INTRODUCTION

The function and behaviour of chromosomes on one hand and the genetic mechanism at the molecular level on the other are well understood. However, inspite of intensive researches with the aid of various methodologies for last few decades, there remains an enormous lacuna in our knowledge on the structure of chromosomes. Physically, a chromosome is made of fine fibres of various dimensions. In so far as the chemical organisation is concerned, in addition to DNA, both histone and nonhistone protein form the bulk of the chromosomes. What still remains to be resolved is the specific association and orientation of the proteins and nucleic acids in the build up of the gross structure of the chromosome.

In a recent book Harris Busch (1965) has classified the nuclear proteins in three groups: the basic nuclear proteins, the acidic nuclear proteins and the nuclear enzymes. From biochemical analysis on Walker tumour and rat liver, Steele and Busch (1964) demonstrated that, of the acidic nuclear proteins, the residual nuclear protein is rich in collagen. The presence of collagen was confirmed by electron microscopic studies. On the basis of the fact that structure and function of chromosomes are same in plants and animals; collagen should be present in plant nuclei, if it happens to be an important and integral part of chromosome.

Hence, an attempt has been made in the present investigation to detect collagen by staining and autoradiographic methods. It has been concluded that, indeed, a detectable amount of collagen is present in plant nuclei.

Of all the chromosomal components, DNA has been attributed with the responsibility for maintaining the linear integrity of chromosome either as 'unineme' or as 'polyneme'. This has been based chiefly on the following lines of indirect and circumstantial evidences:

(a) correlation between chromosome breakage and action spectrum of UV-radiation;

(b) constancy and metabolic stability of chromosomal DNA;

(c) semiconservative replication of chromosome and chromosomal DNA;

(d) electron microscopy of sectioned and surface-spread chromosomes;

(e) action of base analogs, radiomimetic chemicals, DNA precursors, DNA inhibitors and related compounds on chromosome;

(f) action of DNase on chromosome.

However, the concept that protein can possibly maintain the linear integrity of chromosome, has received little attention from the experimentalists, inspite of strong evidences from various sources. Thus, our information on the

action of substances which affect or interfere directly or indirectly with the synthesis and accumulation of protein moiety of chromosome, is very meagre. This lacuna in information has instigated the present investigation on the action of one broad spectrum protein inhibitor, chloramphenicol, one amino acid analog, 5-methyl tryptophan and a specific proteolytic enzyme collagenase on both somatic and meiotic nuclei in vivo.

It has been known for some time that chloramphenicol inhibits protein synthesis (Gale and Folkes, 1953), but it is still not known at which step it acts. The antibiotic does not prevent amino acid activation (De Moss and Novelli, 1956) or esterification to sRNA (Lacks and Gross, 1959), but interferes with some later steps in protein synthesis (Nathaus and Lipman, 1961). Jardetzky and Julian (1964) and Wolfe and Weisberger (1965) reported that chloramphenicol inhibits the binding of messenger RNA to ribosomes. According to Julian (1964) and Traut and Monro (1964) the peptide bond formation is inhibited by chloramphenicol. Recently, Weber and De Moss (1966) reported that the growth of nascent polypeptides is inhibited by chloramphenicol, indicating that the antibiotic in some way interferes with a step common to the formation of all peptide bonds of protein. Hence, it is expected that chloramphenicol would be effective on synthesis of all kinds of proteins.

According to Moyed and Friedman (1959) 5-methyl tryptophan inhibits biosynthesis of tryptophan by blocking the conversion of shikimic acid-5-phosphate and glutamic acid to anthranilic acid. Hence, 5-methyl tryptophan should specifically act on the synthesis of tryptophan-containing proteins. However, it may act on the synthesis of other proteins too (Nisman and Hirsch, 1958; Munier and Cohen, 1959).

According to Nagai (1959) the enzyme collagenase splits specifically the peptide bond between X-glycine in polypeptides which contain the sequence proline-X-glycine-X (in which hydroxyproline may replace proline and X may be any one amino acid in collagen molecule). The choice of the enzyme collagenase was made due to the detection of collagen in plant nuclei. It is expected that the enzyme would act on either the accumulation or the existent collagen.

In order to be certain that chloramphenicol (CAP), 5-methyl tryptophan (5-MT) and collagenase (CLGase) (which would henceforth be referred collectively as "protein antimetabolites") affect the protein content of plant nuclei, a series of experiments on staining reactions for histone and total protein was carried out. It is concluded that all the three protein antimetabolites affect adversely both histone and nonhistone; nonhistone being relatively more susceptible. From a series of preliminary experiments, a range of concentration and period of treatment was selected. This information was used to study their effects on chromosome morphology

throughout the cell cycle. Although all the antimetabolites produced chromosome breaks and gaps in root tip cells immediately after treatment, the aberration frequency became very high after long periods of growth preceeded by short treatment. This suggests that these antimetabolites act on G_1 and possibly G_2 of the previous cycle. Since the aberrations produced are true breaks and gaps and show no rejoining, it is likely that the antimetabolites act on the already existent protein or synthesis and/or accumulation of the structural protein. The antimetabolites also produced chromosome breaks in microsporocytes only when premeiotic interphase cells were treated. The induction of asynapsis is also a common feature. The role of structural protein is discussed.