CONCLUSION

CH was recently isolated from Phomopsis paspali by Pendse (1974) in Indian Drug Research Institute, Poona. The chemical structure was determined immediately but to the best of our knowledge its effects on any biological system are not tested. Therefore, it was decided to do so by using the embryos of Microchyla ornata in early stages of development.

While testing the effects of CH on the entire embryos in early stages of development it was found that the effects were stage dependent, dependent on the duration of the treatment and were reversible on restoration of CH free medium. The vitelline membrane which is semipermeable proved an effective barrier to the transport of the drug. The embryos treated with the vitelline membrane needed more concentration of the drug and more duration of treatment to show any effect. The reversal in this case was also slow. The effective concentration of CH for the embryos with vitelline membrane was found to be 10 µg/ml and for embryos without vitelline membrane was 1.5 µg/ml. The above mentioned observations were in conformity with the observations of Gail and Boone (1971) and Schaeffer et al. (1973b). It was observed that CH brought about inhibition of the development and very interestingly showed disaggregation of embryonic cells. The disaggregation
was observed in the endodermal cells to start with but if treated with higher concentration of CH the ectoderm also disaggregated.

It was thought that the inhibition of the development can be due to either inhibition of cell movement on CH treatment which results in the inhibition of morphogenetic movements, or it is due to the disaggregation brought about by CH.

The histological sections showed the endodermal as well as ectodermal disaggregation. These cells occupied the blastocoel completely changing the architecture of the embryo and stopped developing. This has been shown in Part 1 of the thesis.

The experiments using vital staining technique of Vogt (1929) were designed to test whether the inhibition of morphogenetic movements was brought about secondarily due to the capacity of CH to bring about disaggregation or whether it was the primary effect.

It was confirmed that the primary effect of CH was on bringing about disaggregation of embryonic cells as a result of this the morphogenetic movements were inhibited. It was evident from the results that in the treated embryo in spite
of the disaggregation, the stained region moved as in the control indicating morphogenetic movements. This has been shown in Part 2.

Disaggregation is known to be a phenomenon related to the cell surface. The cells are held together by the property of the cell surface material which is carbohydrate rich. Mucopolysaccharides-glycoproteins are the two components of it. Therefore it was thought likely that CH affects the cell surface material and brings about disaggregation.

Sanger and Holtzer (1972a) had observed 50% reduction in the incorporation of glucosamine which is an essential component into mucopolysaccharides on treatment with CB. This was confirmed by Lee et al. (1973). Eustensen and Plageman (1971) observed reduction in the transport of glucosamine on treatment with CB.

Taking this into consideration, it was thought desirable to see whether CH affects the mucopolysaccharides and glycoproteins in the cells of the embryos of Microbula ornata using histochemical techniques.

PAS staining indicated the reduction in the total content of neutral mucopolysaccharides in treated embryos as compared to the controls. Using AB staining at pH 1.0 and 2.5 it was observed that the sulphated mucopolysaccharides
were not even present in control embryo at that stage and the
treatment of CH has no effect on it. The nonsulphated
mucopolysaccharides were affected after the treatment. This
has been shown in Part 3.

The mucopolysaccharide-glycoprotein surface coat gets
affected after the treatment was confirmed by electron
microscopy by using a specific marker viz. Lanthanum which
selectively gets adsorbed on the cell surface material and
renders it electron dense. The ultra structural observations
indicated the reduction of the cell surface material in
treated embryos as compared to the control. The disaggregation
of cells is evidenced by big empty intercellular spaces in
experimental embryo as against the compact arrangement of cells
and glycogen filled intercellular spaces and the reappearance
of Lanthanum bound cell surface material in the recovered
embryo.

The results in the present investigation indicate
that CH acts through the cell surface material but the exact
mechanism of its action on the cell surface material remains
unknown. Though there is slight difference in the structures
of CH and CB the effects of CH are quite comparable to those
of CB.