GENERAL DISCUSSION AND SUMMARY
GENERAL DISCUSSION

Although our knowledge of the procedures to be adopted in obtaining certain morphogenetic responses in tissue cultures is fairly good, we are yet not in a position to actually predict which particular 'balance' of growth regulators can evoke a specific response at a given site. Admittedly the genetic components of the cells in question may not be very different. Nevertheless questions which arise are why should it be possible to have a particular response on one occasion and no response at all on another occasion even with the same species? Is it that there is a stream of events which must occur sequentially, at a specific time and in a given order to provoke or elicit a typical response? If this be the case we are yet not aware of the exact stream of events or controls and hence our inability to obtain a particular response. This is probably what Mehta (1975) had in mind when he suggested that "Other growth factors, as yet unidentified, might also be involved in hormonal interactions regulating arganogenesis". We do not have any knowledge of these other growth factors. All the advance which we have been able to achieve in trying to unravel these problems are merely approximations. Even in the course of present investigations we have been able to induce callus
formation very easily and in both *Hokarrhena antidysenterica* and *Boerhaavia diffusa* the rate of growth of callus is extremely rapid. And yet, the differentiation is rather poor or none at all from 2nd or 3rd passage onwards. Is it that we have not been able to supply the right factors or is it that the tissues have lost their ability so soon? If so, why? One explanation which comes to mind is that some of the secondary products formed like sterols, alkaloids etc. may have a deleterious effect on the morphogenetic regulatory mechanism. If this is true, the nature of future research will have to take this aspect into account very seriously. The way to do it would probably be to concentrate on dedifferentiation at individual cellular level by shake flask culture, get the differentiation and then grow the embryos. In other words changing to a certain extent the sequences followed in the present investigation. It could be argued that if these secondary metabolites are interfering there is very likelihood that they would also be synthesized at the cellular level. In a suspension culture the chances for 'drainage' of these metabolites away from site as it were would be very much higher and hence lessening of the inhibitory influence. All such ideas need very vigorous testing.

It was noticed that in *Boerhaavia* where abundant rooting
of callus could be easily obtained in several successive passages, the introduction of gibberellins in the medium inhibits rooting. In contrast to this GA₃ inhibits caulogenesis in Holarrhena. Addition to Kn to Boerhaavia rooting callus only brings about more compact callus formation but no shoot formation and the roots continue to grow as usual. Multiple shoots with short internodes and few shoots with comparatively long internodes were observed in media supplemented with Kn. and BAP respectively. Such responses are difficult to interpret.

One other point regarding morphogenesis which remains unanswered is the question of loss of potentiality from passage to passage. It is strong suspicion that this is certainly not a 'loss' but we have yet to recon some nutritional (?) factor, which is being depleted, the effect of which was there in the first one or two passages due to a carry over effect. Likewise, the reduction of alkaloid content in later passages also could be explained along the same lines and a search for such factors should be made as it would be very advantageous.

The real interest in the work on Holarrhena antidysenterica was that the alkaloids are not only effective against amoebic dysentery but also serve as a precursor for the
Biosynthesis scheme of Holarrhena alkaloids from cholesterol.

(After Bennett & Heftmann, 1965.

Aldosterone acetate (Pregn 4 ene, 3, 20, dione, 11, 18 oxide, 19-ol)
synthesis of a steroidal hormone, aldosterone acetate (Pregn-4ene-3,20 diol-11, 18-oxide 19-ol) (Fig 18) which is specific for controlling some sexual processes in animals. Likewise, although today we are not aware of the nature of alkaloids and other components in Boerhaavia we do know that there are effective in eliminating excess of liquids as well as toxic products from kidney tissues, thus helping to rejuvenate an individual. If by tissue culture we could set up the production of both Holarrhena antidysenterica and Boerhaavia diffusa alkaloids it would serve as a boon to pharmaceutical industry.

Some very interesting lines which could be taken up for further work are, the biosynthesis of alkaloids in Holarrhena and Boerhaavia and their variation in content, with the supply of different amino acids precursors and other sterols.

As reported by Bennett and Heftmann, 1965, precursors like cholesterol-4-C14 could be administrated to the leaves of Holarrhena species. The metabolic pathway scheme is shown in Fig. 17. Cholesterol is expected to be a immediate precursor, incorporated into some of the steroidal, alkaloids like Holamine (3α-amino-5pregnen-20-one), holaphyllamine
(3β-methylamino-5pregnen-20-one), and holaphylline
(3β-methylamino-5pregnen-20-one). Progesterone, which is an
immediate precursor from pregnenolone, is rapidly metabolized
while pregnenolone-4-Cl\textsuperscript{4} appears to be slowly metabolized by the
leaves of \textit{Holarrhena} intact plant. To-day because of the lack
of some technical facilities in the laboratory, we could not
undertake work on this aspect.

\textit{Holarrhena} plant posses C\textsubscript{21} steroids and these steroids
are made in plants, as well as in animals, by degradation of
the side chain of a sterol. One of the common plant sterols
which differs from cholesterol only in the side chain is
β-sitosterol. Therefore, β-sitosterol could also serve as a good
precursor.

For such studies tissues grown on a large scale,
desirably in continuous culture, may prove as a better source
of information and studies because precursors could be fed in a
suitable form and the transformations achieved in the culture
in a similar manner as has been possible in the studies on
transformation of progesterone in \textit{Dioscorea deltoidea} and
\textit{Cheranthes cheiri} cultures (Stoehs, et al., 1969; Stoehs, et al.,
1972) or by cell cultures of \textit{Nicotiana tobaccum} and \textit{Sophora}
\textit{anguistifolia} culture (Furuya et al., 1971) or by cell cultures
of Digitalis purpurea (Furuya, et al., 1973).

In our studies, we tried to incorporate 'feed' cholesterol as a precursor to facilitate a higher level of Helarrhena alkaloid synthesis, but as mentioned earlier because of some technical difficulties we could not carry on the experiment further. It is felt that the use of C14 labelled precursors as were employed by Bennett and Heftmann (1965) in vivo studies can also help in vitro studies.

It is only when we have more information on such transformations that one could possibly apply these techniques for the production of compounds important to medicine. This goal would be worth achieving.