

had shown the accumulation of glycolic and  $\alpha$ -ketoglutaric acids in addition to the other acids (Table I).

TABLE I

Different organic acids accumulated in the culture filtrates of cultures grown under light, dark and alternate and dark conditions for 9 days at 30°C

| Control<br>(Normal<br>incubation) | Light  | Dark              | Light-<br>dark | Dark-<br>light |
|-----------------------------------|--|-------------------|----------------|----------------|
| Oxalic                            | Oxalic   |                   | Oxalic         | Oxalic         |
| Citric                            | Citric   | Citric            | Citric         | Citric         |
| Isocitric                         | Isocitric<br>Succinic<br>$\alpha$ -Keto-<br>glutaric<br>Glycolic | Succinic<br>Malic | Succinic       | Succinic       |

Legend:

Light-dark: Light treatment followed by dark treatment.

Dark-light: Dark treatment followed by light treatment.

It has been found that light plays a significant role on the biosynthesis and accumulation of citric acid in the culture of *A. niger* 6N3. Generally, the synthesis and accumulation of citric acid occurs in mold cultures through the Krebs tricarboxylic acid cycle<sup>8-9-10</sup>. Presence of succinic,  $\alpha$ -ketoglutaric, isocitric and glycolic acids in the culture filtrate of cultures grown under light condition probably suggest the presence of some alternate pathways like S.K.I. cycle<sup>2</sup> (Succinate- $\alpha$ -ketoglutarate-isocitrate). Therefore, the low citric acid yield may be due to depletion of the intermediates of TCA cycle to the S.K.I. cycle, necessary for the normal functioning of the TCA cycle. Production of citric acid by fermentation can be kept more or less constant if the fermentation is carried out in the dark as there should be very little chance under such a condition for a deviation in the normal pathway through which citric acid is produced.

The authors wish to express their gratitude to the International Atomic Energy Agency, Vienna, Austria, for financial assistance.

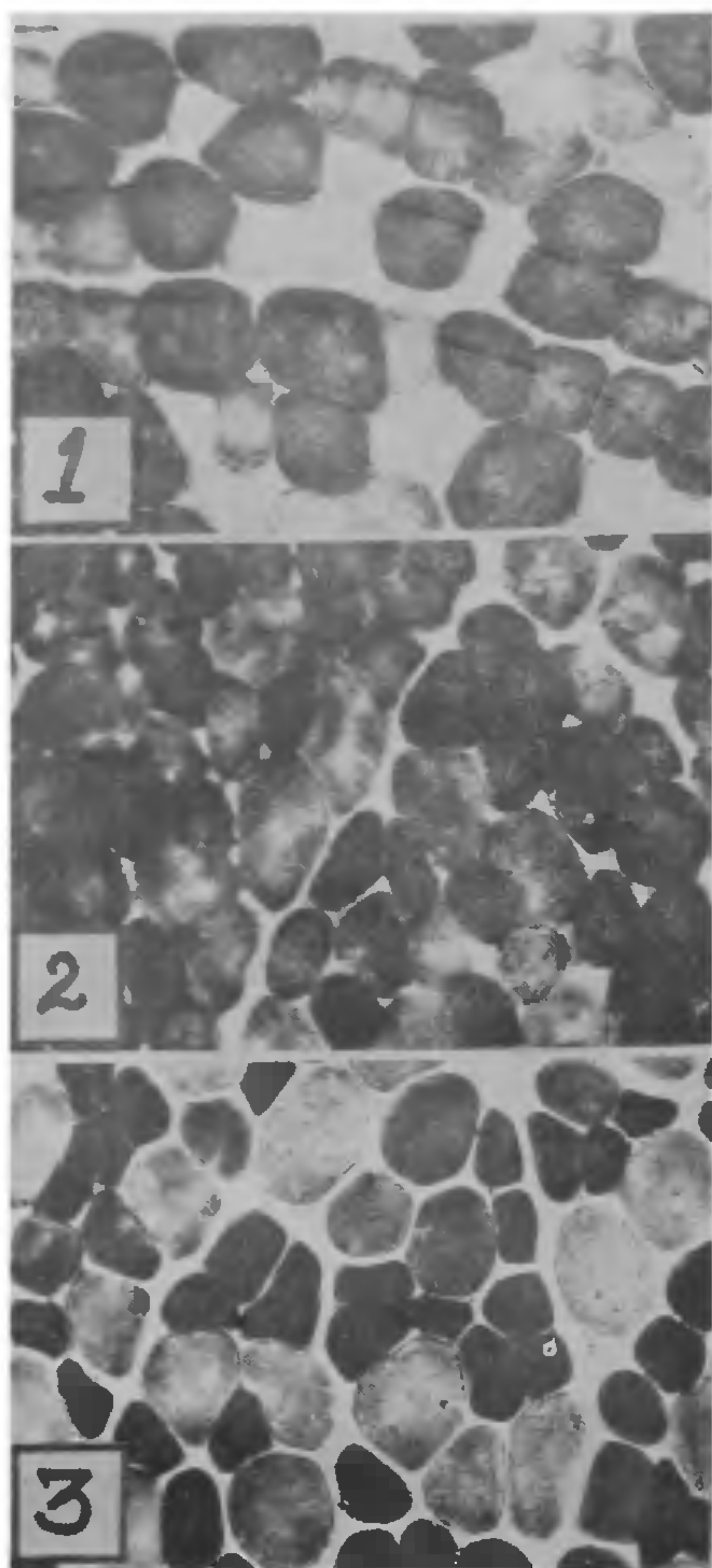
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#### REEVALUATION OF $\alpha$ -GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY IN SKELETAL MUSCLE FIBRE TYPES

CONFLICTING reports have appeared in the literature about the histochemical localization and distribution of lactate dehydrogenase (LDH) and NAD<sup>+</sup>-linked  $\alpha$ -glycerophosphate dehydrogenase (GPD). When assayed *in vitro* white muscle fibres show higher GPD activity, but show lower enzyme activity than the red muscle fibres in histochemical tests<sup>1-4</sup>. It has since been demonstrated, particularly for LDH<sup>5</sup>, that when a sequential staining technique involving the use of phenazine methosulfate (PMS) is used, the white muscle fibres show higher enzyme activity than the red ones. This has led to the suggestion that PMS should be used for the histochemical demonstration of dehydrogenases's activity in locations where diaphorases are a limiting factor<sup>8-10</sup>.

It has also been demonstrated<sup>6</sup> that LDH and possibly GPD also survive formalin fixation, provided the muscle sections have been fixed in formalin vapour. This step is important since without prior fixation, considerable leakage of these two dehydrogenases may take place particularly from white muscle fibres<sup>1,6</sup>. Therefore, using PMS (0.4 mg per ml of the incubation medium<sup>6</sup>) and formalin vapour fixation of the muscle sections, we have reevaluated the histochemical profile of GPD activity in mixed skeletal muscle fibre types. The diaphragm of the rhesus monkey (*Macaca mulatta*) was used in the present study. Formalin vapour fixed sections, 10  $\mu$  thick, were processed for the histochemical demonstration of GPD activity<sup>11</sup>. By varying the concentration of PMS in the incubation medium it was possible to elicit varying histochemical profiles, with higher or



Figs. 1-3. Fig. 1. Histochemical localization of GPD activity in the cross-section of monkey diaphragm using excess PMS in the incubation medium. Note darkly stained white and intermediate fibre types and unstained red fibres,  $\times 50$ . Fig. 2. Same as in Fig. 1, with lesser concentration of PMS in the incubation medium. Note comparatively darker staining of the smaller red fibres as compared to the larger white fibres.  $\times 50$ . Fig. 3. T.S. of monkey diaphragm, stained for lipids with Sudan Black B for the identification of the three basic muscle fibre types. Note the strong, medium and least staining for lipids in the red, intermediate and the white fibres respectively,  $\times 50$ .

lower GPD activity in the white, red and intermediate muscle fibre types (Figs. 1, 2). When excess of PMS (higher than 0.4 mg/ml) is used in the incubation medium, there is suppression of the enzyme activity in the red fibres and a considerably higher activity could be elicited in the white fibres (Fig. 1). Monkey diaphragm served as a useful material for the present study since it contains all the three histochemically demonstrable muscle fibre types (Fig. 3). A more correct profile for GPD activity may be demonstrated in the various fibre types by employing the sequential staining technique (producing a combination of Figs. 1 and 2) as suggested earlier for the correct localization of LDH activity in mixed muscles<sup>7</sup>.

This study was supported in part by a research grant from the U.G.C. to one of us (C. L. T.).

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#### THE CHROMOSOMES OF *COPTOSOMA INDICA* MONTAND (HETEROPTERA—PLATASPIDAE)

AMONG Heteroptera the cytology of the family Plataspidae has received little attention. The species worked out are *Brachyplarys subaeneus*, *Coptosoma biguttula*, *Coptosoma* sp., *C. punctatissimum*, *C. cribrarium*, *C. variegata* and *C. scutellatum*<sup>1-5</sup>. The cytological data suggest that the modal number of chromosomes in the family is 12 (10 + X + Y)<sup>1-5</sup>. The present study is based on an examination of