INTRODUCTION

The organization of various macromolecules in the eukaryotic chromosomes is still an engima. There are two schools of thoughts on the arrangement of nucleo-histones and acidic-proteins in the chromosomes. It is believed by a group of workers that each chromosome is made up of a single chain of DNA extending from one end to the other without any interruption, possibly according to folded-fiber model of Dupraw (1972). According to these workers, the non-histone protein is not involved in maintaining the chromosomal integrity. The other school believes that non-histone proteins constitute a framework, scaffold or a core to which the DNA molecules are attached. The present work addresses itself to this question.

As early as 1947, Mirsky and Ris, reported that fractionation of NaCl solution results in the separation of DNA-histone complex from thread-like structures which are made up of residual proteins and some RNA. These thread-like structures retain the morphology of the chromosome with distinct constrictions. This led them to conclude that the backbone of the chromosome is made of residual protein. Bril-Peterson and Westenbrinik (1963) concluded from an analysis of bull sperm that certain proteins other than protamines are associated with DNA in the sperm and this protein is not basic. On the basis of quantitative cytochemical determination of non-histone protein component of

DNA-puffs of Rhynchosciara, Rudkin (1964) suggested that a non-histone protein might be construed as a structural one, necessary for over all organisation of chromosomal materials. Steffenson (1963) from a very critical study of ^{3}H-thymidine uptake concluded that DNA is localised only in the band and not in the interband. These observations rule out the possibility that DNA is distributed uniformly throughout the length of the basic structure of the chromosome. Later, in 1965, Trosko and Wolff, from a series of enzymatic studies of fixed Vicia faba root tips, suggested that in mitotic chromosomes linear continuity is dependent on protein.

On the basis of electron microscopic observations on isolated chromosomes, Stubblefield and Wray (1971) demonstrated that the mitotic chromosomes consist of distinct axial and peripheral chromatin components. Similarly Paulson and Laemmli (1977) showed that when metaphase chromosomes were stripped of histones and examined by surface spreading, a residual axial structure was found to which the loops of DNA were attached. Adolph et al.(1977 a, 1977 b) showed if the chromosomal DNA was extensively digested with nuclease before removal of histone, it was possible to isolate the residual "scaffold" in a stable form and the scaffold isolated in this way preserved the characteristic paired chromatid morphology. Marsden and Laemmli (1979) observed the thin section of swollen metaphase chromosomes under electron microscope and recorded that loops of

chromatin fibres radiate from the less dispersed centre region of the chromatid. Rattner et al. (1980) studied the meiotic prophase nuclei, which were sprayed on electron microscopic grid. In very early stages of prophase the chromatin is released in linear arrays typical for interphase chromatin material. At pachytene, the loops are evenly distributed along the length of the chromosomes and give a brush-like appearance. Earnshaw and Laemmli (1983) isolated residual protein scaffold on electron microscopic grid from nuclease digested chromosomes. The scaffold is fibrous in nature, but retains the shape of chromosomes and appears to be derived from the kinetochore and the chromatid axis. These findings indicate that the DNA loops are held closed at the chromatid axis by non-histone "scaffolding" proteins.

In essence the question of macromolecular organization of the chromosomes boils down to the existence of a proteinaceous core and its possible role in maintaining the chromosomal integrity.

The present work deals with the effect of three antibiotics which directly or indirectly affect the protein synthesis. The rationale is that if administered at a proper concentration at proper time, the proteinaceous core will be affected by the antibiotic and the effect will be detectable at the level of light microscopy. Three well known antibiotics, cycloheximide (CH), puromycin (PM), and rifamycin (RM)

were used in the present investigation to see their effect on chromosome structure.

The antibiotic CH inhibits protein synthesis in a wide range of eukaryotic cells, fungi, Tetrahymena, mammalian cells and tumour cells in culture (Kerridge,1958, Sisler and Siegel,1967). It is known that CH inhibits translocation of peptidyl-t RNA from the ribosomal A site to the P site (Felicetti and Lipmann 1968, Monro et al. 1968, Culp et.al. 1969), through inhibition of the transfer factor II mediated translocation of peptidyl-t RNA (Mckeehan and Hardesty, 1969, Baliga et al. 1970). Oleinick (1977) suggested that the drug inhibits initiation, elongation and termination and that initiation is the most sensitive phase.

The mechanism of puromycin action was established by Yarmolinsky and Haba (1959), and Kaji (1973). It is an analog of 3' terminal end of amino acyl-t RNA (Yarmolinsky and Haba, 1959) and interacts with acceptor site of the ribosomal peptidyl transferase centre on the larger subunits (50s or 60s). The product of the puromycin reaction (Peptidyl-puromycin) is unable to take part in the next step of protein synthesis.

Rifamycin has the unique property of inhibiting RNA polymerase. It specifically binds to the enzyme and not to the DNA template, and prevents initiation (Wehrli et al. 1968). Rifamycin forms a stable complex with β -subunit of

the enzyme (Zillig et al. 1970) thereby blocking RNA-chain initiation (Sippel and Hartmann, 1968, Lill et al. 1970). Thus, it may be expected that all the three antibiotics will affect the protein in grasshopper system.

In order to ascertain the suitability of drug it is desirable to check its effect at the cellular level with the aid of light microscopy. Thus primary in situ effect may be determined through semi-quantitative assessment of protein stainability. Moreover, the protein stainability as well as the state of DNA could be assessed through both light microscopy and fluorescence microscopy. It is well-documented that cytochemical reactions can be made absolutely specific for strict chemical evaluation.

In order to ascertain that CH, PM, RM affect the protein content of the nuclei, a series of experiments on staining reaction for total protein, histone-protein, non-histone protein was carried out by three established staining methods viz. mercury-bromophenol blue, alkaline-fast green, and acid-fast green respectively.

The mercury-bromophenol blue staining method was first introduced by Durrum (1950) for the demonstration of protein on filter paper spots. It was adopted as a general stain for protein by Mazia, Brewer and Alfert (1953). These authors stated that preparations stained by their procedure followed the Beer-Lamberts laws and that the amount of dye bound was

proportioned to the amount of protein over a wide range. The method was employed by Bonhag (1955) for investigating the composition of the ovary of the milkweed bug. Alfert and Geschwind (1953) demonstrated basic proteins in nuclei which stain with fast green at pH 8.0, after removal of nucleic acids. By the use of model systems, they obtained evidence that these proteins are histones and protamines. In formalin-fixed tissues (Alfert 1956), histones are the only proteins which could be stained with alkaline-fast green. Again histones are high in basic amino acids, they have isoelectric points which are much more acid than other proteins. At the isoelectric point, dye binding by proteins is minimal. The general characteristic of proteins found in tissues are such that, when the tissues are placed at pH 8.0, most of the proteins are above or near their isoelectric point. At pH 8.0, histones are still below their isoelectric point. This means that, by placing tissues at pH 8.0, in an acid dye, such as fast green, the only proteins having groups available to bind the dye are the histones. The colour developed appears proportional to the amount of histone present and can be measured microspectrophotometrically (Jensen 1962). The residual or non-histone proteins remaining in the nuclei after extraction of nucleohistone has been called chromosin by Mirsky and Pollister (1946). Kaye and McMaster-Kaye (1966) used dilute HCl for several hours for

removal of histones, after hot TCA treatment, and then stained non-histone protein with acid-fast green at pH 4.6, which they suggested will be specific for non-histone protein.

In addition to the above mentioned specific protein stains, three different fluorochromes viz. acridine orange (AO), ethidium bromide (EB), and mercurochrome (MC) were used to stain the treated and control cells. Any difference in fluorescence pattern and intensity may reflect a change in the nucleoprotein moiety of the treated cell.

The dye AO exhibits large changes in its emission properties when interacting with nucleic acids with different degree of strandedness. AO staining has been used for differentiation of native double-stranded DNA from denatured single-stranded DNA *in situ* (de la Chapelle *et al*. 1971). Thus it is generally accepted that under strict experimental conditions, AO can be used for differentiation of double and single-stranded nucleic acids. EB is a basic dye, the red fluorescence of which is enhanced approximately 50-fold, when the dye is bound to double-stranded nucleic acids (Lepecq and Paoletti 1966, 1967). It specifically reacts with double-stranded nucleic acids whether DNA, RNA, or DNA-RNA hybrids. Therefore, the amount of dye will be a measure of the double-stranded region of the molecule stained (Moutschsen 1976). The binding constant of EB is limited by the presence of proteins at the surface of nucleic acids by

the folding of DNA and even by unknown configuration (Moutschsen 1976). The fluorescence technique of EB was studied by De (1977) in detail and he concluded that EB fluorescence was highest when all histones were removed.

The use of colour mercurials to demonstrate protein bound sulfhydral (SH) groups has been reviewed extensively by Bennett and Watts (1958). Cowden and Curtis (1970) suggested that mercurochrome appears to be the best dye for localizing SH groups. Jocelyn (1972) and Buys and Osinga (1980) suggested that mercury ion of mercurochrome forms a strong complex with sulfhydral groups of proteins.

Parallel to the cytochemical reaction the effect of antibiotics on the chromosome structure as revealed by induction of structural aberrations can also be accepted as a measure of the effect of the drugs on chromosomal organization. Parchman and Stern (1969) observed stickiness of chromosomes in cultured Lily microsporocytes after treatment with CH. Yoshida et al. (1972) observed fragmentation, non-disjunction of chromosomes in Barley chromosomes after treatment with CH and PM. Similarly, Roman and Georgian (1977) induced both gaps and breaks in chromatids in human peripheral blood culture by rifampicin. Wagenaar (1983) induced irregular anaphase separation in Allium sativum by CH. In addition to these antibiotics, other protein-inhibiting antibiotics and amino acid analogues

also induce chromosomal aberration. Kemp (1964) induced chromosomal aberration by ethionine in Trillium. De and Ghosh (1968) observed breaks, gaps, fragments, stickiness, asynapsis, in chromosomes of Allium cepa after treatment with chloramphenicol and 5-methyl tryptophan. Manna (1969), Bhunya and Manna (1969) and Manna and Bardhan (1972) induced aberrations in bonemarrow cells of mice by chloramphenicol. Ohama and Kadotani (1970) and Hayez-Delette and Freimans (1975) observed chromosomal aberrations in human cells in vivo by bleomycin. Srivastava and Sarma (1980) and Sarma and Abhayvardhani (1981) reported chromosomal aberrations in algal cells by penicillin, streptomycin, tetracycline, mitomycin and polymyxin.

In addition to the study of effect of antibiotics on chromosomes structure, the present report records on improvement as well as analysis of a method of demonstration of chromosome core.

Howell and Hsu (1979) first demonstrated the core by $AgNO_3$ staining at the level of light microscopy. Satya-Prakash et al. (1980) improved the technique by application of alkali-SSC pretreatment. Rufas and Gosálvez (1982), Rufas et al. (1982) and Sentis et al.(1984) used a different protocol for the revelation of core. Later Nokkala (1985), Nokkala and Nokkala (1985) and Haapala (1985) also successfully demonstrated the core by $AgNO_3$ staining. Some workers, however, believe that the core is an artefactual

product of the preparatory methods (Hadlaczky et al.1981b, Burkholder 1982, 1983, Burkholder and Kaiserman 1982).

It is to be appreciated that each step or treatment in a given protocol for demonstration of core has a definite purpose. A replacement or an omission or modification of a single step, if followed methodically or systematically can yield a wealth of information on the actual process which leads to visualisation of the core. Thus the effect of DNase, RNase and trypsin, hot TCA, differential extraction of histones and nucleic acids can all lead to a very meaningful data.

Finally, fluorescence microscope with its innate chemical specificity and capacity to provide additional information can be advantageously used on silver nitrate stained core to yield additional information.

The present work deals with the demonstration of chromosome core structure in the meiotic chromosomes of Acrida turrita. After determining the karyotype of the experimental material, the effect of CH, PM and RM were studied on the basis of protein stainability both by light and fluorescence microscopy. The drugs inhibited protein synthesis and exhibited both meiotic and somatic chromosomal aberrations when administered properly. In addition, the silver staining method was standardised and its various steps were analysed to give an insight to the actual

mechanism for revelation of chromosome core. Finally, a method has been developed to demonstrate the presence of a core of a silver-stained specimen by subsequently fluorochroming with acridine orange as visualization by fluorescence microscopy. Fluorochroming of $AgNO_3$ stained chromosome with AO, shows that the core is discernible at all stages of meiosis by transmitted- light excitation and is surrounded by epichromatin made of DNA.