REGULATION OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION BY CHLOROPLASTS

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ABSTRACT

Addition of intact chloroplasts to mitochondria resulted in uncoupling of oxidative phosphorylation. Mitochondria of plants appeared to be more susceptible to this uncoupling effect than the mitochondria of animals. The uncoupling principle in chloroplasts was identified as the xanthophyll lutein which also induced passive swelling in mitochondria. For the first time, these results provided biochemical evidence that chloroplasts regulated the functions of mitochondria. This study highlights the existence of inter-organellar interaction in the regulation of energy metabolism.

INTRODUCTION

FROM the point of view of energy metabolism the cells of photosynthetic eukaryotes are the most complex, so far encountered. Their development and function involve the interaction of two energy-transducing systems namely, chloroplasts and mitochondria. The question whether mitochondrial oxidative phosphorylation operates or not during active photosynthesis in the light has been raised[1]. The entire energy needs of the mature plant during illumination can be met from photophosphorylation by chloroplasts[2]. Presumably, a further increase in phosphate potential[3] (ATP concentration) by mitochondrial oxidative phosphorylation would exert a feedback effect on both the energy transducing systems. However, a decrease in the oxygen concentration in the cytoplasm is essential for the efficient fixation of carbon dioxide and minimization of photorespiration[4]. This would necessitate that mitochondrial oxidation in the light should proceed without being tightly coupled to ADP phosphorylation. Here we report that chloroplasts uncouple mitochondrial oxidative phosphorylation. The uncoupling principle in chloroplasts has been identified as the xanthophyll, lutein which induces passive swelling in mitochondria. These results appear to provide for the first time biochemical evidence for inter-organellar interaction in plants in the regulation of cellular energy metabolism and function.

MATERIALS AND METHODS

Tightly coupled mitochondria from six-day old mung bean (*Vigna radiata*) seedlings and from rat liver were prepared by differential centrifugation[5,6]. Chloroplasts were isolated from the leaves of alfalfa (*Medicago sativa*) and groundnut (*Arachis hypogea*) by ficoll density gradient centrifugation[7]. The intactness of chloroplasts was confirmed by ferricyanide-dependent oxygen evolution on illumination[8].

The uptake of oxygen was monitored at 30°C polarographically using a Clark-type oxygen electrode in a Gilson K-1CT-C oxygraph[6]. The reaction system to measure the oxidative phosphorylation was essentially the same as described earlier[6] and contained 1 mg of mitochondrial protein in a total volume of 1.4 ml. After adding the substrate (2.5 μmol of glutamate + 2.5 μmol of malate or 2.5 μmol of succinate), 'state 3' oxidation[9] was initiated by adding 300 nmol of ADP.

Mitochondrial volume change was monitored at 25°C by the absorbance decrease at 560 nm[10]. The reaction system contained 25 mM tris-Cl buffer, pH 7.4, 130 mM NH_4Cl and 2 mg of freshly prepared mitochondrial protein in a total volume of 3 ml. To prepare the electron micrographs, freshly prepared rat liver mitochondria (10 mg protein) suspended in 0.5 ml of the swelling medium were incubated with the factor (8 uncoupling units; 50–100 nmoles) for 30 min at 4°C. Mitochondria were then sedimented by centrifugation, fixed with glutaraldehyde, post-fixed with OSO_4, stained with uranylacetate, dehydrated and embedded in araldite. Silver grey sections were analysed in a Philips EM-301 electron microscope at 80 kV[10].

Protein was estimated by the biuret method[11] and chlorophyll by calorimetry[12]. All biochemicals were obtained from Sigma Chemicals Co., MO, U.S.A. All other reagents used were of the purest grade available. Solutions were prepared in double-distilled water in an all-quartz apparatus.

RESULTS

In intact mitochondria, the rate of phosphorylation determines the rate of substrate oxidation[9]. In the presence of substrate (state 2), oxygen uptake is stimulated on addition of ADP (state 3). In state 4 respiration decreases on exhaustion of ADP[9]. A substance which delinks phosphorylation from oxidation (uncoupler) stimulates respiration in the absence of ADP (state 2 or state 4).

Addition of small amounts of chloroplasts (100–300 μg equivalent of chlorophyll) to rat liver or mung bean mitochondria stimulated state 2 (Substrate added) and state 4 (ADP exhausted) oxidation (figure 1). Subsequent addition of ADP failed to stimulate respiration or invoke state 3-state 4 transition indicating loss of respiratory control. Addition of 2,4-dinitrophenol which stimulates respiration by abolishing the respiratory control showed no effect in the presence of chloroplasts. The antibiotic oligomycin inhibits respiration in tightly coupled mitochondria because of its inhibitory action on H^+-translocating ATPase. The inhibition of respiration imposed by oligomycin is relieved by uncouplers like dinitrophenol. It may be seen that chloroplasts reversed the inhibition of oxidation imposed by oligomycin (figure 1A). Respiration stimulated by chloroplasts was sensitive to respiratory chain inhibitors like antimycin A and cyanide, and was insensitive to inhibitors of photosynthetic electron transport like N-dichlorophenyl-N′-dimethylurea. With increase in the concentration of chloroplasts, state 3 (ADP present) oxidation was partially inhibited, particularly with NAD^+-linked substrates. Plant mitochondria appeared to be more sensitive to this uncoupling action of chloroplasts.

Chloroplasts disrupted by freezing (−15°C) and thawing or by sonication also effectively uncoupled mitochondria. Denaturation of chloroplasts by keeping in boiling water for 15 minutes decreased the uncoupling activity by more than 85%.

The photosynthetic electron transport system of chloroplasts has been resolved into two: photosystem I and photosystem II[13]. Uncoupling activity was found to be associated with particles rich in photosystem II. Chloroplasts prepared from plants fixing carbon dioxide by both C_3- and C_4-pathways showed uncoupling activity (data not given).

Since xanthophylls are predominantly associated with photosystem II[14], the pigment fraction was isolated from chloroplasts and was found to uncouple mitochondrial oxidative phosphorylation. The active

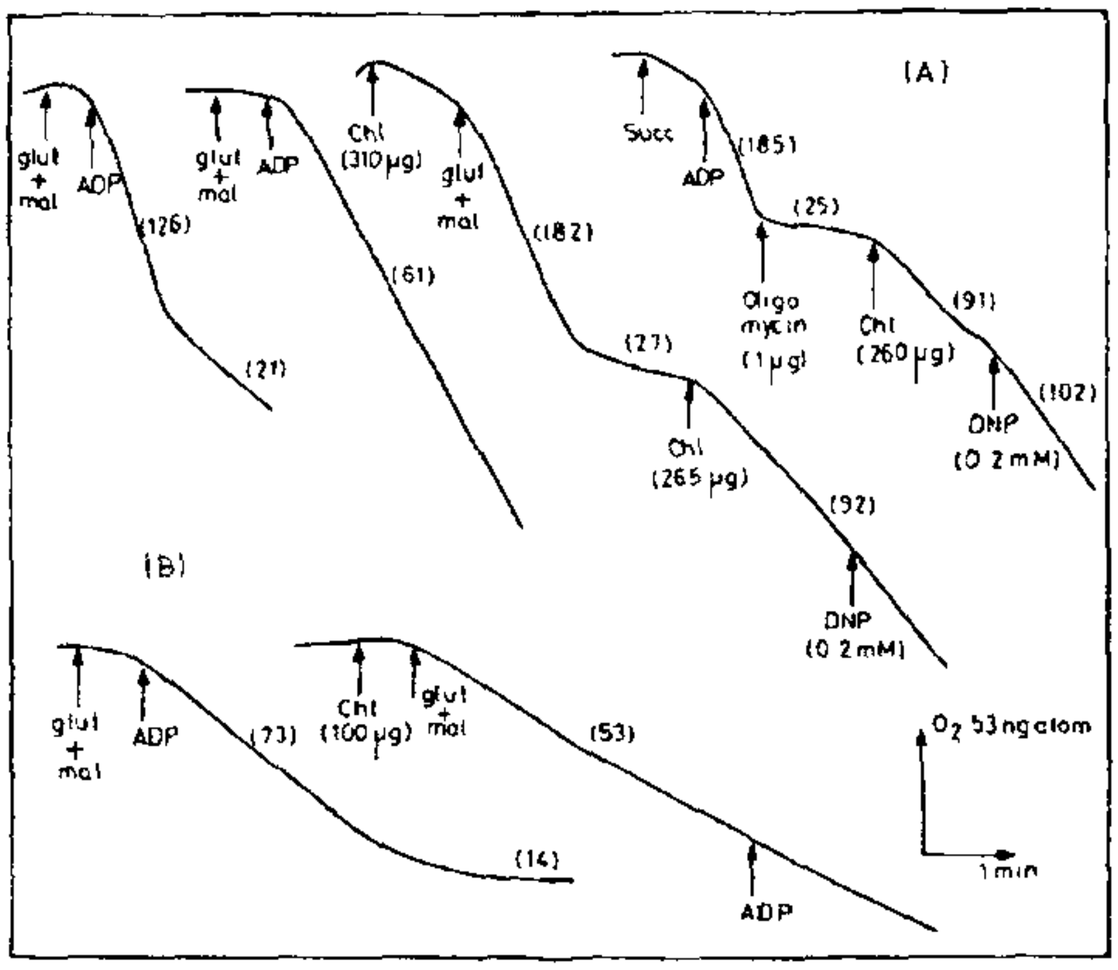

Figure 1. Uncoupling of mitochondrial oxidative phosphorylation by chloroplasts. The effect of addition of groundnut chloroplasts to rat liver mitochondria (A) and alfalfa chloroplasts to mung bean mitochondria (B) are shown. After the addition of substrate (2.5 μmol of glutamate + 5 μmol of malate or 25 μmol of succinate as indicated) state 3 oxidation initiated by the addition of 300 nmol of ATP. Chloroplasts (100–300 μg chlorophyll) were added as indicated. The values in parentheses represent the rate of oxygen uptake in ng atom 0/min per mg protein. The chloroplast suspension medium had no effect on respiratory control. glut = glutamate; mal = malate; Chl = chloroplast; DNP = 2,4-dinitrophenol.

principle was obtained in pure form and identified as lutein[12]. Details are being published elsewhere. The compound uncoupled both plant and animal mitochondria and reproduced all the effects observed with whole chloroplasts represented in figure 1.

To understand the mechanism of the uncoupling action of the compound, we have studied its effect on mitochondrial structure and morphology. The purified factor induced swelling in mitochondria as evidenced by light scattering (figure 2). The volume change induced was proportional to the concentration of the uncoupler added and was not inhibited by respiratory chain inhibitors and ATP.

Electron micrographs of the swollen mitochondria revealed drastic alterations in mitochondrial ultrastructure and morphology. The cristae were distended leaving voluminous matrix space. The outer membrane appeared intact and closely adhered to the inner membrane (figure 3).

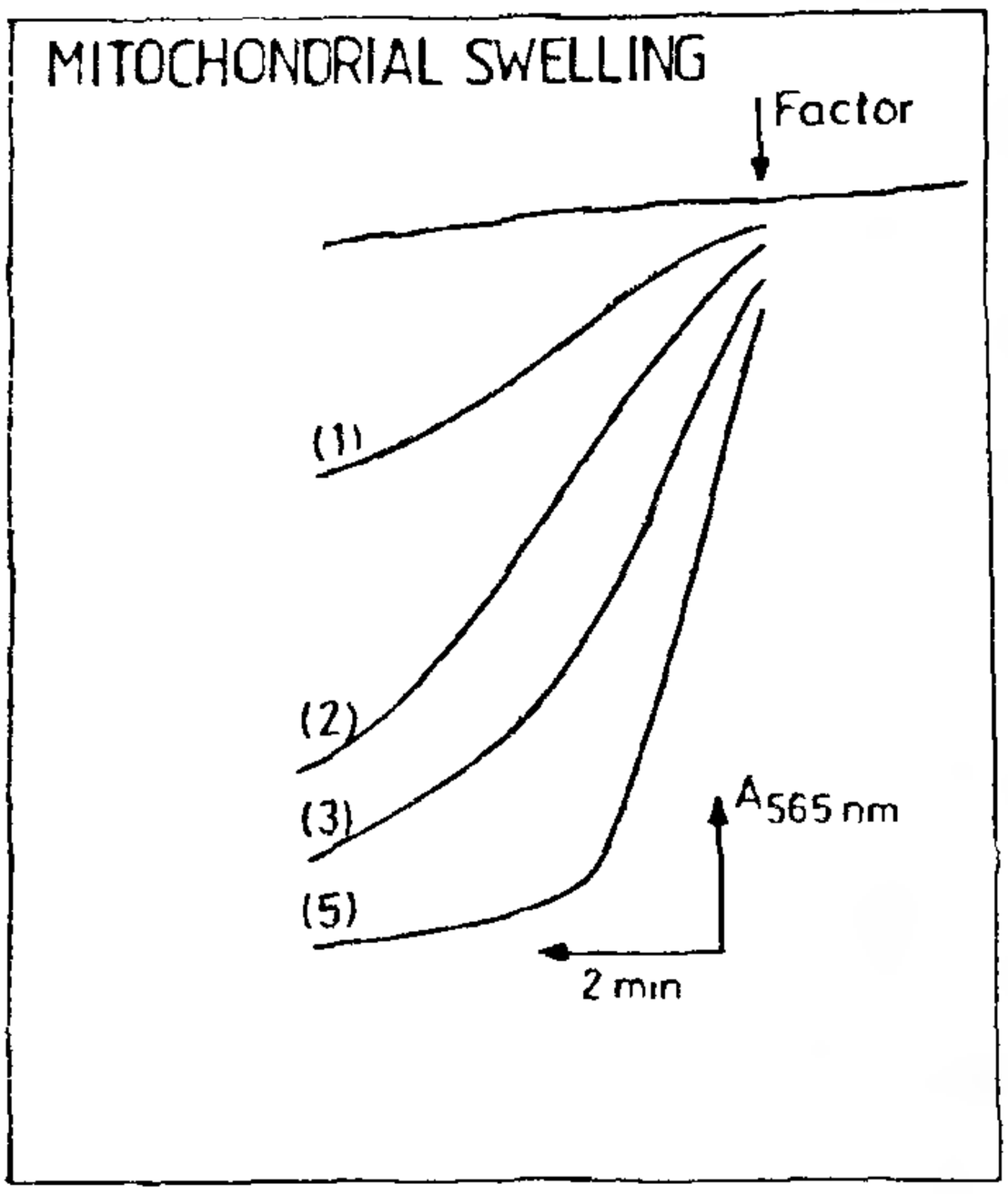

Figure 2. Swelling of rat liver mitochondria induced by the uncoupling factor. Mitochondrial volume change was measured by decrease in absorbance at 565 nm. After spontaneous swelling has ceased, an ethanolic solution of the uncoupling factor was added in the 'sample' cuvette as indicated. Ethanol alone produced no effect. The values in parenthesis indicate the uncoupling units (amount of uncoupler required to abolish respiratory control of 1 mg protein of mitochondria, about 5–10 nmol). Absorbance change was recorded in a Shimadzu UV 200 Spectrophotometer.

DISCUSSION

The interaction between chloroplasts and mitochondria reported in this paper offers biochemical basis for observations earlier recorded in the literature. Thus, light-induced development of the photosynthetic apparatus in jack bean leaves leads to swelling, decrease of respiratory control and increase of respiration in mitochondria[15]. Similarly in cyanobacteria, photophosphorylation turns off oxidative ATP synthesis[16]. Blue light-induced stimulation of respiration has been observed in a mutant of chlorella which lacks chlorophyll but contains carotenoids[17,18]. It may also be mentioned that the chloroplast envelop which is devoid of chlorophyll contains xanthophylls[14]. This would suggest inter organellar transport either by contact or through carrier proteins. The presence of a

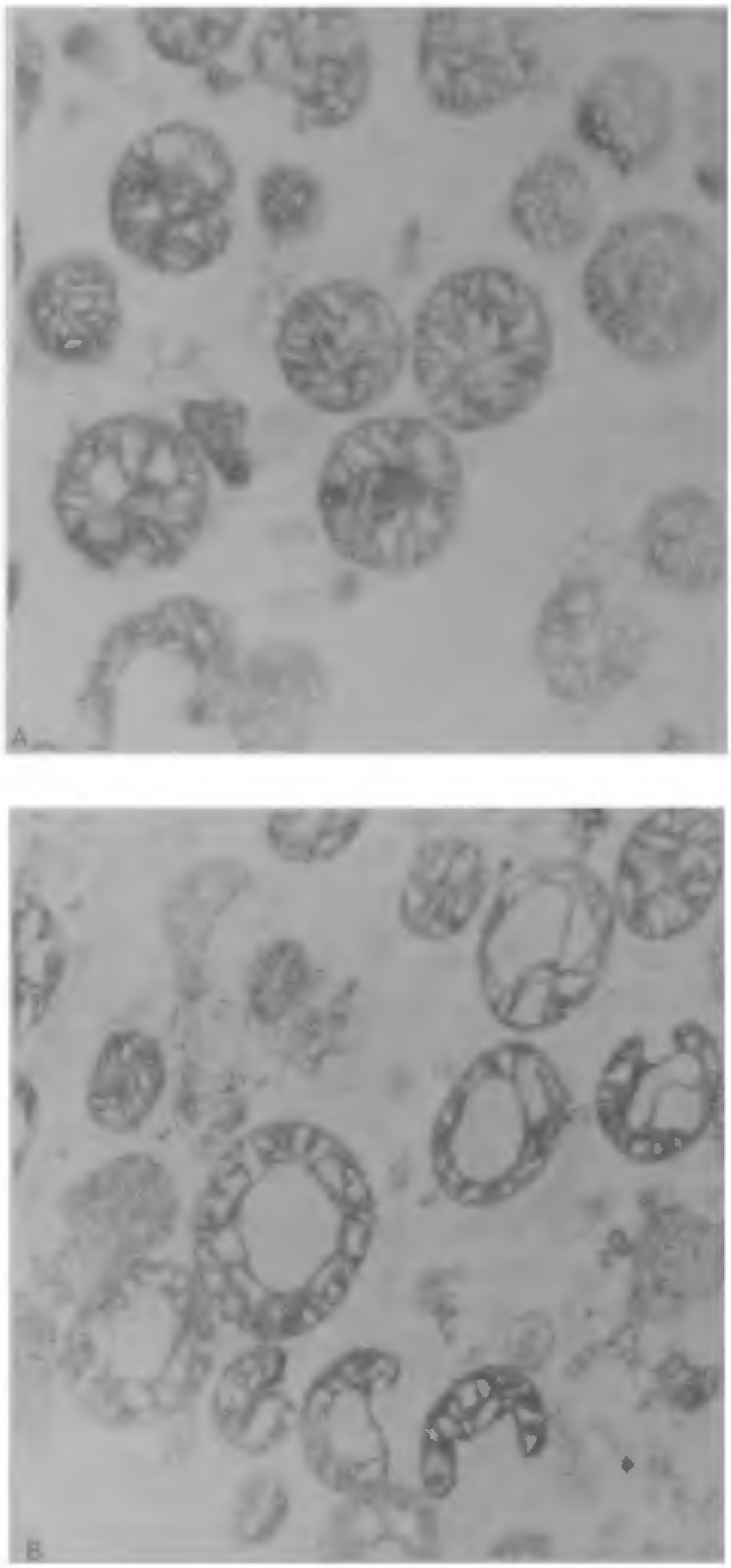

Figure 3. Electron micrograph of rat liver mitochondria showing uncoupler-induced swelling. Details as given in text. Control—A; Swollen—B; × 10,500.

specific peroxidase system which degrades xanthophylls in plant mitochondria[14] presumably ensures the reversibility of the effect. However, the regulatory role of light in this process remains to be elucidated. Our results assign a hitherto undiscovered role for xanthophylls which are ubiquitous in photosynthetic systems.

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ANNOUNCEMENT

ALL INDIA SYMPOSIUM ON RECENT TRENDS IN INSECT ENDOCRINOLOGY

An All India Symposium on Recent Trends in Insect Endocrinology Sponsered by University Grants Commission, New Delhi is scheduled to held from 9–12 September 1985 at the Department of Zoology, Government College, Ajmer under the Directorship of Shri J. N. Mathur.

The Symposium shall have a few major scientific session and invited lectures on the important themes. Papers on any branch of Insect Endocrinology are welcome. The abstract of all contributions and full text of the presented papers are proposed to be published in book form.

For further details please contact Dr Sudhir Bhargava, Symposium-Secretary, "Recent Trends in Insect Endocrinology", Department of Zoology, Government College, Ajmer 305 001, Rajasthan. Last date of receipts of abstract is August 1, 1985.