Short Communications

The release of nucleotides from mitochondria during ultraviolet irradiation*

SARACHEK AND TOWNSEND[1] have reported a swelling of mitochondria of yeast cells during u.v. irradiation and CANZANELLI *et al.*[2] have shown an inhibition of succinic and cytochrome oxidase of irradiated isolated rat-liver mitochondria. Recently, DALLAM AND ANDERSON[3] have reported a lowered oxidative phosphorylation after u.v. irradiation of rat-liver mitochondria, and BRODIE *et al.*[4] have reported complete loss of oxidation and phosphorylation in bacterial particles after irradiation.

In view of the apparent central role of ATP in the coupled oxidative phosphorylation system of mitochondria[5,6] and the correlation between the physical state of mitochondria and their phosphorylative capacity[6], we have measured the release of mitochondrial nucleotides during u.v. irradiation. The results indicate a preferential release of nucleotides and inorganic phosphate from u.v.-treated mitochondria.

Rat-liver mitochondria were isolated and treated as previously described[7]. Mitochondria from one liver were irradiated in either quartz (irradiated) or pyrex (control) tubes. A rotary irradiator containing 14 General Electric 15 W, 18 in. Germicidal lamps arranged in a circle around

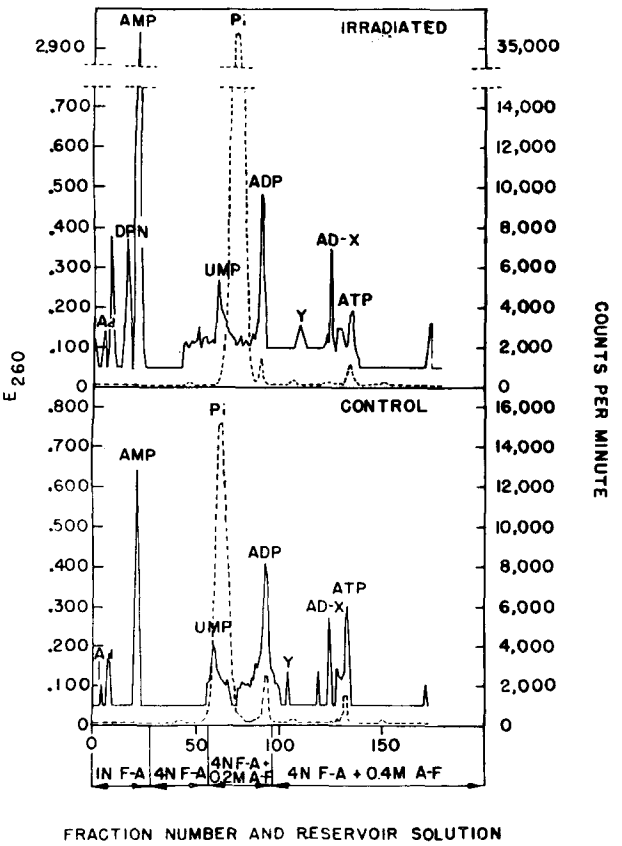

Fig. 1. E_{260} and radioactivity (—— = E_{260}, – – – – = radioactivity) values of chromatographic fractions of $HClO_4$-soluble fraction of rat-liver mitochondria incubated with $H_3{}^{32}PO_4$. The radioactivity scale represents the number of counts/min/50 μl. ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; AD, acid-conversion product of DPNH; AD-X, acid-conversion product of TPNH; UMP, uridine monophosphate; FMN, flavine mononucleotide; FAD, flavine-adenine dinucleotide; F-A, formic acid; A-F, ammonium formate.

* This work was done under the terms of Contract AT(30-1)-911 of the Physiology Department, Tufts University School of Medicine, with the Atomic Energy Commission.

the rotating tubes was employed. The distance between lamp surface and tube surface was 1 cm. Each lamp emitted approximately 62 ergs/m²/sec when measured at 8 ft. Following 45 min irradiation at 0–2°, the particles were reisolated, and the supernatants filtered. The filtrates were deproteinized with $HClO_4$ and, after neutralization with KOH and centrifugation, the supernatants were applied to two columns (10 × 150 mm) of Dowex-1, formate form, 200–400 mesh, 10% cross linkage, washed with water and elution of the various nucleotide fractions accomplished according to HURLBERT *et al.*[8]. The control and irradiated samples were eluted simultaneously from a common mixing flask and the drop rate from the columns maintained at equal rates. Approximately 10-ml fractions were collected and the extinction at 260, 275 and 290 mμ, as well as the radioactive level, of each fraction was determined. Each peak was analyzed qualitatively according to the criteria of BEYER *et al.*[7] and quantitatively by the use of molar absorbancy indices.

Fig. 1 contains typical plots of the simultaneous chromatography of irradiated and control samples. ADP and ATP are easily identified by their labelling with radioactive phosphate. It may be seen that DPN is entirely absent from the control supernatant, but appears in the irradiated. The peaks labelled Ad and AD-X have been reported to represent $HClO_4$-conversion products of DPNH and TPNH respectively[9] and appear in both supernatants although in greater quantity in the irradiated. The UMP level appears to be higher in the irradiated supernatant while the yellow fraction (Y) appears to be approximately equal in both cases. This latter fraction, presumably flavin in character but corresponding to neither FMN nor FAD[7], has recently been implicated in the mitochondrial inhibition of glycolysis[10]. It may be noted that slightly more ATP appears in the supernatant of the control mitochondria. However, the AMP, ADP and inorganic phosphate levels are considerably higher in the irradiated supernatant. This may be interpreted as a hydrolysis of ATP by the irradiated mitochondria and a subsequent release as breakdown products of ATP.

Table I contains the quantities of the various nucleotides which are released as calculated from molar absorbancy indices. It is evident that in all fractions except AP, there is a significantly greater release of nucleotides from the irradiated mitochondria than from the control. The simple addition of these nucleotides to irradiated mitochondria, however, does not restore either the oxidative or phosphorylative capacity of these particles[11].

TABLE I

NUCLEOTIDE LEVELS OF SUPERNATANTS FROM IRRADIATED AND NON-IRRADIATED MITOCHONDRIA

Fraction	*Amount (moles × 10⁸)*		*I/C**
	Control	*Irradiated*	
Ad	4.3	8.1	1.89
DPN	0	25.3	—
AMP	27.8	99.4	3.58
UMP	37.7	53.6	1.42
ADP	33.9	66.6	1.96
AD-X	35.4	47.2	1.33
ATP	39.3	35.8	0.92

* Ratio of values for irradiated and control supernatants

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Received January 17th, 1958