state as well as in solution, is comparable to the electronic spectra of the complexes having the pentacoordinate symmetry[13, 15]. The band at Ca. 13.4 kK observed in the electronic spectra, in addition to the absorption at Ca. 6.1 kK and Ca. 17·1 kK shows the presence of five coordinate cobalt(II) ions.

Nickel(II)–SBTS

The solid state and solution spectra of the complex resemble to the typical patterned electronic spectra of the well-known five coordinate chromophores having a distorted trigonal bipyramidal stereochemistry[14, 16] and differ from those of octahedral complexes in that the low energy band at Ca. 8·9 kK. is considerably less intense than the band at Ca. 14·2 kK.

Copper (II)–SBTS

The electronic spectra of copper(II)–SBTS complex exhibit one broad band at Ca. 14·0 kK along with a shoulder on low energy side and agree reasonably well with the spectra of various five coordinate copper(II) complexes[17,18]. Although in the case of copper compounds on the basis of electronic spectra it is difficult to say about symmetry, but in the light of above discussions (isomorphous nature, non-electrolytic behaviour and i.r. discussions) it appears that copper-SBTS is also probably pentacoordinated.

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A 3-D MODEL FOR MITOCHONDRION BASED ON STUDIES OF ULTRATHIN SERIAL SECTIONS

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ABSTRACT

The model proposed for mitochondria is ellipsoidal and may develop one or two depressions on elongated surface(s) during changed metabolic and/or physiological conditions. This model on being sectioned in various determined planes yields mitochondrial profiles corresponding to those obtained from randomly sectioned *Neurospora* mycelia and from other tissues reported in the literature previously.

MITOCHONDRIA are known to exist in a variety of shapes and sizes and have dynamically changing morphologies. Their size, number, internal fine structure and function have been recognised to be more or less tissue specific. Considerable ambiguity about the shape of mitochondria is apparent from electron microscopic studies. They are seen to range from spheres to extremely anisometric forms and their morphology would appear to be transitory and irregular. This is partly due to the difficulty in controlling the plane while sectioning cells in which mitochondria are suspended.

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The purpose of this report is to present a three dimensional mitochondrial model which on being sectioned through different planes would yield most of the mitochondrial profiles reported by various authors[1–3]. Based on the micrographs (Fig. 1, *a–d*) obtained from sequential sections of a mitochohdrion from growing *Neurospora* mycelia[4], we designed a clay model to attempt to explain the many forms obtained when mitochondria are sectioned.

The model we propose is ellipsoidal, with a pit like depression on one or may be two opposite elongated surfaces (Fig. 2, *a, b*). This depression corresponds to the roughly circular area of low electron density in the replica of isolated mitochondria described by Maser[5]. As can be seen in Fig. 2 (*c–f*) a variety of mitochondrial shapes are obtained when such a model is cut in various determined planes. When the model is placed with its long axis parallel to the horizontal plane and is cut transversely (E–E') a ring shaped mitochonrial section is obtained (Figs. 2*e*, 1*c*). A vertical section (F–F') gives a 'U' or 'V' shaped profile (depending on the shape of the pit), Figs. 2*f*, 1*a* which is not unlike the cup shaped mitochondria described in albino rat testis[2]. Sectioned in a tangential plane (C–C') when the pits are deep, two mitochondria can be seen (Fig. 2*c*). By further

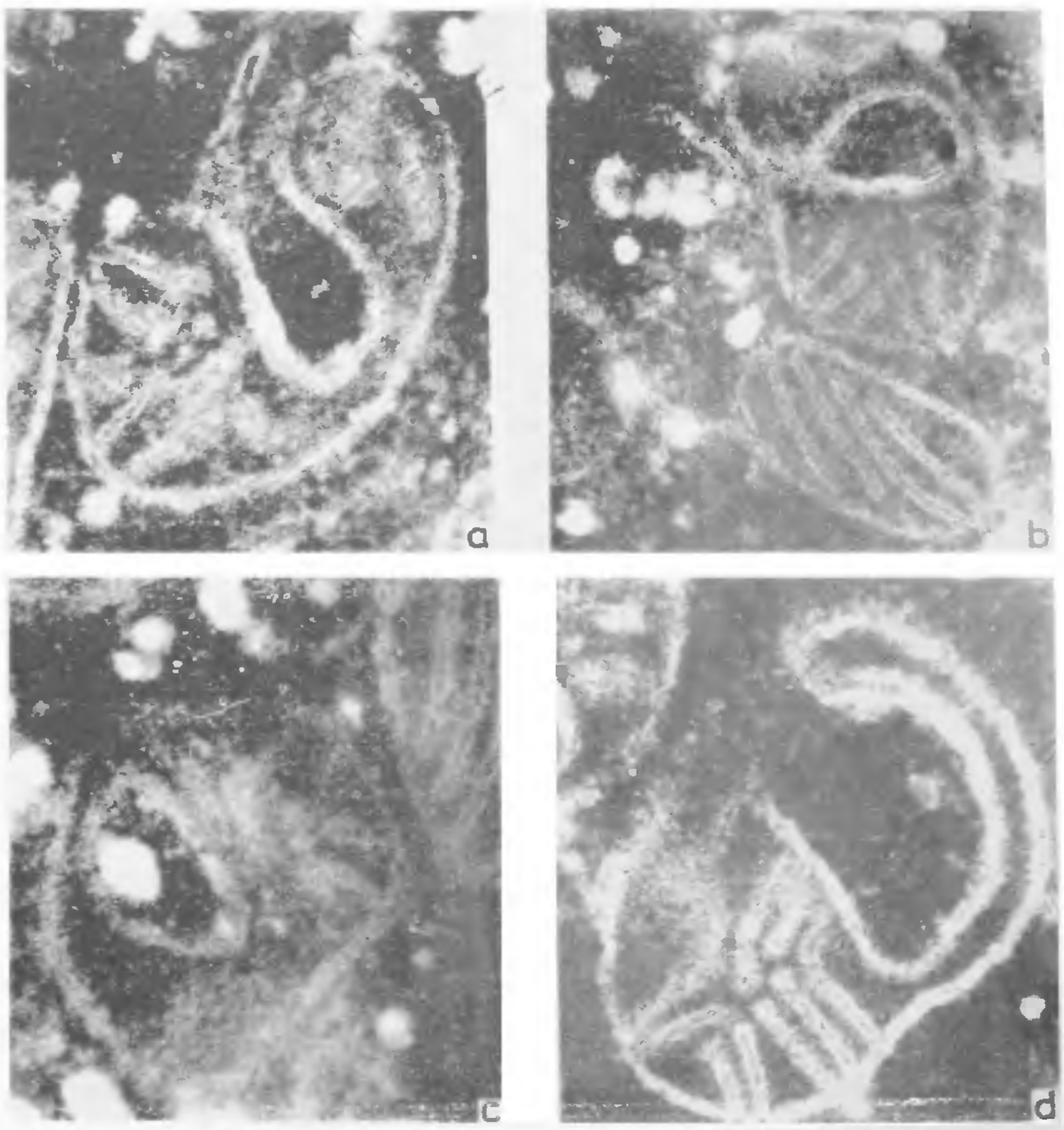

FIG. 1. Assorted mitochondrial profiles from *Neurospora* mycelia in log phase fixed in 3% gluteraldehyde in phosphate buffer (0·1 M, pH 7·3), embedded in GMA. The figure shows (*a*) U-shaped, (*b*) approaching ring shape, (*c*) complete ring and (*d*) 'hooded' mitochondria.

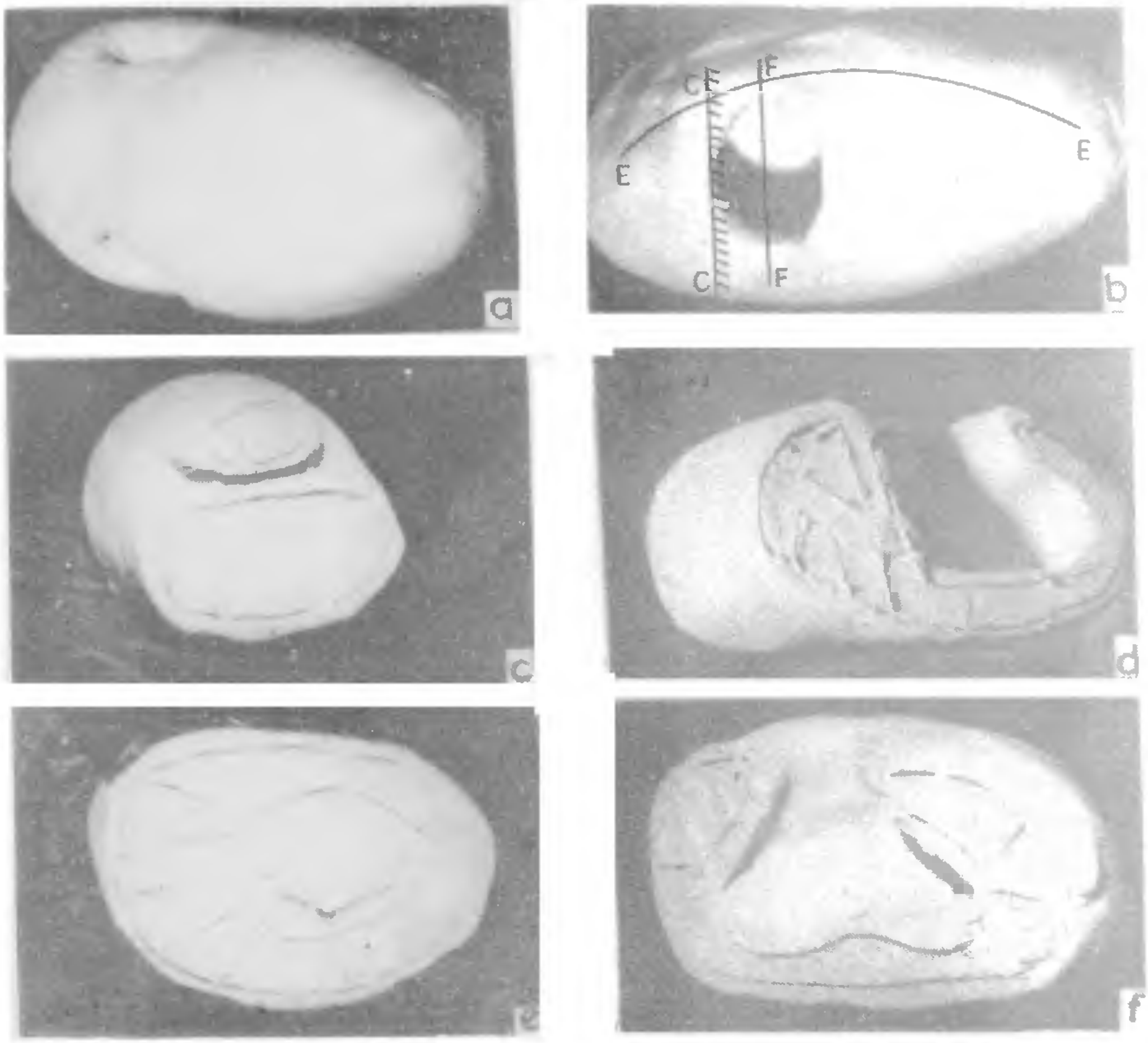

FIG. 2. Clay model showing likely positions where pits develop on the mitochondrion (*a*) and (*b*). Fig. 2*b* shows the planes of sectioning along the lines C–C′, E–E′, F–F′, which yields the profiles shown below in figures *c*, *d*, *e* and *f*.

varying the angle of cutting, other assorted shapes can also be obtained. The 'hooded' mitochondrial section in Fig. 1*d* and to a similar profile reported in bovine oocytes[1].

We maintain that mitochondria are basically only ellipsoidal or rod shaped. There is evidence to show that it is possible for mitochondrial morphology to be altered by changed metabolic and physiological conditions, both when induced[6, 7] and spontaneous[1, 8]. Under changed metabolic and physiological conditions, there appears a constriction(s) on the rod shaped mitochondrion. On being sectioned while in this form, mitochondria yield the various reported shapes including spheres, rods and V-shapes[9, 10]; also refer Fig. 1, *a–d*. Berger[11] has reported both rod and V shaped profiles of mitochondria in rat hepatocyte. The liver being a metabolically very active organ, mitochondria are placed under conditions wherein they have to divide rapidly. At the time of division, with the formation of the pit-like depression on the mitochondria, an increasing number of V-shaped profiles would appear on sectioning. It may be noticed that despite this, the ratio of rod shaped mitochondria to V-shaped ones is 2 : 1, suggesting that the native state of the mitochondria is rod shaped and that during division or stress, the altered mitochondria, which are suspended haphazardly in the cell, yield various other profiles on being sectioned.

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CATALYTIC POTENTIAL OF GLUTAMATE DEHYDROGENASE IN NORMAL AND FATIGUED GASTROCNEMIUS MUSCLES OF FROG

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ABSTRACT

Substrate dependent and co-factor dependent kinetic parameters of glutamate dehydrogenase (GDH) have been investigated in the normal and fatigued gastrocnemius muscles of frog (*Rana hexadactyla*). The fatigue phenomenon seems to induce a mixed type of inhibition by decreasing the maximal velocity (V_{max}) and increasing the Michaelis-Menten constant (Km). The activation energy values for the muscle enzyme were increased on fatigue, suggesting a decreased catalytic efficiency of fatigued muscle enzyme.

INTRODUCTION

WHEN a gastrocnemius muscle is subjected to repeated electrical stimuli, fatigue substances accumulate in the tissue and inactivate the enzyme systems[1,2], inducing fatigue[3]. A significant decrease in succinate, malate, lactate dehydrogenases and pyruvate oxidase[4] and an increase in protease activity levels[5] have been reported during muscular fatigue. This increased proteolysis may affect the existing ammonia metabolism in the tissue. Since GDH is known to play a significant role in the regulation of ammonia metabolism of the tissue, an attempt has been made in the present investigation to study the kinetic parameters of the enzyme so as to assess the specific impact of fatigue on the catalytic potential of the enzyme.

MATERIALS AND METHODS

Healthy medium sized frogs, *Rana hexadactyla*, were double pithed and the gastrocnemius muscles from both the legs were excised with least injury. The muscles were washed 3 to 4 times in amphibian Ringer's medium[6] and then allowed to stand in the same solution for ten minutes to recover from shock effects. One of the muscles was placed in 10 ml of amphibian Ringer's medium and subjected to repeated biphasic direct electrical stimuli of 10 volts at a pulse frequency of 60/min using Inco/CSIO research stimulator Model MR (Ambala-3, India). The stimuli were given continuously until the muscle did not respond to fresh stimuli, exhibiting fatigue phenomenon. The contralateral muscle was also kept in the similar medium and was not subjected to electrical stimulation. Both these muscles were chilled to 5° C to arrest residual metabolism and 10% (W/V) homogenates were prepared separately in 0·25 M sucrose solution using Potter-Elvehjem homogenizer and centrifuged at 2500 rpm for 15 min to remove the cell debris and the supernatants were dialyzed overnight in a dialysis bag at 0° C against the suitable medium (0·25 M sucrose solution). The GDH (EC 1.4.1.3) activity was estimated by the method of Lee and Lardy[7] modified by Pramilamma *et al.*[8] and the enzyme activity is expressed in μmoles of formazan/mg protein/hr. The maximal velocities (V_{max}), Michaelis-Menten constants (Km) were calculated by the method of least squares. The activation energy (E) was calculated as given by Dixon and Webb[9].

RESULTS AND DISCUSSION

After initial standardization, the activity levels of NAD-GDH were determined in normal and fatigued gastrocnemius muscle homogenates of frog. An enzyme concentration of 50 mg and 30 minutes of incubation time were selected for the present study to ensure initial velocity. The substrate dependent activity of GDH was studied at 7·4 pH (sodium phosphate buffer) and 37° C (optimum temperature) with 0·1 mM concentration of NAD (co-factor) and with graded substrate concentrations ranging from 0·5 to 15 mM of glutamate.