused a slight release by itself, greatly protected the mitochondria against the arsenate-induced release; antimycin A and cyanide, in contrast, gave only a weak protection. Of interest is also that 2,4-dinitrophenol, which has earlier been shown by Teply [5] to release the so-called "gel-phosphate" from "cyclophorase" preparations, had only a partial releasing effect under the present

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Table 1. Stimulation of ATP hydrolysis by arsenate.

Concentration of arsenate mM	Additions none	succinate	succinate + antimycin A
		μmoles P_i liberated	
none	0.22	0	1.12
0.1	0.95	0	1.46
1	1.58	0	1.76
3	1.97	0.10	1.80
10	1.88	0.77	2.07

Each tube contained: 100 μmoles KCl, 50 μmoles TRIS buffer pH 7.5, 10 μmoles ATP, 125 μmoles sucrose, mitochondria from 100 mg rat liver (wet weight) and, when indicated, 10 μmoles succinate and 1 μg antimycin A; final volume 2.0 ml. Temp. 30°C. Time of incubation 20 min.

conditions. However this effect could be markedly enhanced by adenosine-5′-phosphate, presumably due to a conversion of intramitochondrial ATP into extramitochondrial (*cf.*Ref.[6]). Finally, succinate greatly inhibited the releasing effect of arsenate, at the same time as it promoted the effect of dinitrophenol. These effects, the possible mechanisms of which will be discussed in some detail, have recently been of great importance as a basis for demonstrating a requirement of high-energy phosphate for the aerobic oxidation of succinate by intact rat liver mitochondria [7].

The above findings will be discussed in relation to the hypothesis that the arsenate-induced ATPase and release of mitochondrial endogenous phosphate are primary manifestations of the general, gradual uncoupling effect of arsenate, observed by Crane and Lipmann [1]. It is visualized that these primary effects reflect an active uptake of arsenate by the mitochondria, this process possibly involving an electron transport via the amytal-sensitive step of the respiratory chain, accompanied by an oxidative arsenylation.

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Table 2. Depletion of intramitochondrial phosphate.

Addition	no arsenate	10^{-3} M arsenate
none	867	185
2 mM amytal	620	541
2 mM KCN	574	298
1 μg antimycin A	561	286

The incubation medium contained: 150 μmoles KCl, 50 μmoles TRIS buffer pH 7.5, 325 μmoles sucrose and ^{32}P labelled mitochondria from 200 mg rat liver (wet weight); final volume 3 ml. Temp. 30°C. Time of incubation 5 min. Values are given in number of counts/sec.