

olefinic C=C), 1090 and 1070 (C-O-C anti-symmet. stretch); NMR (CCl_4) signals at δ 0.93 (t, 6H, $J=7$ Hz, CH_2-CH_3), 2.43 (q, 4H, $J=7$ Hz, CH_2-CH_3), 2.98 (d, 2H, $J=4$ Hz, allylic coupling CH_2-N), 4.0 (d, 2H, $J=4$ Hz, allylic coupling, $\text{O}-\text{CH}_2-\text{CH}=\text{CH}-$) 4.4 (s, CH_2 , benzylic), 5.6 (m, olefinic protons); mass peaks at m/e 233 (0.2% M^+), m/e 91 (100%) and m/e 77 (7%) (Found: C, 77.20, H, 9.6 and N, 6.0%. $\text{C}_{15}\text{H}_{23}\text{ON}$ requires C, 77.25, H, 9.8, N, 6.03%).

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EFFECTS OF DENERVATION ON THE CALCIUM SENSITIVITY OF SUCCINATE DEHYDROGENASE IN THE AMPHIBIAN GASTROCNEMIUS MUSCLE

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ABSTRACT

The effects of calcium and EDTA were studied on SDH activity levels in the denervated and contralateral control muscles. Calcium and EDTA were found to alter the V_{\max} value of the enzyme, the former increasing while the latter decreasing but with no effect on the K_m . Though calcium was found to modulate positively the SDH activity in denervation atrophy, it is possible that the incapacity in *in vivo* may be due to a shift in metabolic involvement of Ca^{2+} towards the membrane properties and function.

INTRODUCTION

THE denervated and dystrophic muscles exhibited an increase in the Ca^{2+} content¹⁻⁴. This calcium accumulation was found to appear early in the progression of the muscle disease⁵. The same muscles were found to be defective in oxidative phosphorylation³. The importance of calcium in muscle physiology has been recognized since the classical studies of Ringer⁶ and Lehninger⁷ indicated that the intracellular regulation of calcium by mitochondria take primacy over oxidative phosphorylation. Since the denervated muscle has elevated calcium levels and low oxidative enzyme activity, it is worthwhile to see how the calcium and oxidative enzyme activity are correlated.

MATERIAL AND METHODS

Rana hexadactyla (60–80 g) were used in the present investigation. Muscle atrophy was induced in the frogs by sciatic nerve section. The thigh of one leg was opened and a length of 1–1.5 cm of sciatic nerve was removed under aseptic conditions. The contralateral muscle of the other leg was considered as control. The frogs were maintained in aquarium tanks being daily fed with cockroaches.

After 30 days of operation, the frogs were double pithed and the gastrocnemius muscle of both legs was isolated and immediately chilled. The tissues were homogenised in 0.25 M sucrose solution to 10% (W/V) and centrifuged at 2500 rpm for 15 min to remove the cell debris. The supernatants were used for the assay of enzyme activity. The succinate dehydrogenase (SDH: EC 1.3.99.1) activity was assayed by the method of Nachlas *et al.*⁸ and the enzyme activity was expressed as μMoles of formazan formed/mg protein/hr. Protein levels were determined by the method of Lowry *et al.*⁹ 2 mM of calcium chloride and 3 mM of EDTA (Ethylene diamine tetra acetic acid) were selected after preliminary standardization.

RESULTS AND DISCUSSION

The activity levels of SDH were 0.112 and 0.173 μMoles in the denervated and contralateral control muscles respectively, showing a drop of 35% in muscle as a consequence of denervation atrophy. Similar decrease in SDH activity of the muscle after denervation has been reported earlier¹⁰. The activity level of SDH in both the denervated and contralateral control muscles was found to be increased by calcium. It increases the maximal velocity from 0.143 to 0.34 μMoles in the control muscle and from 0.119 to 0.330 μMoles in the denervated muscle. The K_m values in the

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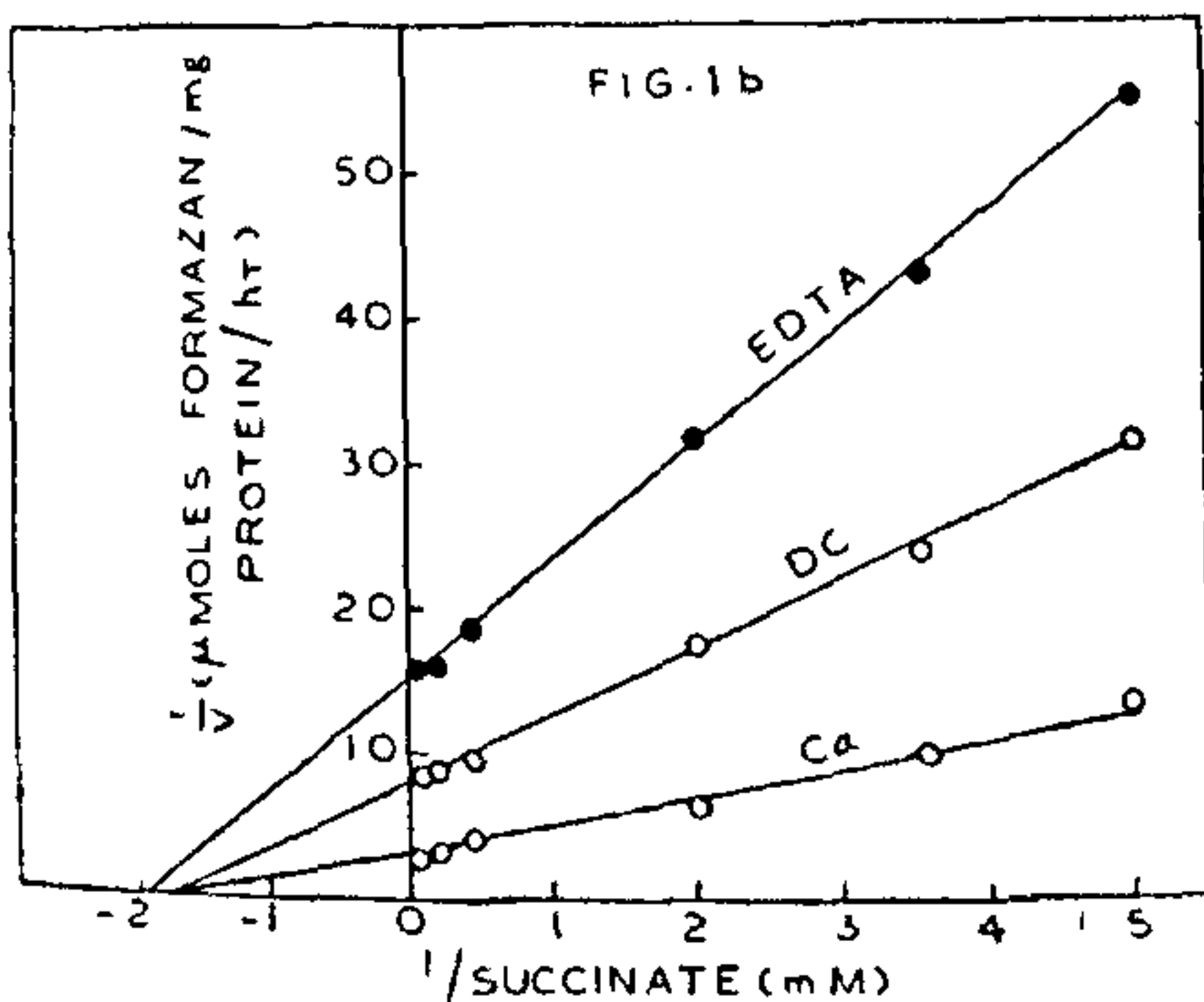
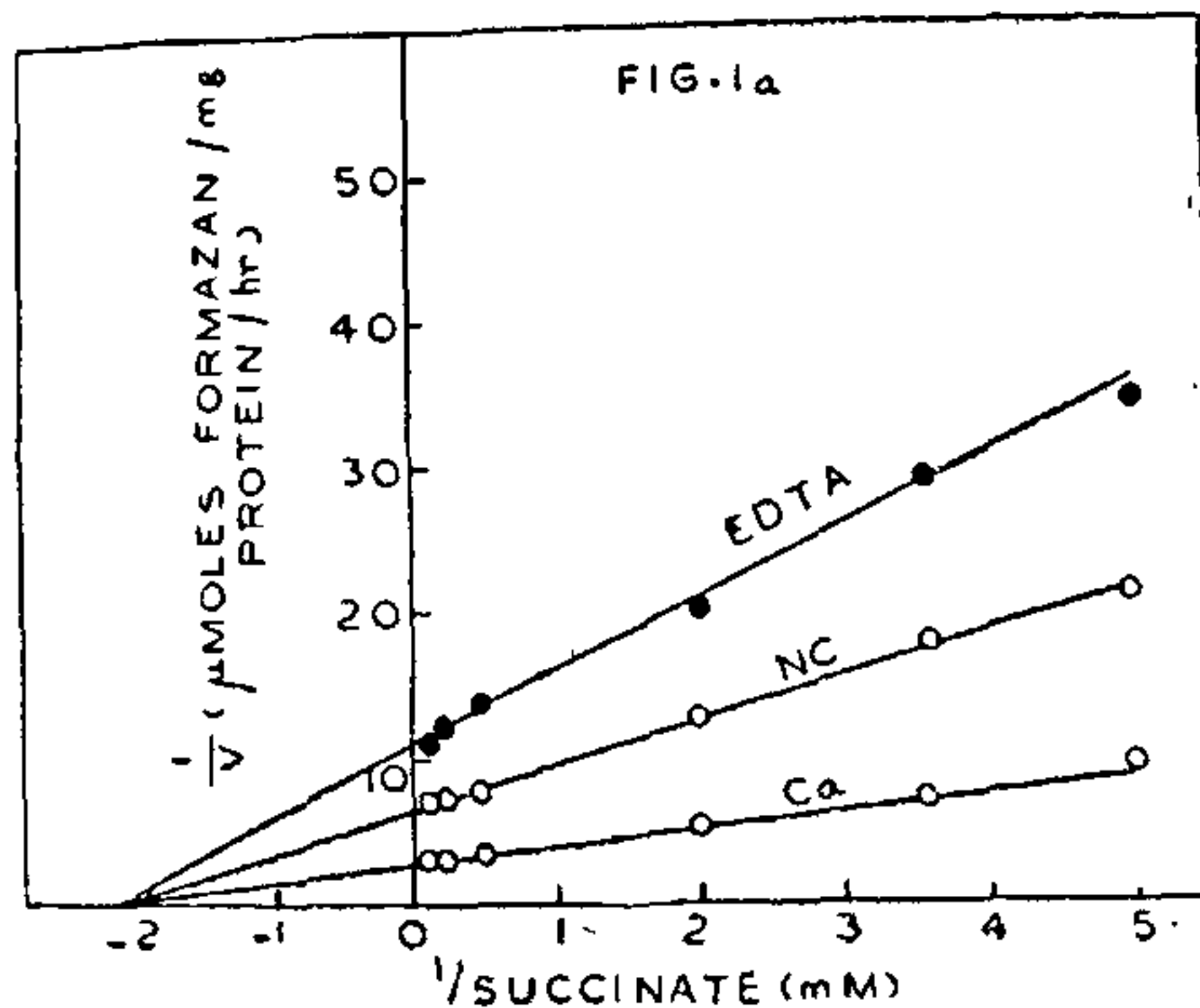
absence and presence of calcium were 0.38 and 0.37 mM in the control muscle and the corresponding values in the denervated muscle were 0.58 and 0.59 mM. This type of activation where only V_{max} was effected but not the K_m indicates that the activation pattern is of non-competitive type (Figs. 1a and 1b). The non-competitive type of activation by

values were found to be 0.38 and 0.39 mM in the control muscle in the absence and presence of EDTA respectively. In the denervated muscle the corresponding values were found to be 0.58 and 0.55 mM. The inhibition by EDTA may be of non-competitive type decreasing the maximal velocity with relatively no effect on the enzyme-substrate affinity (Figs. 1a and 1b).

The activation of SDH by calcium in both the normal and denervated muscles can be attributed to its role in regulating the flow of metabolites across the mitochondrial membrane¹¹. It is likely that the calcium may facilitate the entry of succinate into the mitochondria. The inhibitory action of EDTA may be due to the binding of calcium ions which are shown to positively modulate SDH activity of the normal and denervated muscles.

The per cent calcium activation of denervated muscle enzyme was 177% while the contralateral control muscle enzyme was only 137% indicating that the calcium sensitivity of muscle enzyme increases on denervation atrophy. The per cent inhibition by EDTA was 34 and 45 in the control and denervated muscles respectively. Thus the denervated muscle enzyme was found to be inhibited more by EDTA when compared to that of the control muscle. It was reported earlier that the denervated frog muscle becomes markedly sensitive to extracellular calcium¹². However the elevated free calcium levels reported in the denervated muscle² should have kept up the denervated muscle SDH activity. Probably, the elevated Ca^{2+} content in the denervated muscle might have shifted its involvement in the spontaneous myofibrillar contractions which were found to predominate in the denervated muscle since the denervated muscle was found to need higher amounts of calcium to maintain the muscle membrane properties than the control muscle¹³. Similar accumulation of calcium in sarcoplasm of the muscle was observed during repeated contractile activity resulting in the onset of fatigue¹³. In spite of the high calcium contents, the fatigued muscle was found to have low SDH activity when compared to the normal muscle¹⁰.

Godwin *et al.*¹⁴ showed that the earliest abnormality during the onset of selenium deficiency may be with respect to an ion, the redistribution of which would be expected to cause profound changes in muscle integrity. Hence, it is likely that the shift in the functional involvement of calcium in the muscle for the metabolic enzyme to membrane potentials may be responsible for the lower oxidative enzyme activity or the degenerative processes in general set in the atrophic muscle.



Figs. 1a and 1b. Double reciprocal plots showing non-competitive inhibition of SDH of the control (Fig. 1a) and denervated (Fig. 1b) muscles by EDTA and non-competitive activation of the enzyme by calcium (Ca).

calcium suggests that it reacts with the enzyme at a site other than the active site bringing about conformational changes and making the enzyme active sites catalytically effective. To confirm the role of calcium, the effect of EDTA was studied.

EDTA was found to elicit an inhibition on the activity of the enzyme from both the control and denervated muscles. The V_{max} values for the control muscle enzyme were 0.143 and 0.094 μ Moles in the absence and presence of EDTA respectively. The corresponding values for the denervated muscle enzyme were 0.119 and 0.065 μ Moles. The K_m

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PLANT GROWTH REGULATORS AND SEX EXPRESSION IN FLOWER BUDS OF *MOMORDICA CHARANTIA IN VITRO*

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ABSTRACT

A study on the effect of plant growth regulators on the sex expression of *Momordica charantia in vitro* revealed that α naphthalene acetic acid, kinetin and ethrel induced femaleness by increasing the size of pistillate buds while gibberellic acid and abscisic acid induced maleness by increasing the size of staminate buds. α naphthalene acetic acid and gibberellic acid were more effective than other plant growth regulators in causing femaleness and maleness, respectively. Morphactin caused maximum male sterility.

INTRODUCTION

THE *in vitro* culture of plant tissues has been studied by Gautheret^{5,6} and his coworkers from 1940. The effect of plant growth regulators on sex expression in flower buds *in vitro* is very interesting and recently Galun *et al.*^{3,4} studying the effect of growth regulators like IAA and GA₃ in flower buds of *Cucumis sativus* reported that IAA promote the development of ovary and this effect is antagonised by GA₃. Their interesting findings gave impetus to undertake this problem. One of the advantages of using this type of study is that nutrients and plant growth regulators of known concentration may be substituted for those normally available in the intact plants. Under these conditions, it is then possible to single out any one factor and determine its influence on sex expression under controlled conditions. The present experimental study includes not only α naphthalene acetic acid and gibberellic acid but also other plant growth regulators like kinetin, abscisic acid, ethrel and morphactin.

MATERIALS AND METHODS

The buds (2.5 cm in length) of *Momordica charantia* grown under normal conditions were

dipped in distilled water just after picking followed by 1.5% sodium hypochlorite solution and finally washed with distilled water three or four times. After autoclaving the 2% agar medium appropriate amounts of α naphthalene acetic acid (α NAA), gibberellic acid (GA₃), kinetin (KN), abscisic acid (ABA), ethrel (Eth) and morphactin (CFI) were weighed and mixed in culture medium. The culture medium was the same as used by Henderson *et al.*⁷, with slight modification, i.e., KN and IAA were not used as the constituents of culture medium. Each plant growth regulator was prepared at 5 ppm concentration. Plant regulator free basic medium was taken as control. The agar nutrient medium either with or without plant growth regulators was poured in deep sterile petridishes to a 5.00 mm height and 3.00 mm of lower ends of flower buds were embedded in the culture medium. The flower buds were incubated at $22 \pm 2^\circ \text{C}$. Eight replicate cultures were used for each treatment. The reduction in opening of staminate flowers from staminate buds was taken as a criterion for pollen sterility. This was further confirmed by using the safranin stain, given to the pollen.