crude extract per ml) at a conc. of 150 mg (wet weight) per ml for 120–180 min. Samples of the digested suspension were diluted eight times with 1 molar mannitol and examined by light and phase contrast microscopy. Protoplasts can easily be differentiated on the basis of morphological characteristics (figures 1 A and B). The number of protoplasts as determined by differential cell count using a haemocytometer were expressed as a percentage of the total cell count. (table1)

Table 1 shows that the yield of protoplasts from *S. cerevisiae* after the treatment with the crude digestive juice for 135 minutes, lies between 60 and 70%.

This yield of protoplasts could be increased by pretreatment with thiol reagents if desired[1]. It was also found that the increase/decrease in the concentration of crude enzyme also decreases protoplast yield.

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STUDIES ON THE TOXIC EFFECT OF *CALOTROPIS GIGENTEA* LATEX ON *ASPERGILLUS JAPONICUS*

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PLANT latices are known to show potent antimicrobial activity[1,2]. A hydrocarbon degrading strain of *A. japonicus* was isolated[3], which could transform the components of *Euphorbia nerifolia* and *Calotropis gigentea* latices[4,5]. Cell lysis took place within 72 hr, during transformation of *C. gigentea* latex. In the present investigation we demonstrate that one of the toxic effects of *Calotropis* latex is lysis of mitochondria of *A. japonicus*. Further, *in vitro* studies indicated that succinate dehydrogenase (SDH) activity was inhibited by two lipid components isolated from the *Calotropis* latex.

The cultivation of *A. japonicus* and the growth conditions are the same as described earlier[5]. The preparation of cell free extract (CFE) was essentially the same as reported earlier[6], except that Tris-HCl buffer (pH 7.2) was used for the preparation of CFE. In order to isolate mitochondria, the mycelia were frozen and subjected to mild grinding with glass powder. The extract was then suspended in TSE buffer pH 7.5 (0.44 M sucrose, 0.2 M EDTA, 10 mM Tris-HCl)[7]. The preparation was centrifuged at 1,000 g for 10 min to remove cell debris and the resulting supernatant was centrifuged at 34,000 g for 45 min. The mitochondrial pellet thus obtained, was washed twice with TSE buffer and recentrifuged at 34,000 g for 45 min. SDH (EC 1.3.99.1) activity in mitochondria was assayed from the supernatant after the mitochondrial pellet was disrupted by sonication (six 30 sec. bursts with 30 sec intervals). The method employed for assaying the enzyme activity was that of Arrigoni and Singer[8].

Intracellular lipids were extracted as reported by Gunasekeran *et al*[9]. Total lipids were estimated by the method of Bragdon[10]. Qualitative and preparative thin layer chromatographic (TLC) analysis was performed on 0.25 mm and 0.5 mm thick silica gel G (Ranbaxy) plates respectively, using a mobile phase of heptane:ether:acetic acid (80:20:2). The bands were visualized by exposing the plates to iodine vapours.

Upon incubation of mitochondrial pellets (obtained from glucose grown cells) with latex, SDH activity appeared in the supernatant (table 1). The ratio of SDH activity in the supernatant to that in the mitochondrial pellet increased with time suggesting that mitochondria were lysed in the presence of latex. The corresponding ratios in the control set did not show a significant increase, indicating that lysis of mitochondria in buffer was negligible.

Latex also inhibited SDH activity *in vitro*. A 56% inhibition of SDH activity was observed when 0.4% (v/v) latex was added to the assay system. The lipid content in *C. gigentea* latex is about 2.9 g%. Two lipid spots, having R_fs 0.64 (lipid 1) and 0.77 (lipid 2) were detectable by TLC in untransformed latex and in cells of *A. japonicus* grown in latex. They were, however absent in extracts of glucose grown cells. Therefore it was of interest to see the effect of these two lipids on SDH activity. Both the lipids were purified separately by preparative TLC. Lipid 1 (9.04 mg/ml) causes 22% and lipid 2 (9.7 mg/ml) 19% inhibition of SDH activity within 24 hr when incubated with CFE at 4°C

Table 1 *Mitochondria isolated from glucose grown cells incubated in TSE buffer*

Sample	Succinate dehydrogenase activity u/ml.	Ratio of activity in supernatant / activity in pellet
Supernatant 0*	0 (0)	0 (0)
Pellet 0	5.6 (8.6)	
Supernatant 12	1.6 (4.9)	0.575 (0.775)
Pellet 12	2.8 (6.4)	
Supernatant 24	2.3 (6.8)	0.709 (3.26)
Pellet 24	3.2 (2.1)	

* Time in hours. Units for succinate dehydrogenase: Amount of enzyme which brings about decrease of 0.01 OD at 600 nm/min at 30°C. Values within brackets refer to mitochondria incubated in the presence of latex. While values outside brackets refer to those in the absence of latex.

Table 2 *In vitro inhibition of succinate dehydrogenase by lipids*

Cell-free extract (2 ml)* incubated with	Specific activity	% inhibition
1 ml CH_3OH (Control)	0.23	—
1 ml CH_3OH Contg. lipid 1	0.18	22
1 ml CH_3OH Contg. lipid 2	0.1815	19
1 ml CH_3OH Contg. lipid 1 and 2	0.12	45
0.1 ml CH_3OH Contg. lipid 1 and 2	0.12	45

* Succinate dehydrogenase was assayed after cell free extract was incubated for 24 hr as mentioned.

(table 2). A 1:1 mixture of lipids 1 and 2 (9.4 mg/ml) produced about 45% inhibition. Decreasing the concentration of the lipid mixture by 10-fold did not affect the degree of inhibition. Addition of the lipid mixture (4 mg/3 ml) to the assay system showed 44% inhibition of SDH activity. Lipids 1 and 2 were identified as a terpenoid ester and a fatty acid ester, respectively on the basis of TLC and colour reactions[11].

Ghai and Modi[12] reported competitive inhibition of glucose-6-phosphate dehydrogenase and malic enzyme by fatty acids and β-carotene during ripening of mangoes. Vincenzini *et al*[13] reported inhibition of isocitrate lyase by octonate and oleate, in germinating seeds, in which case the inhibition was neither specific nor proportional to the concentration of the lipid. In the present study, the lipid mixture could inhibit enzyme activity when added directly to the assay system. The inhibition was slightly greater when the enzyme preparation was preincubated with the lipids. The presence of both the lipids is necessary to cause the same extent of inhibition as that brought about by untransformed latex. Probably, inhibition of the SDH activity and mitochondrial lysis contributes to latex toxicity towards *A. japonicus*.

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