Reports and Letters

Lowered P/O Ratios with Mitochondria Isolated from Livers Showing Cloudy Swelling

Morphological changes in the mitochondria of cells with "cloudy swelling" (the earliest histological evidence of cellular degeneration) have been demonstrated repeatedly. A reciprocal relationship between the shape of mitochondria and the rate of oxidative phosphorylation has been shown recently (*1*). Mitochondria isolated from normal cells were used to establish this interdependence; swelling was induced either by osmotic means or by incubation under conditions in which the oxidative phosphorylation was uncoupled.

It was of interest, therefore, to study the formation of high-energy phosphate bonds in cells affected by a typical cloudy swelling such as can be produced with certain bacterial toxins. Evidence has been presented in a previous paper (*2*) that, in cloudy swelling induced by diphtheria toxin, the easily hydrolyzable phosphate is decreased. Further experiments have shown that repeated injections of 2,4-dinitrophenol, a compound well known to uncouple oxidative phosphorylation *in vitro* (*3*), produced cloudy swelling of liver and kidneys in the rat (*4*). Analogous results were obtained by injecting rats with thyroxine (*5*), which also uncouples oxidative phosphorylation (*6*). This report is concerned with the phosphorylation quotients observed with mitochondria isolated from livers that showed cloudy swelling (*7*).

In order to produce cloudy swelling of the liver, rats were injected intraperitoneally with *S. typhi murium* toxin (the smallest dose that would kill such animals in 4 days) and guinea pigs were injected subcutaneously with diphtheria toxin (1 MLD/250 g of body weight). Animals were used 24 hours after the toxin injection. In all cases, the livers were examined histologically to confirm the occurrence of cloudy swelling in the treated animals. Liver mitochondria were prepared in 0.25*M* sucrose-0.005*M* versene (ethylenediaminetetraacetic acid, adjusted to *p*H 7.4 with NaOH), essentially by the procedure of Schneider (*8*).

The results of the phosphorylation experiments are shown in Table 1. They are expressed as P/O ratios (moles of inorganic orthophosphate which disappear per atom of oxygen consumed).

The complete reaction system contained the following: mitochondria derived from 500 mg of fresh tissue; 250 μmoles of sucrose (contributed from the added mitochondria); 15 μmoles of versene (including the amount of the mitochondrial suspension); 60 μmoles of potassium phosphate buffer at *p*H 7.4; 75 μmoles of KCl; 20 μmoles of $MgSO_4$; 40 μmoles of KF; 90 μmoles of succinate or 30 μmoles of α-ketoglutarate; 3×10^{-2} μmoles of cytochrome *c*; 3 μmoles of adenosine-5′-phosphate; 78 μmoles of glucose; and 20 mg of a hexokinase preparation (*9*) in a final volume of 3 ml. A Warburg bath was used for incubation at 25°C for 20 minutes, with air as the gas phase; CO_2 was absorbed with KOH. Inorganic orthophosphate in the trichloroacetic filtrates was estimated according to Fiske and Subbarow (*10*).

It can be seen that with both succinate and α-ketoglutarate, the phosphorylation quotient was lowered when mitochondria from livers showing cloudy swelling were used. The lowering of the P/O ratios has been observed with mitochondria prepared from livers of rats treated with *S. typhi murium* toxin and also from livers of guinea pigs injected with diphtheria toxin. In the latter system, the inhibition of phosphorylation is more marked with α-ketoglutarate as a substrate than it is with succinate as a substrate.

Table 1. Phosphorylation quotients with mitochondria from livers showing cloudy swelling as compared with the controls. The figures represent the mean ± standard error and those in parentheses give the number of observations.

Animal	Substrate	P/O	
		Controls	Treated animals
Rat	Succinate	1.8 ± 0.07 (5)	1.3 ± 0.09 (5)
Rat	α-Ketoglutarate	3.2 ± 0.07 (10)	2.5 ± 0.16 (10)
Guinea pig	Succinate	2.0 ± 0.08 (5)	1.7 ± 0.07 (5)
Guinea pig	α-Ketoglutarate	3.1 ± 0.21 (4)	1.7 ± 0.14 (4)

The hydrolysis of adenosine-5′-phosphate, adenosinetriphosphate, and glucose-6-phosphate by the mitochondria was also studied; no increased rate of hydrolysis was found with the mitochondria from pathological livers. Thus, the low P/O ratios do not appear to be attributable to an increased dephosphorylation.

A. Fonnesu
Clara Severi
Institute of General Pathology, University of Milan, Milan, Italy

References and Notes

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2-Methyl Hydrocortisones: A New Series of Steroids with Enhanced Potency and Prolonged Action

Steroid chemists have recently developed a number of synthetic analogs of the adrenocortical steroids, several of which have been found to possess remarkable biological properties. The 9-α-halogen steroids developed by Fried and Sabo (*1*) have been found to possess a greater biologic potency than their nonhalogenated analogs with respect to all properties thus far studied. The sodium-retaining activity of these halogenated corticoids has been found to be enhanced out of proportion to the increase in "glucocorticoid" activities. The Δ-1 series of corticosteroids developed by Herzog *et al.* (*2*) have been found to possess greater biologic activity than their natural analogs with respect to properties dependent on the presence of an 11-oxygen group (for example, anti-inflammatory activity). On the other hand, those properties that do not depend on the presence of an 11-oxygen group (for example, sodium-retaining activity) have not been enhanced by the Δ-1 modification (*3*).

Very recently, the development of still another series—the 2-methyl analogs of