RNA is in fair agreement with the value of 29 calculated from the parameters of the hypothetical prolate ellipsoid deduced from the light-scattering and electron-microscopy results and used to fit the data.

From these results it can be concluded that RNA of the ascites-tumor cell consists of compact globular molecules, best described as prolate ellipsoids of revolution. This would account for the low viscosity of its solutions and the high sedimentation coefficient observed. These results, however, have to be considered only as preliminary and subject to some uncertainty since RNA is unstable and subject to degradation both upon freezing and thawing and upon standing in solution at room temperature. Further work is in progress with the aim of eliminating these complications.

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The nature and action of mitochrome

POLIS AND SHMUKLER[1] recently described the preparation from disintegrated mitochondria of a purified protein which activated the latent adenosine triphosphatase (ATP-ase) and uncoupled oxidative phosphorylation in freshly prepared mitochondria. Since the absorption spectrum of this preparation resembled that of a haem compound, the authors gave it the name mitochrome. These findings are of considerable interest, since it seems probable that mitochrome is responsible for the development of ATP-ase and loss of phosphorylative activity during the aging of mitochondria.

To study the relationship between this haem compound and the uncoupling of oxidative phosphorylation by the mitochrome preparation, the latter was prepared[1] and tested as an inhibitor of the ATP-inorganic phosphate exchange reaction in the presence of various substances known to react with haem compounds in general and mitochrome in particular. Table I shows that mitochrome inhibited the exchange almost completely, while the other substances were without effect, either in the presence or in the absence of mitochrome. The results indicate that under our conditions the exchange reaction is independent of the state of oxidation or reduction of components of the respiratory chain, suggesting that a haem is not directly involved in the exchange reaction. Since $Na_2S_2O_4$, $K_3Fe(CN)_6$, CO and KCN had no effect on the inhibition of the exchange reaction by the mitochrome preparation, whereas these compounds caused marked changes in the absorption spectrum[1], it became doubtful whether the haem could be responsible for the inhibitory effect. It was also found that the inhibitory activity was not lost if a fine suspension of heat-treated (10 min at 100°) mitochrome was used. It may be concluded therefore that neither protein nor haem but another component of the preparation must be the active inhibitor.

It was found that this substance was readily extracted by isooctane, which caused little precipitation of protein or alteration in the pigment as evidenced by the unchanged spectrum of mitochrome after extraction. The isooctane was evaporated in the absence of air and the residue dissolved in 96% ethanol. It can be seen from Expt. 2 that the isooctane extract caused a marked inhibition of the exchange reaction and that the extracted mitochrome had no effect. In other experiments, it was found that this inhibition could be abolished by albumin and that the isooctane extract also strongly inhibited the phosphorylation coupled to the oxidation of succinate.

TABLE I

EFFECT OF MITOCHROME AND OF SUBSTANCES REACTING WITH HAEM COMPOUNDS ON THE ATP-INORGANIC PHOSPHATE EXCHANGE REACTION

Incubation medium: KCl, 98 μmoles; $MgCl_2$, 6 μmoles; ethylenediamine tetraacetate, 1.5 μmoles; ATP, 9 μmoles; tris(hydroxymethyl)aminomethane-acetate, pH 7.0, 75 μmoles; phosphate, pH 7.0, containing ^{32}P, 10 μmoles. Mitochrome (in Expt. 1, 0.16 mg protein, in Expt. 2, 0.33 mg protein) where indicated. Final volume, 1.45 ml. The reaction was started by the addition of 0.05 ml liver mitochondria, isolated in 0.25 M sucrose and the exchange[2] measured over 15 min at 20°. The values are corrected for ATPase activity. In Expt. 2, the mitochrome preparation was extracted twice at 20° with 3 vol. isooctane by shaking by hand.

Expt.	*Addition*	*μatom P exchanged/mg protein/h*		*Inhibition (%)*
		No mitochrome	*Mitochrome*	
1	None	4.1	0.4	90
	$Na_2S_2O_4$ ($10^{-3}M$)	3.5	0.2	94
	$K_3Fe(CN)_6$ ($10^{-2}M$)	4.3	0.2	95
	KCN ($10^{-4}M$)	3.7	0.4	89
	CO (100% in gas phase) + $10^{-3}M$ $Na_2S_2O_4$	3.5	0.2	94
2	None	3.3	0.2	
	Ethanol (2.4% final concn.)	3.3		
	Isooctane extract of mitochrome in 2.4% alcohol (final concn.)	0.1		
	Isooctane-extracted mitochrome		2.8	

Preparations[3] of cytochrome $a + a_3$, allowed to age at 4° for several days, contained a pigment with the following absorption peaks: oxidized, 410 mμ; oxidized + KCN, 418 mμ; reduced, 422 mμ; reduced + CO, 418 mμ. Mitochrome has broad peaks in the same regions and showed the same spectral shifts with CO and KCN. Acid-acetone extracts[4] of mitochrome and of the aged preparation of cytochrome $a + a_3$ were made and the haems chromatographed on paper using lutidine-NH_3[5]. Both haems migrated with an R_F of 0.85 and as a single spot when mixed. Alkaline-pyridine extraction[6] of the acid–acetone-extracted mitochrome yielded a reduced pyridine protohaemochromogen with absorption peaks at 556, 523 and 416 mμ. Thus, mitochrome contains the haems from cytochromes b*, a and a_3, but does not show the characteristic spectra of these cytochromes. The formation of the mitochrome-like pigment in the preparation of cytochrome $a + a_3$ was accompanied by the release of an isooctane-extractable substance with the same inhibitory properties as the substance extractable from mitochrome.

From these results it can be concluded that the component[s] in the mitochrome preparation which is [are] responsible for its spectrum is [are] derived from cytochromes, that the component[s] responsible for its effect on the exchange reaction and oxidative phosphorylation can be separated from the pigment and that the inhibition must be ascribed to material contained in the mitochrome preparation soluble in organic solvents.

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* Cytochrome b bands were observed following the acetate precipitation in the mitochrome preparation procedure, but rapidly disappeared on standing in the ethanolamine buffer.

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