Brief Notes

Oxidation and Phosphorylation in Liver Mitochondria Lacking "Polymerized" Ribonucleic Acid.*

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The administration of cortisone to rats and rabbits has been found to cause profound changes in the hepatic parenchyma; *i.e.* . . . increase in cell size, accumulation of glycogen, and greatly decreased cytoplasmic basophilia which has been found not to be decreased further by treatment with ribonuclease (1–4). Microscopic examination has also indicated a decrease in numbers of mitochondria and alterations in their morphology (5). Mitochondria isolated by differential centrifugation of homogenates of such livers in either saline (4) or 0.25 M sucrose (6) have been found to be completely devoid of ethanol-precipitable ribonucleic acid (presumably highly polymerized forms of RNA). In addition the microsomes from such livers were found to contain little or no ethanol-precipitable RNA. These effects of cortisone are reversible; within 8 days after cessation of treatment the liver picture is essentially normal (4).

The apparent lack of polymerized RNA in such mitochondria is a point of special interest. Although it has often been suggested that the RNA found in isolated mitochondria derives from contaminating microsomes (*cf.* 7, 8), even the most careful subfractionations of the gross mitochondrial fraction from normal liver (8–10) have shown that significant quantities of RNA were still present in those fractions with high succinic oxidase activity. Because the mitochondrial fraction from livers of cortisone-treated rats contains no ethanol-precipitable RNA, the opportunity to determine whether the presence of this form of RNA is necessary for some of the most characteristic enzymatic functions (11) of mitochondria was presented. This report concerns the succinic oxidase activity and oxidative phosphorylation in such preparations.

The livers of normal and cortisone-treated rats (4) (Wistar strain, males, 150 to 200 gm.) were fractionated in 0.88 M sucrose as described by Hogeboom, Schneider, and Palade (12), a procedure which has been shown to preserve mitochondrial structure. The nuclear and microsomal fractions were not washed, as is usually done, but were analyzed and assayed directly so that aberrations in sedimentation characteristics of the cortisone-treated liver fractions would not cause "redistribution" effects. The mitochondrial fraction was washed twice in 0.25 M sucrose. The fractions were analyzed for total N, total RNA-ribose (13), and for succinic oxidase activity (14). RNA was extracted from each fraction with hot 10 per cent NaCl, and subsequently precipitated with two

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volumes of ethanol. The precipitate was dissolved in water and reprecipitated with one-third volume cold 20 per cent trichloroacetic acid (TCA). This precipitate was heated for 30 minutes at 80°C. with five volumes of 5 per cent TCA. Ribose determination was performed on the hydrolysate. This procedure effectively separates polymerized RNA (precipitable out of salt solutions by ethanol) from unpolymerized RNA and simpler nucleotides (15, 16).

was present in the normal mitochondrial fraction isolated by the procedure used, as has of course been found by many workers. In addition no polymerized RNA could be detected in the microsome fraction of cortisone-treated livers in this experiment.

A far more crucial test of the functional integrity of isolated mitochondria is the ability to carry on oxidative phosphorylation coupled to the oxidation of intermediates of the Krebs cycle and

TABLE I

Analysis of Liver Fractions of Normal and Cortisone-Treated Rats

Fraction	Total N	RNA as ribose	Succinic oxidase activity
	mg./gm. wet weight	*mg./gm. wet weight*	*μl. O_2 uptake/gm. wet weight/60 min*
Normal			
Whole homogenate	37.4	3.72	36,200
"Nuclei"	4.3	0.92	6,200
Mitochondria	8.9	0.25	26,200
Microsomes	4.3	1.22	<900
Supernatant	20.0	1.25	<900
Cortisone-treated			
Whole homogenate	31.0	1.82	29,200
"Nuclei"	3.8	0.30	3,400
Mitochondria	6.6	0.00	20,600
Microsomes	7.7	0.00	<1,200
Supernatant	12.1	1.21	<1,800

The results of this comparison are shown in Table I. It is seen that the total succinic oxidase activity of the cortisone-treated livers is slightly less than that of normal livers. However, the distribution of succinic oxidase among the fractions is approximately normal, the great bulk being present in the mitochondrial fraction in both normal and cortisone-treated livers. It is striking that, although the mitochondria of cortisone-treated livers have succinic oxidase activity not far removed from normal, no polymerized RNA could be detected even when very large aliquots were tested, whereas this form of RNA

other substrates (17, 18). To date, oxidative phosphorylation with maximum P:O ratios has been observed only in reasonably intact mitochondria (17). The mitochondria from normal and cortisone-treated livers were isolated from 0.25 M sucrose homogenates, and then washed twice with 0.25 M sucrose. These preparations were then assayed in the presence of glutamate or succinate as substrate, Mg^{++}, NaF, phosphate, adenosinetriphosphate (ATP), glucose, and purified yeast hexokinase. Oxygen uptake and phosphate uptake data were used to calculate the P:O ratio (moles P reacting with adenosinediphosphate

(ADP) per atom oxygen taken up). Data from a typical experiment are shown in Table II. The P:O ratios of the two types of preparations were not found to be grossly different in a series of measurements.

These experiments obviously do not test directly the question of whether RNA is an integral component of mitochondria from normal livers. However, from these findings the presence of polymerized RNA appears not to be essential for the function of either of the two complex and characteristic mitochondrial enzyme systems tested.

The procedure used to determine RNA (15, 16) measures high molecular weight polynucleotides, since only in such form is the sodium salt insoluble in ethanol. It is conceivable, and in fact indicated by other experiments, that low molecular weight, less highly polymerized RNA, containing ribose and phosphorus and giving high absorption at 260 mμ is present in the cortisone-treated mitochondria, since determination of RNA by the Schneider procedure (19) (extraction with hot trichloroacetic acid, following removal of mononucleotides with cold TCA) (see Table III) reveals the presence of a very significant amount of such material. These findings

TABLE II

Oxidative Phosphorylation Test

Warburg vessels contained 0.0025 M ATP, 0.01 M glutamate or succinate, 0.025 M phosphate pH 7.4, 0.005 M $MgCl_2$, 0.01 M KF, 1×10^{-5} M cytochrome *c*, purified yeast hexokinase, 0.05 M glucose, and the washed mitochondria derived from 0.5 gm. wet weight liver in total volume of 2.0 ml. Incubated in air at 15° for 47 minutes.

	RNA-ribose	O_2 uptake	P uptake	P:O
	μg.	*μatoms*	*μatoms*	
Normal	101			
Glutamate		6.82	16.9	2.48
		6.40	14.1	2.20
Succinate		7.70	6.31	0.82
Cortisone-treated	0.0			
Glutamate		6.30	13.9	2.20
		6.71	15.6	2.32
Succinate		5.50	4.65	0.85

TABLE III

Forms of Mitochondrial RNA

Type of experiment	Ribose	
	Amount per gm. liver obtained by	
	TCA	10 per cent NaCl
	μg.	*μg.*
Normal (average of 8 experiments)	908	727
Cortisone (average of 4 experiments)	448	0

therefore suggest that cortisone treatment, either directly or indirectly, affects the degree of polymerization of RNA.

The lack of polymerized RNA in the microsomes is also of interest because of recent work suggesting a role of the microsome fraction in protein synthesis (20), and the already considerable evidence relating RNA and protein synthesis.

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