ASSAYS OF SOME MUTAGENIC AND TERATOGENIC EFFECTS OF THE BACTERIUM, PSEUDOMONAS AERUGINOSA IN TREATED MOUSE SYSTEM

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Who thought for the development of

Science and Technology in India

and

Peace and Prosperity of the world

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ASSAYS OF SOME MUTAGENIC AND TERATOGENIC EFFECTS OF THE BACTERIUM, PSEUDOMONAS AERUGINOSA IN TREATED MOUSE SYSTEM

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INTRODUCTION

Microbes as genatoxic agents have not yet received due attention though Manna (1969, 1971, 1973, 1980a, 1982) has been advocating the same over and again. In the field of mutagenesis they constitute "Living mutagens" introduced by Manna (1971, 1980a, 1982) because Viruses (Hamper and Ellison, 1961), Ricketsia (Halkka and Heinomen, 1968), Mycoplasm (Paton et al., 1965), bacteria (Manna and Bhowmic, 1969) and the fungus, Aspergillus fumigatus (Manna and Patel, 1981) were shown the chromosome breaking agents in treated or injected cells of animals. Customarily the mutagens were put under two categories as physical agents and chemical agents while the "genetic mutagen" discovered by Mc Clintock (1951) did not receive proper attention. At present mutagens could be put under four categories viz. (1) Physical agents, (2) Chemical agents, (3) Genetic mutagen, and (4) Living agents.

Mutations play the key role in speciation and evolution. The artificial induction of mutation with the help of ionizing radiations has very intensively been studied

after the pioneering discovery made by Muller (1927) in Drosophila, chromosomal mutation in maize by Stadler (1928) and genetic transformation in Nicrotiana by Goodspeed and Olson (1928). The chemical agents inducing mutation in both plant (Oehlkers, 1943) and animal (Auerbach and Robson, 1944) were discovered later. In the recent past we have reasonably been conscious about the environmental mutagens as the environmental pollutions are increasing in alarming rate as a result of use and abuse of atomic energy, large scale industrialization, agricultural practices, public health measures, personal beautification, drug habit and so on. Mutagens present in our environment, according to the source, could be of natural origin like cosmic rays, Ozone, mineral like asbestos, natural gases etc. but the man made ones would out-number them which might be thousand fold or more (Sharma, 1981). Environmental chemical mutagens are ever increasing and each year few thousands are added. Attempts have been made to put the chemical mutagens under some major groupings by different workers (Biesele, 1958; Sharma and Sharma, 1960; Kihlman, 1966; Vogel and Rohrborn, 1970; Sharma, 1981; Sharma, 1984 ; Catcheside, 1948; Sparrow, 1951; Hollaender, 1954; Muller, 1954a, b; Giles, 1954; Ray-Chaudhuri, 1961; Bacq and Alexander, 1961; Evans, 1962; Lea, 1962; Wolff, 1963; Savage, 1970; Bender et al., 1974; Searle, 1974; Cattanach, 1982 etc.). Further because of the favourable chromosome number, morphology and easier handling, Tradescantia, Vicia, Alium, Datura etc. among plants and Drosophila, grasshoppers, heteropteran bugs and in vivo and in vitro cells of mammals including man have more extensively been used to study the various fundamental problems of ionizing , non-ionizing radiations, odd chemicals etc.

Some mutagenicity testing methodologies

Since the discovery of the artificial induction of mutation by Muller (1927), a good number of test systems deploying microorganisms to laboratory animals and numerous end points from point mutations to tumor induction have been developed in the last quarter of a century to cope with the present need. The study of chromosome aberrations still remains the standard and dependable method while most other methods introduced in testing the mutagenecity had variable claims (Hsu, 1982). However, it is now advocated that more than one test to be conducted in claiming the mutagenic potentiality of an agent. The useful informations could be obtained from the books of Vogel and Rohrborn (1970), Chemical Mutagenesis in Mammals and Man; Hollaender (1971a, b, 1973, 1976, 1978) - Chemical Mutagens, Principles and Methods for their Detection (Vols. 1-5); Hsu (1982) - Cytogenetic Assays of Environmental Mutagens; Sharma, Environmental Chemical Mutagenesis (1984) etc. All these methodologies were deployed for testing the mutagenic potentialities of mainly hundreds of chemicals and ionizing radiations. They have seldom been used in testing similar properties of living agents (Bartsch, 1970; Manna, 1973, 1980b, 1982).

Most of the mutagenicity testing methods have briefly been presented in the following :

1. Host-mediated assay :

Ames (1970) has been credited to introduce the enteric bacterium, Salmonella as a testing model for chemical mutagen in which a potential chemical is injected with an indicator micro-organism and the mutation frequency is

measured. In recent year Preston and Brewen (1982) evaluated the use of host mediated assay method and opined that it provides several advantages.

2. Extra karyotic mutation :

Plastids and Mitochondria of Eukaryotic cells contain DNA. The mitochondrial genetic material called as "Chondriome" and that of plastid as "plastome" like "genome" were studied for induction of mutagenic changes (Arnold, 1970). However, it is yet to be established as a standard method for extensive use.

3. DNA repair :

Some studies have been carried out on base damage repair of DNA, DNA cross-links, DNA-protein cross links which could be stimulated by DNA damaging agents. Painter (1982) opined that DNA repair in higher organisms might induce chromosome breakage and sister chromatid exchange.

4. Multiple loci methods :

The method in mouse is quite useful but the economic considerations limit its applicability (Ehling, 1970). Anyhow, in this method mutation to recessive allele could be detected in homozygous test stock developed for specific gene (Cattanach, 1971). When the homozygous stock with specific loci was treated with some mutagen, the character would not be seen if there was a mutation.

Somatic chromosome aberrations :

The bone-marrow cells as a means of testing clastogenic

agents is very well known (Datta et al.,1970; Manna, 1985). Other somatic tissues like spleen, thymus, lymph nodes, cornea etc. have also been used but among all of them bone marrow has been found most ideal in in vivo condition.

The effects of various mutagens have also extensively been studied in in vitro cells of mammals by different workers (Schoeller and Wolf, 1970; Chu, 1970). Even the ascites tumor cells like bone marrow cells were deployed in mutation research (Adler, 1970). The human peripheral blood lymphocyte culture for assaying the cytogenetic effect of environmental mutagens has usually been recommended (Buckton and Evans, 1982) because the testing would not be possible in in vivo condition. Assays of chromosome aberrations in mammalian short-term cultured cells as a useful means in mutagenicity testing has been advocated by Au and Hsu,(1982). Beside mammalian cells, cultured neuroblast cells of grasshoppers or chromosomes of German cockroach, Blatella germanica, mosquitoes, some blow flies, house flies etc. have also been used (Gaulden and Liang, 1982).

Regenerating liver cells :

Regenerating liver cells of mice (Manna and Ghosh, 1984) and rat (Parida et al., 1984) have also been found a suitable model in assaying the genetoxic agents. It was shown that the regenerating liver cells were more sensitive than the bone marrow cells (Manna and Ghosh, 1984).

Flow cytometry :

Chromosome aberration study induced by mutagens happened to be the common practice but it has the limitation

of finding adequate number of dividing cells all the time. Thus in recent years attempts were made to develop computer oriented image analysis mechanism. The machine was originally designed for karyotype reading but it became obsolete with the innovation of chromosome banding techniques. By the joint efforts of physicists, biophysicists and engineers, the flow microfluorometer (FNF) was developed. The original name is now changed to flow cytometry. It has the advantage of measuring large number of cells (10^5) within in a few minutes. That would enable statistical analysis of the data of cellular differences. Efforts have been made to use the mechine in breakage analysis, programme of isolated chromosome, the principle of flow analysis etc. Anyhow, the technique has not been commonly used because of some limitations and other considerations.

Premature chromosome condensation (PCC) :

The cell fusion technique to study the regulation of cell progression through the reproductive cycle was developed by Rao and Johnson (1970). The fused mitotic cell with synchronus population of G_1, S and G_2 stage would lead to rapid condensation of the interphase chromatin into chromosomes which has been termed as "Premature Chromosome Condensation (PCC). On the other hand as the induction of PCC resembled a process similar to that of a cell entering into mitosis, it was also termed as "Prophasing" (Matsui et al., 1972). The ability of mitotic cells to induce PCC of interphase nucleus showed no specific barrier and cell fusion could be accomplished with a variety of fusogens like viruses (e.g. Sendai virus, Newcastle disease viruses), Lysolectin and Polyethylene glycol etc. Earlier worker could not envisage how this phenomenon could be deployed in

detecting environmental mutagens, but it is now well understood. The study has uneven progress and the present state of knowledge has been reviewed by Hittelman (1982).

Sister chromatid exchange (SCE) :

Since the innovation of BrdU-dye techniques for sister chromatid exchange (SCE), replacing radio-isotopes this method has very extensively been used to characterize the impact of mutagens and carcinogens on chromosome. BrdU methodology for the detection of SCE has been reviewed by Latt et al. (1982) and he has listed a large number of chemicals used by various workers showing induction of elevated sister chromatid exchange. The chemicals were put into two categories as (i) agents showing strongly positive effect and (ii) agents exhibiting mixed SCE induction behaviour. The SCE as a means of mutagenicity testing in plant material specially in Vicia faba has also been reviewed by Kihlman (1982). In fact, many workers suggested that SCE is a very dependable method for the detection of odd mutagens. However, Hsu (1982) opined that regardless of the materials and the end points used, there are basically only two types of cytogenetic test systems viz.the classic chromosome aberration study and the more recent sister chromatid exchange (SCE). The procedure for SCE staining is more cumbersome than that of chromosome aberration test and he recommends that SCE can be applied as an additional criterion. Kligerman (1982) in his review on the cytogenetic assays of genotoxic agents in aquatic environment, recommended the use of SCE method. In some cases it has been shown that the frequency of chromosome aberrations and that of SCEs was not complementary to one another after the treatment of same dose of an agent. Anyhow, Bloom (1982) recommended that the SCE method could be applied to Avian embryos which

showed the possibility for studying SCE in the prenatal period.

Heritable translocation test :

Though various types of chromosome aberrations could be induced by different mutagens of which the reciprocal translocation has been used in mammalian mutation research. This was, however, found not to be associated with any phenotypic change. It was associated with semi-sterility in both sexes, having small size of testes and multivalent meiotic association, lethality etc. The progress has been efficiently reviewed by Cattanach (1982).

Micronuclei test (MNT)

MNT has been strongly advocated because of the rapidly and simplicity of the method. It has been advanced by Heddle (1973, 1981). Matter and Schmid (1971), Schmid (1973, 1975a, b, 1976, 1982); Evans (1976) after it was originally discovered by Schroeder (1966, 1970). Micronuclei are supposed to be formed by the acentric fragment, dicentric or multicentric bridges, laggards, asymmetrical exchange etc. They could be detected for few hours after the last mitosis. MNT has been found very helpful for assaying very large number of cells within a short time but the symmetrical exchanges and all form of chromosome aberrations would not be detected by this methods which could be done by the chromosome aberration study.

Biochemical detection of altered DNA :

That the genetic differences and mutations could be

determined in a precisely defined region of the genome. was claimed by molecular biologists. This has been studied on the genetic apparatus in microbiol system as in Escherichia and Saccharomyces and even in cultured mammalian cells. The limitation of the technique has however, been discussed by Klamerth (1970). The mechanism has been reviewed by Painter (1982).

Teratogenic assay :

It has been shown by Ehling (1970) that mutation might induce various teratogenic defects of which the skeletal deformities induced by dominant mutation has been studied in more details. The dominant mutation associated in lethal effect in mice was studied by Grüneberg (1954). Besides the mutagenic hazards, the teratogenic effect of ionizing radiations during development is well-known in various laboratory mammals like rats, mice and even in man. The teratogenic effects have been reviewed by Hicks and D' Amato (1966). The causative factor has been suggested to be due to somatic cell mutation. Radiation induced fetal damages have also been reviewed by Jacobsen (1970).

The use of drug thalidomide created a great controversy when numerous cases of congenital malformation in the offspring were reported whose mothers used the drug. Beside thalidomide, other drugs which have been found to be associated with congenital malformation are insulin (Wickes, 1954), sulphonylureas, coricosteroids and A.C.T.H., Antithyroid drugs, Progestogens, androgens and oestrogen, antibiotics, atebrine, salicylates, phenmatrazine, serotonin, cyclizine etc. In fact the detection of good number of congenital malformation and other birth defects are due to

increase of drug habit and use of chemicals in human society. Besides the study of the clastogenic, mutagenic and tumorogenic effects of thousands of chemicals, the teratogenic effect has also been studied. Among drugs and chemicals as many as 30 antibiotics have been tested on different laboratory mammals like rats, mice, rabbit, hamster etc, with respect to their teratogenic effects and in most cases the results were positive (See Roy, 1982). The effects were seen in different organs at different stages of development of the embryos. The teratogenic effect of antibiotics has also been studied in birds. The antibiotics used were Olivomycin (Klimova, 1972), Phleomycin (Lee et al., 1972), Actinomycin-D (Cury and Montenegro, 1969), Mitomycin-C (Singh and Singh, 1976) etc.

The teratogenic effects of Azo Dyes have been reviewed by Beck and Lloyd (1966). Trypan blue an azo compound found to be potent teratogenic agent causing congenital anomalies in Rodent when injected in critical time. Similar effect was also found in chick. Even it was found in medaka fish, Oryzias latipes (Briggs and Wilson, 1959), though the effect on cold blooded vertebrates has not extensively been studied.

Besides the tumoragenic and mutagenic effects of viruses, their teratogenic effects have also been reported by several workers (Greenberg et al., 1957; Holowach et al., 1957; Hill et al., 1958; Manson et al., 1960; Potter, 1961; Corston and Mirchandani, 1964). Brown (1966) reviewed the viral aetiology of congenital anomalies associated with rubella, influenza, cytomegalo, hepatitis, coxsackie, poliomylitis, ECHO mumps, measles, Viricella and encephalitis viruses. The teratogenic effects during growth and development of infants with congenital rubella have specially been reviewed

by Desmond et al. (1970).and Hanshaw (1970) that of cytomegalovirus infection. The infection of rubella and cytomegalo virus might alter the fetal anlage possibily by somatic mutation as a result of which malformation in the offspring has been observed. Therefore, viruses are very potential teratogens. Any irregularity during developmental process might lead to the malformation. Generally among various possibilities, the chromosomal abnormalities were found to be major cause for origin of malformation. Chromosome anomalies associated with various human birth defects are well known at present.

Effects of meiotic chromosomes

While chromosome aberration in somatic cells might imply somatic mutation, similar effect in germinal cell would suggest hereditary implication. The method of handling male germinal chromosomes has now been very much improved. The time-table of spermatogenic cycle in rat using PAS reaction was determined by Leblond and Clermont (1952). Much improvement has been made specially in mouse. Adler (1982) gave the detail time-table of spermatogenesis and spermiogenesis of mice basing on the data of Oakberg (1960) and showed how it was useful in determining the stage sensitivity to X-ray and chemical mutagens.

The recent methodological improvements have removed the earlier difficulties in handling mammalian oocytes and they have now been used in cytogenetical assay of mutagens. It is known that females at birth are endowed with the complete set of oocytes and the last oogonial cell division occurs sometimes before the birth. The stage at which oocytes remain at birth except rat and mouse is known as diffuse diplotene. Payne and Jones (1975) developed the

technique of mass isolation and culture of mammalian ova. By this technique the cytogenetic effects of several chemicals and X-rays could be studied by different workers (see Brewen and Preston, 1982).

The timing of meiotic stages in mammalian females germ cells and oocytes in mutagenicity testing is equally important. Kuhlmann (1970) discussed the problem. The process of oogenesis in mice taking as a model, he recognized three major stages along with timing : (a) Primordial germ cell - 8 days, (b) Oogonia - day post coitum ± 1 day and oocytes - 14 days. However, Brewen and Preston (1982) reviewed the process and put eight distinct morphological stages in the adult ovary.

Dominant lethal test :

Dominant lethal test has specially been elaborated in mice by Röhrborn (1970). Though originally discovered by Snell (1935) and Hertwig (1935). The test has been considered to be a very crucial one. The lethal mutation induced by some mutagen in the male parent when mated to normal females, the mated female in case of lethality induced might show (1) Pre-implantation loss or (ii) Post-implantation loss (Röhrborn, 1970). The detection of post-implantation loss is relatively simple which is generally assessed at late period of gestation by exposing the uterus of pregnant mother. The dead embryos leave a scar mark while the living ones show buldges. Soares (1972) also developed a technique of detecting the dead implants after parturition by exposing the uterus of the female mice at the end of the weaning period of the litters. However, the term 'dominant lethal' appeared to be misnomer

because the effect could be followed in F_2 generation as well (Patel, 1982; Roy, 1982; Banerjee, 1982).

Sperm head abnormalities

It is claimed that the head morphology of sperms is under genetic control (Wyrobek et al., 1975, 1976; Wyrobek, 1979). It has been shown by different workers that frequency of sperms with abnormal head morphology was increased in epididymal sample of mutagen treated male mice than that of control.

Chromosome aberration in F_1 and F_2 generation :

Beside the heritable translocation referred to before, it was found by Manna and his collaborators (Roy, 1982; Patel, 1982; Banerjee, 1982)the occurrence of various types of chromosome aberrations in embryos and adults of F_1 and F_2 generation of mutagen treated lines. This has been explained due to the transmitation of mutagenic factor from parent to following generations through some mutated loci of chromosome and the said loci were supposed to control in normal conditions the structural intrigrity of chromosome (Manna, 1984). However, similar studies were not carried out in other laboratories. Perhaps it was thought that the mutation induced by the testing agent was 'dominant lethal'.

Drosophila list

Drosophila has been found to be a good genotoxic material which has been reviewed by Vogel and Sobels (1976). It provides the advantage for the study in gene mutation and chromosome aberrations (Sobels, 1974).

Though there are several methodologies referred to before, the present author deployed same of them (vide infra) for reasons like limitation of time, facilities etc.

Physical agents :

Among different physical agents, the effects of ionizing radiations on chromosomes extensively have been studied. They could broadly be put as (a) the mechanism of structural rearrangements, (b) qualitative and quantitative analysis of aberrations and the verification of 'Target' hypothesis, (c) effect of modifying factor and radioprotection, (d) radio-sensitivity and radio-resistance, (e) biochemical mode of action, (f) therapeutic uses, (g) determination of finer structure of chromosomes, (h) tracer study, (i) crop improvement and preservation, (j) live stock improvement etc., (k) lethal effect, and (l) heritable translocation study. We refrained from going into the details because it is little beyond our present scope and efficient reviews have been made time to time (Lea, 1962; Bacq and Alexander, 1961; Evans, 1962).

Effect of chemical agents :

The progress in the field of chemical mutagenesis is not only very exhaustive like that of radiations but also more varied and innumerable number of agents. Because of the limitation of the present scope, no review of works has been attempted by author and the effects of various chemicals on chromosomes have also been reviewed time to time by different workers (Darlington, 1953; Eigsti and Dustin, 1955; Biesele, 1958; Sharma and Sharma, 1960; Wilson, 1960; Freese, 1963; Von Rosen, 1964; Kihlman, 1966;

Auerbach, 1967; Manna, 1969, 1971; Vogel and Rohrborn, 1970; Fishbein et al., 1970; Hollaender, 1971a, b, 1973, 1976; Hollaender and de Serres, 1978; Sharma, A.K., 1981; Hsu, 1982; Sharma, A., 1984). There has been several attempts to put the chemical mutagens according to their chemical properties and/or cytological effects. Bauch (1947), classified the mitotic poisons as, (a) spindle poison, (b) cell division poison and (c) chromosome poison; Ahlstrom (1951), as (a) spindle poisons, (b) interphase poison, (c) karyoclasic poison and (d) other poisons; Dustin (1952) as (a) spindle poison, (b) perfect radiomimetic poison and (c) partial radiomimetic poison; D'Amato (1954) as (a) inhibitors of cytokinesis, (b) inhibitors of spindle formation and (c) preprophase inhibitors; Biesele (1958) as (i) prophase poison, (ii) chromosome poison, (iii) metaphase and later stages poison; Sharma and Sharma (1960) as special drugs, antibiotics, plant product; plant hormones, vitamins, growth regulators and organic compounds, inorganic salts, metals and gases; Wilson (1965) as (i) prophase poison, (ii) spindle poison, (iii) mitotic inhibitors, (iv) chromosome breaking effects; Kihlman (1966) as (i) DNA precursors and related compounds, (ii) Antibiotics, (iii) Alkalyting agents, (iv) Ribose compounds and (v) Miscellaneous chemicals and so on.

The present thrust on the study of the environmental mutagens has led to the discovery of nearly a million chemical agents. Various gene-tox programmes have been set up in different countries for their detection and management. The environmental mutagens have been put under various categories basing on some parameter. Barthelmess (1970) put the chemical compounds in human environmental

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sources as (i) medical drugs, (ii) stimulants, (iii) food, (iv) dyes, (v) cosmetics, (vi) pesticides and (vii) veterinary drugs. Sharma (1981) discussed the sources as natural and man-made ones and other problems of pollution.

The effect of chemical mutagens on dividing cells could broadly be generalised for the following types of action (1) inhibitory effect at prophase and at other stages, (2) accelerating effect of the cell division, (3) prophase poison, (4) metaphase poison, (5) nucleotoxic effect, (6) cytotoxic effect, (7) inhibition of cytokinesis and so on.

The effects of chemicals like that of physical agents have been studied generally for (a) the qualitative and quantitative assays, (b) the biochemical mode of action, (c) dose-dependent effect, (d) chemoprotective effect, (f) antagonistic and synergistic effect, (g) intra- and inter-specific chemosensitivity etc. Some amount of differences could be attributable on the effect of chemicals on chromosomes as compared to that of ionizing radiations. They are : (a) the effect could be immediate to variable length of time; (b) the aberrations were mostly chromatid type;(c) the breaks were nonrandomly distributed between and within chromosomes and so on.

Genetic mutagen

Not very extensive study has been carried out in this field. It was shown that "Dotted" gene (Dt) located in short arm of chromosome-9 induced the gene a located in chromosome-3 to mutate to A in maize (Singleton, 1965). Earlier McClintock (1951) reported that another genetic

mutagen known as Ac-Ds system in which Ac stands for activator, DS for producing chromosome breaks in maize. However, the progress is very limited in comparison to that of physical, chemical and living mutagens.

Living mutagens : Since the present author used the Bacterium Pseudomonas aeruginosa as a living mutagen, clastogenic, mutagenic and teratogenic potentialities of various microbes have briefly been reviewed in following :

Effect of viruses : The pioneering discovery of chromosome aberrations by Hamper and Ellison (1961) in cultured cells of hamster induced by the Herpes simplex virus opened up a new field of investigation. Viruses (= Poison, Latin) are the smallest and simplest among microbes which range from 0.01 to 0.3 micron in diameter and are strictly parasitic to animal, plant or bacterial host. They lack biosynthetic and energy yielding enzymes and possesses only one kind of nucleic acid either RNA or DNA and not both. Rarely some viruses appeared in symbiotic form in close association with bacteria.

During the first quater of the present century, it was shown that sarcoma in fowl could be induced by the viral agents by Rous (1910, 1911). Since then virus induced tumor has been reported in different vertebrates organisms including man. However besides beguin tumor the malignant tumor in man of viral origin has not yet been reported (see Brachet, 1954). It was also claimed that the interferon of the viral agent possibly induces the structural changes of the salivary gland chromosomes of the Dipteran species - Rhyncosciara angelae (Diaz and Pavan, 1965; Pavan and Basile, 1965). The occurrence of pycnotic nuclei at Pachytene in the germ cells of leaf-hopper, Idiocerus was

suspected to be due to viral infection (Halkka and Heinonen, 1968). Though the tumorogenic property of viruses in different groups of vertebrates was discovered early (Rous, 1910, 1911), while the study of clastogenic effect induced by the viral agents mostly in mammalian species including man began much later (Hamper and Ellison, 1961) the latter has advanced very rapidly (see Aula, 1965; Bartsch, 1970; Manna, 1973, 1980, 1982). Soon after the discovery of the clastogenic effect made by Hamper and Ellison (1961), Nichols and his collaborators carried out a series of studies on different aspects of virus induced chromosome aberrations (Nichols, 1963, 1966, 1968; Nichols et al., 1962, 1964, 1965). He (Nichols, 1966, 1968) advocated the role of viruses in somatic mutation and carcinogenesis as he found the similarities between the chromosome aberration induced by viruses and some nucleic acid components. He (Nichols, 1978) also studied the genetic effect of virus transformation. Good many other workers have also studied the chromosome aberrations induced by different viruses, their vaccine and so on which have been briefly summarized hereunder.

According to Zinsser Microbiology (Joklik and Willett, 1976), animal viruses are grouped into A) DNA containing viruses comprising six major families :

i) Poxviridae
ii) Herpesviridae
iii) Adenoviridae
iv) Papovaviridae
v) Iridoviridae
vi) Parvoviridae

of which the chromosome breaking effects of Irido- and Parvo viruses have not been reported, and B) RNA containing viruses comprising ten major families :

i) Picornaviridae
ii) Togaviridae
iii) Reoviridae
iv) Orthomyxoviridae
v) Paramyxoviridae
vi) Rhabdoviridae
vii) Retraviridae
viii) Bunyaviridae
ix) Arenaviridae
x) Coronaviridae

of which viruses belonging to last three families, Bunyaviridae, Arenaviridae and Coronaviridae have not been reported for their chromosome breaking property.

A. Effect of DNA viruses :

Proxiviridae : Different forms were found to induce various types of chromosome aberrations including gross effects in the leucocyte culture of patients infected with small pox (Aula, 1965; Schuler et al., 1968) as well as in cultured cells infected with vaccinia viruses. The effect of viruses and their vaccines on chromosome was also studied in vivo bone marrow cells of mice (Barinsky and Dementiev, 1968; Michaylova and Gorsunova, 1969a, b, 1975, 1979), in cultured kidney cells of monkey (Boiron et al., 1966), embryonic cells of bat (Huang, 1967), and Chinese hamster (Mazzone and Yerganian, 1963), cultured cells of man (Aya et al., 1967; Miles and O'Neill, 1969; O'Neill and Rapp,

1971), HeLa cells and HEP-2 cells (Sablina and Bocarov, 1968). The chicken pox virus induced predominantly break type chromosome aberrations in patients and in culture human cells on infection (Aula, 1965; Grover et al., 1968; Renda, 1968), but Harnden (1964) did not find substantial amount of increase in chromosome aberrations.

Herpesviridae : Besides the original studies of the chromosome aberration induced by Herpes simplex virus by Hamperand Ellison (1961, 1963), other workers showed that the breaks were somewhat localized and nonrandom in occurrence (Huang, 1967; Stich et al., 1964a, b). The G_2 phase of the host cell was found most susceptible (O'Neill and Rapp, 1971). Further the pulverization effect caused by the Herpes simplex virus was found similar to that of the cytomegalo virus (Huang, 1971).

Adenoviridae : The adanoviruses, the causative agents for respiratory trouble, tumorus changes etc. (Yerganian et al., 1965; Stoltz, et al., 1967) were also found to induce various types of chromosome aberrations (Aula et al., 1968). Both diffuse and break type aberrations were induced by adenotype 2, 4, 12, S.A. 7 and S.V. 15. A comparative study of the virus induced chromosome aberrations in hamster and human cell revealed that the aberrations were of similar nature and somewhat localized. Dolimpo et al. (1968) added that some of the viruses also induced numerical changes in chromosomes. The breaks induced by Adenotype-2 were of chromatid type, localised and nonrandomly distributed (Huang, 1967; ZurHausen, 1967), though some metaphase plates were also grossly affected.

Papovaviridae : They include tumor forming DNA viruses. Both structural and numerical changes was observed in mammalian chromosomes papillome-shope virus was infected. Polyoma virus induced structural changes in chromosomes of embryonic cells of hamster after neoplastic transformation (Dulbecco, 1965; Vogt and Dulbecco, 1963). Chromosome aberration was reported in human diploid cells (Chumakov et al., 1964; Moorhead and Saksela, 1965) and in the bovine lung fibroblast culture (Ponten and Lithner, 1966) when they were infected with SV40 (Simian virus). Wolman et al. (1964) claimed that the breaks were localized besides pulverization and heteroploid changes. Yerganian (1966) in Armenian Hamster and Lehman and Defendi (1970) in Chinese hamster observed the chromosome aberrations induced by SV 40 virus. The breaks were chromatid type and non-randomly distributed between and within chromosomes in both human and hamster cells (Weinstein and Moorhead, 1965; Yerganian, 1966).

B. Effect of RNA viruses :

Picroviridae : Poliomyelitis viruses of man and monkey were found to induce break and gross type chromosome aberrations after infection with type-1 (Bartsch et al., 1967) but the infection with type-3 failed to induce the specific form of chromosome aberration (Aula and Nichols, 1968). Mice immunised by poliovaccine type-2 contained chromosome aberration in the form of breaks, constriction, heteroploidy, pulverization, pycnosis etc. (Michaylova and Gorsunova, 1969). Further, infection with Columbia S.K. virus induced both breaks, constriction and heteroploidy in cell culture (Nachtigal et al., 1966).

Togaviridae : Arboviruses and rubella viruses belonging to Togaviridae were reported to induce chromosome aberrations by different workers. The flavirus, the mosquito-borne yellow fever virus was found to induce chromosome aberration in mice (Barinsky and Dementiev, 1968) and in man (Harnden, 1964). Even the vaccination against yellow fever virus induced various types of chromosome aberrations in some individual (Harnden, 1964). The tick borne encephalities virus of arbo group induced heteroploid changes but the effect of individual chromosome structure was negligible in human cell strain and in BHK-21 cells (Tolstova et al., 1966).

Paramycoviridae : In this family, Sharma and Polasa (1978) reported not only the chromosome breaking effect of Honkong strain of influenza virus but also the dominant lethal effect in mice. The parainfluenza virus type-1 and 3, newcastle disease virus, mumps and measles viruses were found to induce the chromosome breaking effects by different workers. Gross type chromosomal aberration in HeLa cell for the treatment of mumps and parainfluenza virus 1 and 3 was reported by Cantali et al. (1966). The pulverization effect induced by parainfluenza virus (Sendai) was seen in human chromosomes (Salkinder and Gear, 1964; Aula and Saksela, 1966; Kato and Sandberg, 1968a, b; Stenman and Saksela, 1969) and in chromosome of kidney cells of rabbit. It was reported before the genomic changes induced by parainfluenza virus (Homma et al., 1968). The infection of newcastle disease virus of chick to HeLa cells and normal human cells (Canteli et al., 1966; Szanto et al., 1967) induced chromosomal pulverization and heteroploidy. Patients infected with mumps and measles showed various

types of structural changes in chromosomes (Renda, 1968; Aula, 1965).

Rhabdoviridae : The infection with rabbies virus induced diffuse type chromosome aberration (Mechajlova and Gorsunova, 1969) amd there was no localized breaks (Marquez-Monter et al., 1967). The diploid cell strain infected with this group of viruses was also shown to have chromosome breaking effects (Libíková and Motajová, 1967). The chromosome breaking effect induced by rubella has been studied in human cell by different workers (Boue et al., 1964; Chang et al., 1966; Kuroki et al., 1966). It was also studied for teratogenic effects (vide infra).

Reoviridae : The acute and chronically infected cultured cells with reoviruses induced both breaks in chromosome and heteroploidy (Nachtigal et al., 1966). The neonetal mice infected with reovirus type-3 induced heteroploid change of chromosome number (Keast, 1966).

Orthomyxoviridae : The human influenza virus did not induce marked chromosome breakages when strain A (PR-8) was introduced to the human embryonic lung culture (Gavrilov et al., 1965), in human diploid cell line.

Retroviridae : The oncogneic RNA viruses of this family causing tumors in chick, mice etc. were found also to be the potential chromosome breaking agent by different workers as it was reported for several strains of Rouse Sarcoma virus (Ahlström et al., 1964; Nichol et al., 1964; Kato, 1967; Boue et al., 1968), the murine leukomia virus, Friends strain (Sofuni et al., 1967) etc. Tsuchide and Rich (1964) found only the pulvarization and heteroploid

changes of chromosome. In cultured cells of monkey treated with Moloney virus induced transformation of cell but very little structural changes in chromosomes (Boiron et al., 1966).

Miscellaneous group : Hepatitis virus because of their indefinite nucleic acid constitution has been put into the miscellaneous group (Joklik and Willet, 1976). They were found to induce chromosome aberrations by different workers. The leucocyte culture of patients suffering from acute hepatitis contained chromosome aberration in the form of structural changes, pulverization and genome modificiation (Aya and Makino, 1966; El-Alfi et al., 1965; Kerkis et al., 1967). Various types of chromosome aberrations were found in bone marrow of mice due to the hepatoencephalotrophic viral infection (Barinsky and Dementiev, 1968). In other mammals chromosome breaks and mitotic suppression were noticed for the infection of hepatitis virus (Mella and Lang, 1967; Yoshida et al., 1967; Matsaniotis et al., 1968). Some workers found various types of chromosome aberrations in vivo cultured cells of dog infected with canine hepatitis virus.

The clastogenic property of viruses belonging to different groups referred to above is beyond any doubt. The aberration induced by viruses occurred predominantly in the localized from of chromatid breaks, constrictions, gaps, etc. As mentioned before since the nature of chromosome aberrations induced by viruses and that of nucleic acid analogues were of similar nature, it was implicated with the etiology cancer (Nichols, 1966, 1968; Boue et al., 1968). Even the regionwise localization of breaking points of some mammalian chromosome including man induced by viruses

and chemical mutagens was noticed (Cohen and Shaw, 1965; Aya and Makino, 1966; Huang, 1967). Manna (1980a) showed the basic similarity of chromosome aberration types induced by viruses, bacteria and human cancer. It was also claimed that breaks induced by viruses undergo delayed restitution.

The precise mechanism of virus induced chromosome aberrations is yet to be found out. ZurHausen (1967) opined that the viral proteins having effect on enzyme but it did not receive much attention. Bartsch et al. (1966) suspected the lysosomal involvement from their study on poliomitilis virus induced breaks. Several workers (Nichols et al., 1964; Cohen and Shaw, 1965; Bartsch et al., 1965) opined that the inhibition of cellular bio-synthesis, specially DNA by the viruses could be of special significance. The last was more attractive because virus induced change of cellular DNA, RNA and Protein synthesis have wider acceptability and it can explain virus induced gross effects, pulverization, inhibition of mitosis and even numerical changes (Bartsch, 1970). Nichols (1978) advocated that the genetic effect of the transforming viruses in the infected cell might help to prove into their role in the genetic alteration of virus induced oncogenensis. The recent report on the virus induced sister chromatid exchange has provided a further evidence of the mutagenic potentialities of viruses. Unfortunately the interest in the study of the virus induced cytogenetic changes has declined in recent years. The mutagenic potentiality determined by dominant lethal test on mice has only been carried out by Sharma and Polasa (1978b). Anyhow, the mutagenic potentiality of viruses needed be established by more works.

Effect of Rickettsia :

Rickettsiales are simple, obligate intracellular parasites and exceptional condition they can grow in cell free nutrient broth containing body fluid. According to Cruickshank (1970), class Microtabobiotes beside viruses, species belonged to order Rickettsiales. This causative agent of scarlet or typhus fever is said to have influenced the course of human history in Prague and London (see Joklik and Willett, 1976). The Rickettsia, the causative agent for scarlet fever were discovered by Dr. Howard Ricketts. Three genera (i) Rickettsia, (ii) Rochalima and (iii) Cosiiella belonged Rickettsiales. They are pleomorphic rod-shaped coccoid organisms, measuring 0.3-0.8 x 0.6 m μ. In ultrastructure and chemical composition, they show resemblances to gram positive bacteria. Unlike viruses, they possess both DNA and RNA and their respiratory and synthetic capabilities are present in the cell. They are smaller than bacteria and larger than viruses.

Among the three genera the chromosome aberrations induced by Rickettsia have only been suspected in meiotic cells of male gryllid, Acheta sp. There were no true break type aberrations and only some sticky and clumping effects were seen in first spermatocyte metaphase chromosomes (Halkka, 1967; Halkka et al., 1970). No effect on mammalian chromosomes has so far been known. The teratogenic effect induced by Rickettsia has also not been reported. Therefore, the Rickettsia as mutagen to mammalian system remained practically unexplored.

Effect of Bacteria and Fungi :

It is well known that the fungi are higher in order than bacteria but we have taken the liberty of reviewing fungi induced cytogenetic effects before that of bacteria in order to keep the continuity of the presentation our relating to cytogenetics effect of the bacterium P. aeruginosa, the subject matter of the thesis.

Effect of Fungal agent and Fungus :

The classification of fungi is a debatable issue. The classifications presented by Joklik and Willett (1976) and by Cruickshank (1970) are not in full agreement. According to Cruickshank, among four classes of fungi viz. Fungi imperfecti, Basidiomycetes, Ascomycetes and Phycomycetes, the Fungi imperfecti have characteristically no sexual stage and consist mainly the pathogenic moulds, yeasts, yeast-like fungi and dimorphic fungi. It was placed in between bacteria and fungi proper. So far as the present author is aware no mutagenic property of species belonging to Fungi imperfecti applying various mutagenicity testing methods has been carried out. The family Moniliaceae belonging to Fungi Imperfecti contained some highly pathogenitic genera like Aspergillus and Penicillium. The mutagenic effect of penicillium itself though not tested directly, its toxin and the antibiotic penicillin have been found to produce clastogenic effect in treated mice (Manna and Bardhan, 1973b). Among about 160 species of Aspergillus recognised (Raper and Fennel, 1965), A. fumigatus, A. flavus, A. parasiticus, A. condidus, A. ochracious etc. are human pathogenic forms. Aspergillus contamination degrades food material (Thom and Raper, 1945; Park and

Park, 1977). The metabolites produced by them are known collectively as mycotoxins like aflatoxins, rubratoxins, ochratoxins etc. Among them aflatoxins (produced by Aspergillus and other organisms) have been proved to be mutagenic by different mutagenicity testing protocols like liver microsomal assay in rodent (Matzinger and Ong, 1976; Kuroki et al., 1979), and man (Buening et al., 1978), agar diffusion assay (Awerbuch and Stark, 1979); modified liquid culture method of microbial assay (Mitchel, 1978); DNA repair method in Chlamydomonas (Schimmer and Werner, 1974) and other microbial method deploying E. coli (Goze et al., 1975); Salmonella (Wong et al., 1977) and Drosophila (Fahmy and Fahmy, 1978) etc.

The chromosome breaking properties of aflatoxins in human (Dolimpo et al., 1968), hamster (Korte et al., 1979) rat (Kuroki et al., 1979) and rat-kangeroo cells (Green et al., 1967) have also been found positive. Beside aflatoxins the chromosome breaking properties of other mycotoxins like citrinin on rat, Chinese hamster cells and man (Thust and Kneist, 1979) and patulin on bone marrow of Chinese hamster (Korte, 1980) were found also positive. The treatment of aflatoxin B_1 in male mouse induced heritable chromosomal changes in F_1 (Leonard et al., 1975) while in rats micro-doses had no affect on sperm count and sperm head morphology (Egbunike, 1979). Among mycotoxins the relative efficiency of clastogenic property was more for patulin than aflatoxins and the order was PA> AFB_1> AFG_1 (Korte, 1980). The application of the aflatoxin and mitomycin in the cell-line of rat Kangaroo induced chromatid type gaps and breaks and the long arm of the autosome and the heterochromatic X-chromosome were found more sensitive (Green et al., 1967). The chromosome breaking effects of aflatoxins in root tip cells of Vicia faba

(Lilly, 1965), onion (Reiss, 1971) etc. were found positive. The mycotoxins were also found to inhibit mitosis (Lilly, 1965) and normal growth of parts (Reiss, 1977).

Beside the clastogenic and mutagenic property of some mycotoxins, specially the Aflatoxin B_1 referred to before they also produced teratogenic effects. Aflatoxin B_1 when added to culture medium, it inhibited blastoid transformation or to the breeding medium of the frog, Xenopus, the mortality rate of the tadpoles was increased, inflicted some eye abnormalities and impaired other organogenesis (Aleksandrowicz et al., 1975). Teratogenic effects in rats was also noticed of for the treatment of Ochratoxin A (Brown et al., 1976). Therefore, among various mycotoxins, the aflatoxin B_1 was shown more clearly to have mitogenic, clastogenic, mutagenic, carcinogenic and teratogenic properties.

The carcinogenic property of aflatoxins have widely been tested. A. flavus can liberate aflatoxin and aflatren. The later, when treated to mice caused behavioural changes (Gallagher and Wilson, 1979). Some species like A. niger, A. glaucus, A. flavus etc. were also found to produce various antibiotics and mycotoxins (Jacobs and Gerstein, 1960). Similarly antibiotics produced from Penicillium are citrinin, patulin, Ochratoxin, sterigmatocystin, rubratoxin, sinigrin, aflatren, territrem A and B, funitremergen A, B, C, D etc. of which patulin from Penicillium patulum, Citrinin from P. citrinum and Ochratoxin from A. ochracious deemed to have mutagenic property.

Though a number of fungal toxins were found to induce cytogenetic effects in treated specimens, it was Manna and his collaborators (Manna, 1980, 1982; Manna and Patel, 1981; Kundu, 1983; Manna and Chakrabarti, 1984) reported the spores of different species of Aspergillus acted as mutagenic agent in treated mice. Manna and Patel (1981, 1984, unpublished) and Patel and Manna (1984) after making the initial discovery of bone marrow chromosome aberrations in spores of Aspergillus fumigatus treated mice, they continued the study on various aspects to probe that the spores as mutagen in treated mice deploying various metagenicity testing methods. Earlier, crude mycelial extract of A. fumigatus tested of chicks and laboratory mammals was found to be neurotoxic, hematoxic and histotoxic effects (Henrici, 1939). From the culture of A. fumigatus, antibiotic substances like fumigacin (Waksman et al., 1943), fumagillin (Houson and Edle, 1954), fumigatin (Oxford, 1942), gliotoxin (Jacobs and Gerstein, 1960), etc. were isolated. However, the chromosome breaking effect of the crude extracts of the mycelium or of the antibiotic agent of A. fumigatus was not studied. Manna and Patel (lit cit) established reasonably the mutagenic property of the spores of A. fumigatus and the results are summarised below :

The isolated spores of A. fumigatus after intraperitoneal injection to mice induced higher frequency of various types of chromosome aberrations assessed at 18 intervals between 5 min and 90 days which were in the form of sub-chromatid, chromatid and rarely iso-chromatid breaks, acentric fragment, translocation, gaps and constrictions grouped as individual types and stickiness, pycnosis, polyploidy etc. grouped as gross types. The treatment of heat-killed spores, however induced lower frequency of

aberrations but it was still higher than that of control. The mitogenic effect was proved by the study of the mitotic frequency in bone marrow cells of treated specimens against control. The treatment of spores showed in the male epididymal suspension significant depletion in sperm count and higher frequency of sperms with abnormal head morphology. The mutagenic properties of spores of A. fumigatus in treated mice was established by the study of the lethal effect in F_1 generation evaluated at 15 day of gestation and after parturition by mating spore injected male parent to separate sets of 3 virgin females each week for 5 weeks. The mutagenic index was significantly high in treated series than control. Further, the chromosome aberration study in liver and spleen cells of F_1 embryos of 15 day gestation and in bone marrow of F_1 individuals of different age revealed various type of abnormalities like their treated male parent but none of the aberration was specifically heritable one like heritable reciprocal translocation found by others for treating chemical and physical agents (Cattanach, 1982). Even in F_2 generation the lethal test and the chromosome aberration study showed some positive effect indicating thereby the mutagenic factor was transmitted in successive generations.

The study of the effect of spores of other species of Aspergillus, A. niger (Kundu, 1983; Kundu and Manna, 1984a, b, 1985, unpublished) and A. flavus (Chakrabarti and Manna, 1984, 1985 and unpublished) in mouse systems appeared also to be mutagenic revealed by different tests the study of which is in progress. Thus fungi imperfecti and their products appeared to be mutagenic to mammalian system.

Effect of Bacteria

Among microbes of lower order, bacteria other than viruses constitute a very important group. They may grow and attach to one another in cluster, chain, rods, filament while higher bacteria - Actinomycetales form a mycelium. Bacteria and blue green algae are prokaryotic (Pro=primitive, karyote = nucleus) cells, having no nuclear membrane while the naked DNA or the chromosome (genophore) is not associated with basic protein but with a semi-rigid cell wall of unique composition. The size of the cell ranged between 0.4-1.5 micron in diameter and in many form the rigid cell wall gives them the characteristic shape. Morphologically they are classified as (1) higher bacteria - simple branching filamentous actinomycetes and (ii) lower bacteria - spherical cocci, rod-shaped bacillus, comma-shaped virbio and spirally-twisted spirilum and flexius, filamentous spirochaetes. Genetically they are classified according to the nucleolide compositions of DNA (Joklik and Willett, 1976) while the biological classification is based on Linnaean Scheme (Cruickshank, 1970).

According to Cruickshank (1970), bacteria, class - schizomycetes comprise five order as (1) Actinomycetales, (2) Mycoplasmatales, (3) Spirochaetales, (4) Eubacteriales and (5) Pseudomonadales.

The chromosome aberrations induced species belong to Actinomycetales considered as fungus by some workers for forming mycelium and possessing special cell for reproduction have not been reported but a number of antibiotics isolated from them showed the chromosome breaking properties when tested in plant and animals (Wilson, 1960; Gebhart, 1970; Manna, 1980b, 1981b).

Spirochaetales :

Spirochaetes are either pathogenic or commensalic-forms, having slender flexuous spirals of 4-16 μ in length and 0.1-0.6 μ thick. So far as the present author is aware the chromosome breaking properties of spirochaetels or their products have not been tested on any higher organisms.

Mycoplasmatales :

Mycoplasma (Pleuro-pneumonia-like or PPLO) are prokaryotes and placed in the next higher category of viruses. Their sizes ranged between 0.5-1.0 μ in diameter, lacking a rigid cell wall, mostly parasitic to man and animals, one saprophytic species found in the soil or sewage (Cruickshank, 1970). Except for the absence of cell wall, its fine structure is similar to that of bacteria (Joklik and Willett, 1976). It can act as the causative agent for human atypical pneumonia. The progress in the study of mycoplasma induced chromosome aberrations has been very limited but the effects has been observed both in insect and mammals including man. It appears that Paton et al. (1965) were pioneers to report the chromosomal changes in cultured human cells infected with mycoplasma. MacPherson (1966) studied the chromosome damages in cultured human triploid cells line infected with mycoplasma. Fogh and Fogh (1967) also reported the PPLO infected Fl-human amnion cells with pulverization and other gross type chromosomal effects. Kindsin et al. (1971) showed the effect of T-strain of mycoplasma for inducing chromosome aberration. Chromosomal aberrations were also reported in the cultured cells of Rat Kangaroo (Lastikka et al., 1976). Besides the chromosome breaking property, some forms caused changes in nucleic acid metabolism of host

cells (Levine et al., 1968).

Like the effect of rickettsia, the mycoplasma induced chromosome damages in the form of laggard, acentric fragment, stickiness, translocation, inversion, aneuploidy, polyploidy, cytoplasmic budding without chromosome and so on were reported in the male germinal cells of the water-bug, Nepa and a gryllid, Achela (Halkka and Heinonen, 1968). However, there was some doubt about the identification of the parasite. Anyhow, there was little doubt about the clastogenic potentiality of mycoplasma because it has been observed by different workers and in different materials while their mutagenic property is yet to be established.

Halkka and Heinonen (1968) suggested that the argenine degradation and cleavage of thymidine caused by the infection of mycoplasma in the host cell was responsible for the chromosome damage. Since arginine is a component of cellular histone, its degradation might affect the histone of chromosome. However, as the type of chromosome aberration induced by mycoplasma showed basic similarities with those of viruses and bacteria, it is possible that some common mechanism might be involved.

Eubacteriales :

Nine families under this order have been recognized of which the chromosome breaking activities by any species belonging to the families Bacteroidaceae, Brucellaceae and Acromobacteraceae in host cells have not been reported. On the other hand some species or their product belonging to the remaining 6 families were found to show some chromosome

breaking activity carried out by Manna and his collaborators.

Corynobacteriaceae :

No chromosome breaking activity induced by any species in this family has been reported but the treatment of the culture of Corynobacterium diphtheriae to cultured mammalian cell yielded notable cytotoxic effects between 24 and 96 hr, but similar treatment of C. pseudodiphtheriae failed to show any notable changes upto 7 days (Holowiecka and Donuata, 1969). On the other hand, Manna and his collaborators (Manna, 1980; Das, 1976) observed break, acentric fragment, gap and even translocation, pulverization, stickiness etc. in chromosomes of bone marrow cells of mice injected with diphtheria toxin. The treatment of TABC vaccine into mice also yielded higher frequency of chromosome aberrations than that of control.

Micrococcaceae :

Out of 4 genera in this family the chromosome breaking activities and mutagenic properties of Staphylococcus aureus in experimental mice have ellaborately studied by Manna and his collaborators (see Manna, 1971, 1973, 1980, 1982; 1985; Manna and Bhowmick, 1969; Manna and Chakrabarti, 1970, 1975; Chakraborti, 1974; Hazra, 1980; Hazra and Manna, 1980, 1981; Manna and Hazra, 1981; Manna and Ghosh, 1984, unpublished; Ghosh, 1985) using log culture, isolated bacteria suspended in saline, culture filtrate and heat-killed samples of the bacterium. This species is a gram-positive cocci, grows in clusters and acts as the causative agent for superficial inflamatory lesions with pus formation. The studies carried out by Manna and his collaborators in mice (lit. cit.) showed positive clastogenic and mutagenic effects against controls for : (1) The

chromosome aberrations in bone marrow cells fixed at different intervals between 5 min and few days, (ii) nonrandom distribution of breaks within and between chromosomes, (iii) inhibitory effect on mitotic frequency at different intervals, (iv) micronuclei test, (iv) dose-dependent response on chromosome aberration frequency, (v) chromosome aberration frequency in bone marrow and in regenerating liver cells of the same partially hepatectomised and their alterations by the conjoint treatment of penicillin, (vi) chromosome aberration at various intervals in germinal cells of male mouse, (vii) sperm depletion test, (viii) abnormal sperm head morphology test, (ix) lethal test in F_1 generation by mating treated male parent with different set of virgin females, (x) chromosome aberrations in F_1 generations in 15 day old embryos and adults of different age, (xi) lethal test in F_2 generation against relevant controls and so on. Even the results were positive when the bacterial sample was injected to other mammals like cat, dog, rat, syrian hamster, monkey etc. indicating that S. aureus is a potential mutagen in mammalian system.

The mutagenic potentiality of another species, Sarcina (Micrococcus) lutea in this family Micrococcaceae has recently been studied by Manna and his collaborators in mice system (Manna, 1982; Manna and Goswami unpublished; Goswami and Manna, 1982; Goswami, 1985) using different mutagenicity testing methods. The mutagenic potentiality of S. lutea in treated mice was determined by (a) the study of bone marrow chromosome aberrations at various intervals by the treatment of log culture, isolated S. lutea suspended in saline, log culture filtrate and heat killed bacterial suspension, (b) the aberration frequency

in the chromosome of different stages of meiosis in testes cells was also higher than that of control, (c) MNT was positive and showed more or less a dose dependent effect, (d) there was mitotic retardation in the bone marrow cells of treated mice than that of control, (e) the sperms count in the epididymes of treated specimens was lower than those of the controls (f) the frequency of sperms with abnormal head morphology was higher in the treated specimens, (g) the lethal test conducted by mating male parents treated with log culture of Sarcina lutea to different sets of virigin females revealed significantly higher mutagenic index assessed at 16th day gestation and at the end of weaning period after gestation in the treated series than control in seven weeks mating programme, (h) mating of F_1 brother and sister of seven week treated lines revealed also higher frequency of lethals assessed on 16th day of gestation and after parturition in F_2 generation than that of control and so on.

Neisseriaceae :

In this family among two genera Neisseriea and Veillonella recognised by Cruickshank (1970), the chromosome breaking activity of Neisseria meningitidis has been studied by Manna and Gupta (1979). The bone marrow cells of mice treated with log culture sample of Neisseria meningitidis had higher frequency of chromosome aberrations assessed after a few minute to a few hours of treatment. However, the treated specimens did not survive beyond 12 hr. The treatment also showed mitotic retardation in bone marrow cells and the frequency of chromosome aberration was reduced when the bacterial sample treated mice were injected with penicillin.

No lethal test could be conducted as the treated specimens died shortly.

Lactobacillaceae :

In this family among 4 genera, Diplococcus, Streptococcus, Peptostreptococcus and Lactobacillus only two species S. pyogenes and S. faecalis were tested for their cytogenetic effects in the treated mice (Manna and Gupta, 1977). The intraperitoneal injection of log culture, isolated bacteria in saline suspension, culture filtrate of S. pyogenes and S. faecales into mice yielded higher frequency of bone marrow chromosome aberrations at five intervals between 10 min to 12 hr than that of control. The effect was little more for the treatment of S. pyogenes than that of S. faecales. In both the cases the treated mice did not survive beyond 12 hr for which other mutagenicity testing was not possible. It was also seen that the frequency of aberration either in S. faecales or in S. pyogenes treated mice was greatly reduced when penicillin was injected to them.

Bacillaceae :

In this family the clastogenic effects have not been tested in mice after treating log culture of any species of the two genera Bacillus and Clostrodium. However, the injection of the tetanus toxin was found to induce good amount of bone marrow chromosome aberrations in the treated mice (see Manna, 1980a; Das, 1976).

Enterobacteriaceae :

In this family among 8 genera, the chromosome breaking effects of Salmonella typhi and paratyphi and of Klebsiella pneumoniae on mice have been studied. The treatment of log culture of S. typhic and paratyphi induced various types of chromosome aberrations in the bone marrow cells of the treated mice. The treated specimens however, did not survive long (Manna and Das, S.K., 1971) for which no other testing was possible. It was also found that the vaccine of TABC when injected into mice also induced higher frequency of chromosome aberrations than that of control.

The injection of the log culture, culture filtrate, and isolated bacteria in saline suspension of K. pneumoniae into mice also induced some amount of chromosome aberrations but the frequency was not very high as compared to that of control. Unlike other bacterial species, the treatment was not very toxic for which aberration frequency was studied upto 48 hr. The chromosome aberration appeared after an hour of the injection and interestingly there was some acceleration of mitotic frequency in treated series than control (Manna and Gupta, 1977; Gupta, 1976). There was also a reduction of the frequency of chromosomes aberration when penicillin was injected into the K. pneumoniae treated mice.

Azotobactericeae :

Postgate (1982) put Xantobacter autrotopriccus in this family. The culture of this nitrogen fixing bacterium

produced higher frequency of chromosome aberration in the bone marrow cells of the treated mice fixed at various intervals (Sadhukhan and Manna, 1984). Preliminary tests indicated that the X. autrotopiccus could act as a potential mutagen to mice system.

Pseudomonadales :

Under this order 2 families Spirillaceae and Pseudomonadaceae are included (Cruickshank, 1970).

Spirillaceae :

In this family 2 genera Vibrio and Spirillum are included but the higher chromosome breaking potency of the cholera toxin and vaccine has only been studied in treated mice (Manna, 1980; Das, 1976).

Pseudomonadaceae :

Pseudomonas aeruginosa is the bacterial species which has been studied by the present author to show its clastogenic, mutagenic and teratogenic potentiality in mice systems by different methods. More than 140 species belonging to the genus Pseudomonas have been recognized but only the species, P. aeruginosa (Bacillus pyocyaneus) was first isolated about 100 years ago. It was recognized as the causative factor of the bluish-green discolouration of surgical dressings (Eykyn, 1985). The Pseudomonas infection could easily be recognised by its characteristic small and flat greenish colonies. P. aeruginosa (= pyocyaneus) is pathogenic to man (Cruickshan, 1970). They are frequently present in small number in normal intenstinal flora of man

and animals. They can also be found on skin of healthy man and could be isolated from sewage. The other species are generally saprophytic in nature living in water and in soil. They are also pathogenic to plants and less commonly to animals. P. aeruginosa is a gram negative non-sporing bacillus measuring between 0.5 micron to 1.5 micra and moves by a single polar flagellum. Most peculiarly it is the only species that produces the chloroform soluble pigment 'pyocyanin' but there are however, some strain difference with regard to the pigment producing capacity. The strain differences were detected by serologic typing, phage typing and pyocin typing methods (Joklik and Willett, 1976). The cell-wall is similar to that of Enterobacteriaceae. They are very adaptive and can utilise over 80 different organic compounds for growth. Though anaerobic in habit but can utilise nitrate as an electron acceptor (Cruickshank, 1970). Certain biological activity of the extracellular slime glycolipoprotein of P. aeruginosa even after pruification and enzymatic treatment, could be retained (Sensakovic and Pksquale 1975). The role of Pseudomonas in plant diseases was also observed by Paton (1960). The germination, growth and production of the plant was found to be inhibited possibly by the production of the antibiotic, Pyocyanin (Brathwaite et al., 1982). The antifungal activity of fluorescent pigment produced by the species has been shown by Misaghi et al. (1981).

The common of infection of Pseudomonas in man is seen at the sites of burns and wound, in corneal tissue, urinary tract, malignant tissue etc. lungs, (Eykyn, 1985; Cruickshank, 1970). The subcutaneous infection of P. aeruginosa in rabbits, guineapig and mice might produce

fever and local abscess. The major problem of antibiotic therapy for the treatment of P. aeruginosa infection has been discussed recently by Eykyn (1985) and he opined that the species is opportunist per excellence and it seldom causes infection in patient with normal host defences.

Pseudomonas has been found to be tumourogenic agent in the fish, climbing perch (Anabas) as the epithelial carcinoma was developed due to the mixed infection of P. fluorescens and Micrococcus variance (Pal and Pal, 1984; Pal et al., 1978). On the other hand P. aeruginosa was claimed to have tumour inducing properties in mice (Gaydos et al., 1976).

The mechanism by which P. aeruginosa produces diseases in man has not been considered. A number of enzymes and toxins including endotoxins in addition to the slime seemed to produce pathological effect. It is suggested that two types of proteases produced by P. aeruginosa could be responsible for haemorrhagic skin lesion and eye infection. They might cause destruction of tissue. The phospholypase and the glycolipid have been found to produce haemolysin. Three exotoxins, A, B, C which are lethal to mice and dogs have been identified. Exotoxin-A has been partially characterised as a protein but it was not the causative factor for the necrosis in the skin. The toxin produced by P. aeruginosa is not heat labile and it can withstand the temperature over 100°C. Pyroverdine, the iron-binding fluorescent pigment of P. fluorescens has been stated to have a role in iron transport (Meyer et al., 1978).

Some genetical study has been carried out in P. aeruginosa. The relationship between mutated amidasis and hydroxyurea has been studied (Brown et al., 1979). However, very little work was done at the cytological level outside our laboratory. Scharmann, Jacob and Porstendoerfer (1977) studied the cytotoxic effect of the leucocidin from P. aeruginosa on human polymorphonuclear leucocyte which induced characteristic morphological alteration within a few minute of exposure to leucocidin. However, Manna and his earlier collaborator (vide infra) carried out quite an extensive study on the cytogenetical effect of this bacterium in treated mice, More data, both confirmatory and new and results of new line of investigations for establishing firmly the bacterium as the clastogenic, mutagenic and teratogenic agent in treated mice have been presented in this thesis.

Beside P. aeruginosa, P. fluorescencs (tentatively identified) isolated from tumor site of the climbing perch when injected intraperitoneally into mice after culturing induced various types of chromosome aberrations (Manna and Pal, 1982). It was also shown by Pal and Pal (1984) that the mixed culture of the bacteria, P. fluorescens and Micrococcus varience (?) after isolating from the tumorous fish infected into normal Anabas specimens, some died and others developed ulcer at the point of injection as well as in other regions. Some of the surviving fish also developed lyphoma in dermal layer while in others some epidermal papilloma and melanocarcinoma appeared. Similarly subcutaneous injection of the mixed culture of these two bacteria into mice caused death of most of the specimens while two individuals after 2 month showed the preneoplastic changes in liver, five individuals after 5th month developed

adeno-carcinoma in the lung and one had cholangio-hepato-carcinoma (Pal and Pal, 1984). Very recently Manna and Biswas (1986) reported the increase in the frequency of the micronucleated peripheral erythrocytes of the fresh water fishes, Catla catla, Labeo rohita, Cirrhina mrigala and Oreochromis mossambicus at 24 hr after intraperitoneally injecting them with log culture of P. aeruginosa.

As mentioned before an elaborate cytogenetic studies has been carried out by the present author using pure culture of P. aeruginosa as a continuation of the work carried out by Manna and Dey (Manna, 1980, 1982; Manna and Dey, 1979; unpublished; Dey and Manna, 1978). The present author undertook the problems with a view to extending the data as well as making new experiment to establish the bacterium as a clastogenic, mutagenic and a teratogenic agent avoiding the repeatation of the studies carried out earlier Manna and Dey (lit. cit.). The studies carried out by them were the bone marrow chromosome aberrations at some 14 intervals between 10 min and 192 hr induced by the I.P. injection of log-culture, saline suspensed isolated bacteria, culture filtrate and heat killed suspension of P. aeruginosa into separate sets of mice. The average frequency of aberration taking all intervals for both individual and gross type aberration was 34.0%, 30.4% and 27.50% for log culture, isolated bacteria in saline and culture filtrate treated series respectively. On the otherhand in the heat-killed bacteria sample treated mice it was 14.08%. There was also mitotic retardation in the bone marrow cells of mice treated with saline suspended P. aeruginosa. Some preliminary studies on depletion in sperm count, induction of dominant lethal in F_1 and F_2 generation etc. were also carried out.

The present author has reinvestigated the clastogenic potentiality of the bacterium by study of chromosome aberrations in the bone marrow cells of treated mice at different longer intervals, carried out MNT, studied meiotic chromosome aberrations and also mutagenic potentiality by generation study of lethal effect and chromosome aberration, biological changes for sterify teratogenic and histogenic potentialities as given in the following chapters :-

CHAPTER - I

Cytogenetic Assays of Pseudomonas aeruginosa Treated and Control Mice in Parental Generation

A. Studies on Somatic cells :

a) Effect on bone marrow chromosome at various intervals.

b) Micronucleus Test.

B. Study on Male Germinal Cells :

a) Effect on chromosomes at different stages of meiosis.

b) Effect on sperm count.

c) Effect on sperm head morphology.

C. Histological Studies :

a) Effect on Testis

b) Effect on Ovary

c) Effect on Uterus.

CHAPTER_ II

Cytogenetical Assays in First Filial (F_1) Generation

A. Lethal Test :

a) At 15 day of Gestation and at the end of the weaning period of the mother after parturition

B. Somatic Chromosome Aberration in F_1 Generation

a) In embryos of 15 day gestation.

b) In F_1 individuals of 3 month old

C. Meiotic Chromosome Aberration in 3 Month Old F_1 Males

D. Sperm Count in 3 Month Old F_1 Males

E. Sperm Head Morphology in 3 Month F_1 Males

F. Teratogenic Effect

a) In F_1 fetus at 15 day of gestation

b) In 3 month old F_1 individuals organs.

G. Histological Effects :

a) Effect on testis

b) Effects on vary

c) Effects on uterus.

CHAPTER _ III

Cytogenetic Assays in Second Filial (F_2) Generation

A. Lethal Test :

a) At the end of weaning period of the mother.

B. Somatic Chromosome Aberration in F_2 Generation

a) In embryos of 15 day gestation

b) In F_2 individual of 3 month old

C. Meiotic Chromosome Aberration in 3 Month Old F_2 Males

D. Sperm Count in 3 Month Old F_2 Males

E. Sperm Head Morphology in 3 Month Old F_2 Males

F. Histological Studies :

a) Effect on Testis

b) Effect on ovary

c) Effect on uterus.

CHAPTER _ IV

Teratogenic Effect in P. aeruginosa Treated and Control Pregnant Mice

A. Effects of the Log-culture of P. aeruginosa Injected on 1 to 5 days in Females Showing Vaginal Plug against Nutrient Broth Injected Control and Examined at 15 Day Pregnancy

B. Histological Studies of Uteri of Control and of Treated Series Mothers

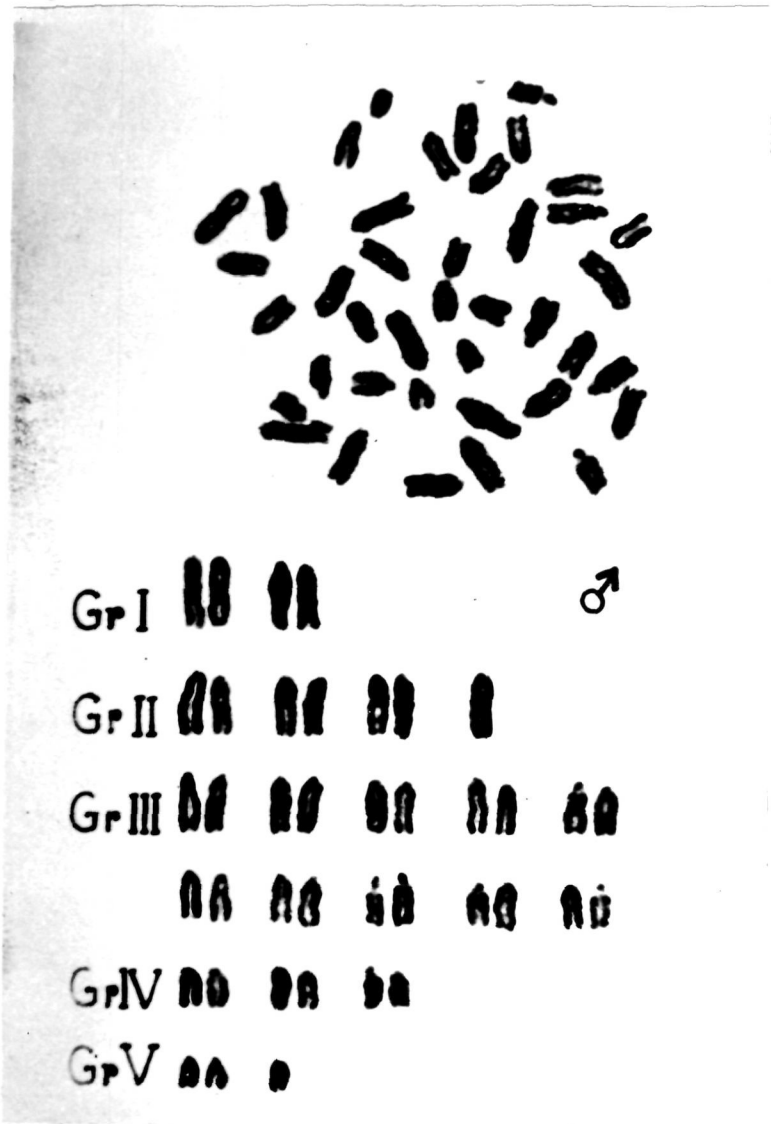
CHAPTER - I

Cytogenetic Assays of P. aeruginosa Culture Treated and Control Mice in Parental Generation

A. Studies on Somatic Cells

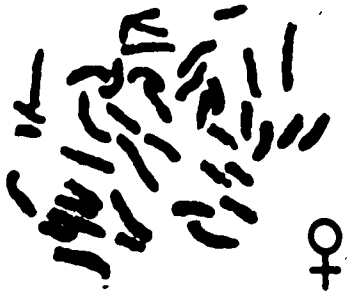
a) Effects on bone marrow chromosome at various intervals :

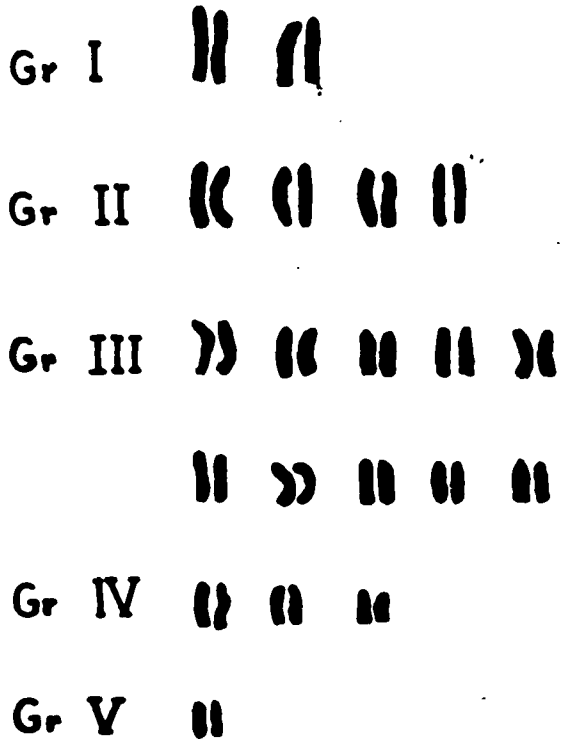
It has been mentioned that the effects of log-culture, isolated bacteria in saline suspension and culture filtrate in separate sets of treated mice were studied in the bone marrow chromosome at 14 different intervals between 10 min to 192 hr against suitable control by Dey (1981). The effect of heat killed bateria was also studied in few intervals after injection. The present author also studied the effect of the log-culture of P. aeruginosa in treated mice each week for 7 weeks with a view to extend the data and to know how long the effect could continue and whether the aberration effect was present during the mating of the treated male with virgin females in connection with the lethal testing programme. It was to know whether the aberration types were more or less the same at all fixation intervals and so on.



a) Photomicrograph of a normal diploid metaphase complement in bone marrow of male mice.

b) Karyotypic arrangement in 5 groups.





a) Photomicrograph of a normal diploid metaphase complement in bone marrow of female mice.

b) Karyotypic arrangement in 5 groups.

MATERIAL AND METHODS

Laboratory bred Swiss strain of albino mice, Mus musculus of both sexes and of about 3 month old were used unless otherwise mentioned. Bone marrow cells of control and of treated specimens were fixed for 7 weeks at an intervals of 7 days beginning with the 1st week after the treatment. Two males and 2 females each in control and treated series were used at each week interval. Generally 100 metaphase spreads per individual were assessed to determine the aberration frequency and the data of 4 individuals of control and treated specimens of each week were combined and presented in the Table 1. The method of collection of the bone marrow cells and the cytological preparations of the slides of the control and treated samples were the same. Specimens of both control and treated series were intraperitoneally injected with 0.04% colchicine solution at the rate of 1 ml/100 gm body weight one and half hour before the collection of the tissues unless mentioned otherwise.

Collection and fixation of bone marrow cells :

Following the standard practice, the femur of each specimen after cervical dislocation was removed quickly and the surface was cleared by the surgical guage. The bone marrow from the femur of both control and treated specimens was collected in separate centrifuging tubes immediately after cutting two ends of the femur and pushing warm (37°C) 1% sodium citrate solution through one cut end by the hypodermic syringe and collecting the fluid

along with bone marrow comming out through the canal at the other end of the femur. The process was repeated to make sure that the maximum number of cells were flushed out. The collected bone marrow cells in citrate solution were brought into suspension by repeated flushing the fluid by a micro pipette. The centrifuge tube was then kept in a thermostatic water bath (37°C) for about 15 min. The tube was then taken out, flushed again and centrifuged for about 10 min at 1000 rpm. The supernatant fluid was discarded and the cells collected at the bottom was fixed in aceto-alcohol (1:3) by resuspension for at least 15 min.

After fixation, the tissue was again centrifuged at 1000 rpm for about 10 min and the supernatant fixative was removed. The fixed material was resuspended in left over aceto-alcohol fixative.

Preparation of slide and staining : Clean grease free slides kept in a jar containing 50% alcohol and stored in a refrigerator were taken out. A drop or two of the fixed bone marrow cell suspension was put at one end of the chilled slides and the slide was touched immediately to a flame to catch fire. It was left in slanting position to burn out the alcohol when the cells spread over the slide along with the down-ward flow of burning alcohol and fixed on the surface. After further drying in air, the slide was stained in diluted Giemsa stain (one part Giemsa stain mixed with 20 parts distilled water with pH 7.2 adjusted by phosphate buffer) for about one and half hour. After staining, the slide was rinsed in water, dried in air when it became ready for observation.

Control sereis :

In control series each specimen was intraperitoneally injected with freshly prepared nutrient broth at the rate of 1 ml/100 gm body weight and the assessment of chromosome aberration if any was made from the tissue fixed at the desired interval (Table 1).

Treated series :

The freshly prepared nutrient broth was inoculated with a loopful of the stock culture of P. aeruginosa and the bacteria were allowed to grow upto the log phase in medium. A regular recording of time against growth of the bacterial cells by colorimetric reading was made. The number of bacterial cells at log phase was determined by the standard bacteriological dilution plating method and it was approximately 17×10^7 cells per ml. The log phase culture of P. aeruginosa was intraperitoneally injected at the rate of 1 ml/100 gm body weight of the mouse. The bone marrow cells of the specimens were fixed at the desired intervals corresponding with the control (Table 1). Almost the same number bacterial cells were injected in the present study as was used by Dey (1981).

In order to verify if the chromatid breaks were nonrandomly distributed or not, 40 chromosomes of the diploid complement were arbitrarily put in 5 groups and their proportionally with regard to number and relative percentage length were determined according to the method described elsewhere (Crippa, 1964; Manna, 1971; Manna and Das, 1972).

RESULTS AND COMMENTS

As compared to the treated series, the the frequency of chromosome aberrations in the bone marrow cells of control specimens fixed in 7 weeks was relatively low and some of the aberration types were not regular in appearance (Table 1).

In the treated series the different types of aberrations were put arbitrarily into two categories as individual types and gross types (PM 1-10; Figs. 1-20). In case of individual types some chromosomes of the whole complement were affected (PM 1-9; Figs. 1-11 and 14-20) while in gross type the whole complement was affected (PM 10). Sometimes, there were overlapping cases (PM 7-9). In scoring the data if a plate contained both types of aberrations, it was considered under one category and not in both. The individual type aberrations were in the form of chromatid break (PM 1-4; Figs. 1-11), acentric fragment (PM 4; Figs. 12, 13, 17), translocations (PM 7-8; Figs. 14-17, 20) etc., representing the breaks with the possibility of genetic implication and gap (PM 5; Fig. 19) and constriction (PM 5; Fig. 18) as physiological type. Some plates contained more than one individual type aberrations. The occurrence of isochromatid breaks or constriction was rare. The translocations occurred in the form centric fusion, terminal chromatid fusion, ring etc. (Table 1). Besides them there were good many cases of precocious centric dissociation which occurred differentially in different groups of chromosome (Table 2). An analysis of the data (Table 1) would show that among the individual types, the occurrence of chromosomes with precocious separation of chromatids was highest of all and the next one was the translocations.

Explanation of Photomicrographs

Photomicrographs (PM) of affected metaphases in the bone marrow cell of mice for the injection of culture of Pseudomonus aeruginosa

PM 1. A faint distal chromatid break in a group I chromosome.

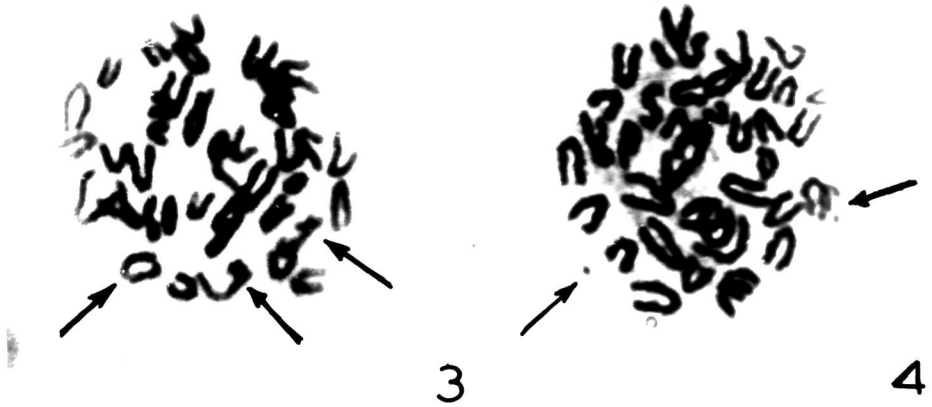
PM 2. A centric fusion and a mid-region chromatid break in a group I chromosome.

PM 3. A ring, a fragment of unknown origin and a chromatid break in a group II chromosome.

PM 4. A mid-region chromatid breaks in a group III chromosome and a fragment.

PM 5. Isochromatid constriction, gap etc.


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Explanation of Photomicrographs

Photomicrographs (PM) of affected metaphases in the bone marrow cells of mice, for the injection of *Pseudomonas aeruginosa* culture

PM 6. A distal chromatid break in group I chromosome.

PM 7. A centric fusion and partly stickiness in chromosomes.

PM 8. The exchange or association of two chromatids and general stickiness.

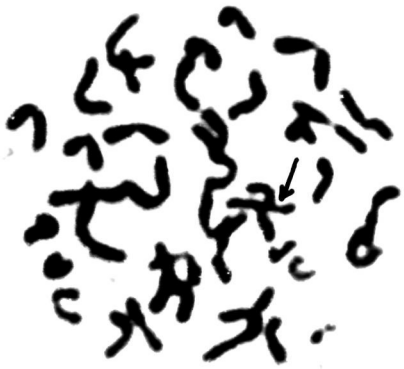
PM 9. A proximal chromatid break in a group III and centromeric dissociation in group V chromosome and some with beaded structure.

PM 10. A polyploidy metaphase.

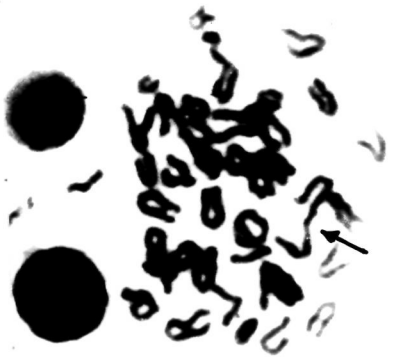
PM 11. Metaphase with the stickiness in chromosomes.



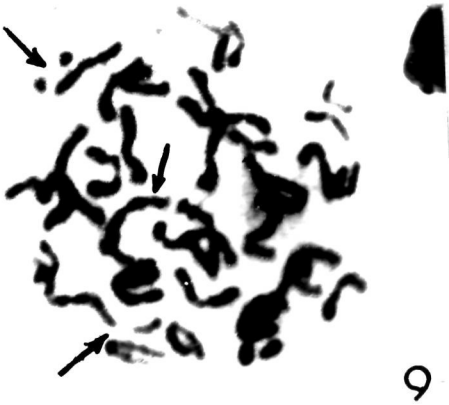
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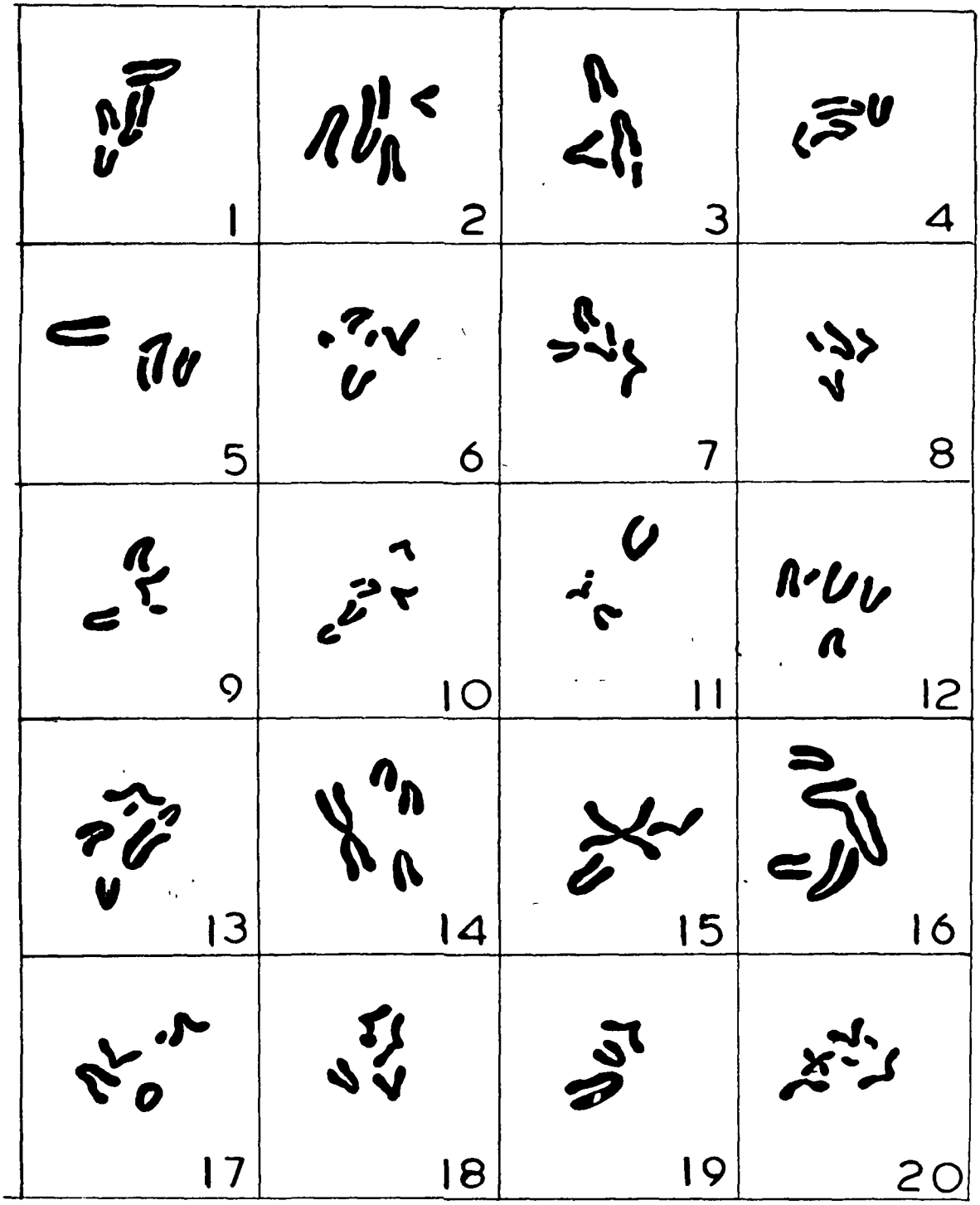
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Explanation of figures

Camera lucida drawing of the affected part of metaphase plates showing different types of aberrations.

Fig. 1. A proximal chromatid break in a group I chromosome.
Fig. 2. A mid-region chromatid break in a group I chromosome.
Fig. 3. A distal chromatid break in a group I chromosome.
Fig. 4. A proximal chromatid break in a group II chromosome.
Fig. 5. A mid-region chromatid break in a group II chromosome.
Fig. 6. A distal chromatid break in a group II chromosome.
Fig. 7. A proximal chromatid break in a group III chromosome.
Fig. 8. A mid-region chromatid break in a group III chromosome.
Fig. 9. A chromatid break in the distal region of group III chromosome.

Fig. 10. A proximal chromatid break in a group IV chromosome.
Fig. 11. A mid-region chromatid break in a group IV chromosome.
Figs. 12 and 13. A fragment of unknown origin in each
Figs. 14 and 15. A centric fusion between two chromosomes in each.
Fig. 16. End to end association between two chromosome.
Fig. 17. One ring chromosome and an unknown fragment.
Fig. 18. A chromosome with constriction in one arm.
Fig. 19. A gap in the middle region of a chromosome.
Fig. 20. A centric fusion between two chromosome and the centric dissociation in a group V chromosome.


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Each individual type aberration was not regular in appearance and their number fluctuated erratically at different fixation intervals. If the individual type aberrations were taken together, the net frequency-increase in treated series over control was 23.0% in 1st week, 21.50% in 2nd week, 26.0% in 3rd week, 18.25% in 4th week, 32.85% in 5th week, 34.0% in 6th week, 16.75% in 7th week and 24.53% in the average of all weeks taken together (Graph 1). Therefore, though the data were fluctuating between 16.75% (7th week) and 34.0% (6th week), the difference in all invervals was significantly high indicating that the treatment of log culture of P. aeruginosa continued to induce high frequency of aberration than that of control.

Since there were good number of centric dissociation cases and the genetic significance of which was uncertain, their the frequency at different week if eliminated, the net increase in the individual type aberration frequency would come down to 9.75% in 1st week, 10.25% in 2nd week; 8.25% in 3rd week; 6.0% in 4th week, 9.25% in 5th week, 5.5% in 6th week and 9.0% in 7th week. Even then the frequency of aberration was significantly high implying that the clastogenic potentiality of the bacterium continued as long as 7th week after the injection.

The frequency of gross type aberrations like individual types also fluctuated in different weeks and there was no regular mode (Table 1). The net increase in the gross type aberration frequency over control was 12.5% in 1st week, 5.75% in 2nd week, 5.25% in 3rd week, 17.25% in 4th week, 6.6% in 5th week, 12.75% in 6th week and 16.25% in 7th week.

If data of both individual and gross type aberrations were combined, the net increase in aberration frequency in the treated series over control was 41.50% in the 1st week, 38.50% in the 2nd week, 38.25% in the 3rd week, 39.0% in the 4th week, 42.50% in the 5th week, 57.25% in the 6th week, 31.50% in the 7th week and 41.14% in the average of all weeks taken together. The combined aberration frequency data of the control and of treated series were subjected to statistical analysis and in every week the difference was significant at various level.

If the aberration data of all weeks were combined, out of 2800 metaphase in control series examined in seven weeks, there were 11 chromatid breaks, 5 fragments, 17 translocation or association, 7 gaps and constriction and 50 centric dissociation making a total of 92 or 3.28% individual type of aberrations. On the otherhand out of the 2800 metaphases examined in the treated series, there were 73 chromatid breaks, 31 acentric fragments, 147 translocation and association, 13 gaps and constriction and 507 centric dissociation making a total of 779 or 27.82% which was much higher than that of control (Table 1). The net increase was 24.54%. Further, if the cases of centromeric dissociation were excluded, the frequency of individual type of aberrations would become 1.5% in control series and 9.71% in the treated series. Thus the net increase was 8.21% which indicates that the treatment induced much higher aberration frequency. Further in the combined data of 7 weeks, out of 2800 metaphases there were 104 or 3.71% gross type aberrations in the control series against 21.57% in the treated series. If both individual and gross types were combined the average frequency was 7.82% in the control series against 48.96% in the treated series making an average net increase of 41.14%.

Comment :

The present study revealed that the aberration effect continued in quite high frequency till 7th week, the last fixation interval. Therefore, it would imply that the aberration effect was possibly due to some genetic changes induced by the bacterium and the fluctuation in the frequency at different intervals might be due to increase and decrease of the population of the cells incorporating the mutated chromosome. Manna (1984) postulated a hypothesis on the genetic control of chromosome aberration in which it has been advocated that there might be some loci in the chromosome which under normal condition maintain the structural integraty but when mutated due to the effect of some mutagen it might lose control. This would lead to the occurrence of simple various type aberrations. This hypothesis has also been applied to explain the chromosome aberration observed in F_1 and F_2 generations of the treated series. The present study has also subscribed to the same view. That the behaviour of chromosome is under genetic control has been advocated by Gupta (1985a, b) though it was concerned with meiotic pairing (vide infra) but we postulated its bearing on the appearance of chromosome aberration in treated parent and in the treated line of F_1 and F_2 as compared to the respective control.

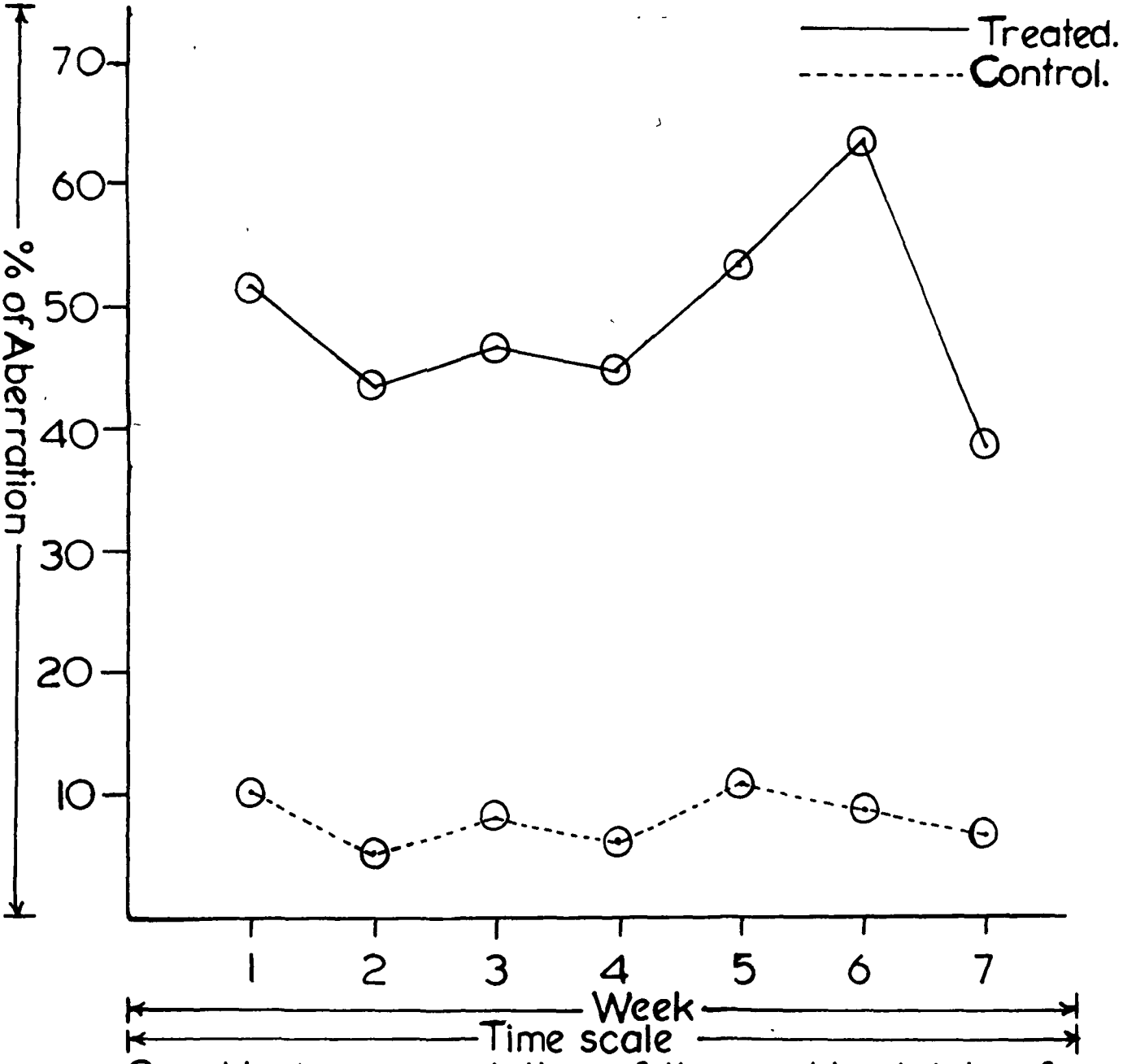
Table 1

Chromosome aberrations in bone marrow cells of mice intraperitoneally injected with log-culture of bacterium, P. aeruginosa (Control data given in breackets). 100 cells/inciv. were counted and 2 males and 2 females. Fixation were sacrificed in both control and treated series

Fix Time	Total cells count	Individual type aberrations: Break type: Chro-mat.	Frag.	Trans Asso.	Physio.type: Cons & Gap	Part. group C.D.	Total	Gross type aberrations: Num. change	Others	Total	Grand total	Net incr.%
1st wk	400	6	4	31	-	63	104	50	52	102	206	41.50
	(400)	(-)	(1)	(1)	(-)	(10)	(12)	(6)	(2)	(8)	(40)[a]	
2nd wk	400	3	4	36	2	50	97	55	34	89	174	38.50
	(400)	(3)	(1)	(2)	(-)	(5)	(11)	(2)	(7)	(9)	(20)[b]	
3rd wk	400	9	8	23	-	79	119	33	33	66	185	38.25
	(400)	(1)	(-)	(4)	(2)	(8)	(15)	(9)	(8)	(17)	(32)[c]	
4th wk	400	11	4	16	-	52	83	22	73	95	178	39.00
	(400)	(1)	(-)	(5)	(1)	(3)	(10)	(5)	(4)	(9)	(22)[d]	
5th wk	400	16	6	19	3	106	149	30	33	63	212	42.50
	(400)	(3)	(-)	(2)	(1)	(12)	(18)	(15)	(9)	(24)	(42)[a]	
6th wk	400	5	5	18	1	122	151	52	60	112	263	57.25
	(400)	(2)	(1)	(2)	(2)	(8)	(15)	(10)	(9)	(19)	(34)[b]	
7th wk.	400	23	8	4	7	35	76	7	70	77	153	31.50
	(400)	(1)	(2)	(1)	(1)	(4)	(9)	(14)	(4)	(18)	(27)[e]	
	2800	73	39	147	13	507	779	249	355	604	1371	41.14
	(2880)	(11)	(5)	(17)	(7)	(50)	(92)	(61)	(43)	(104)	(219)[a]	

a = $P < 0.001$, b = $P < 0.01$, c = $P < 0.02$, d = $P < 0.05$, e = $P > 0.05$

Graph - 1



Graphical representation of the combined data of frequency of chromosome aberration in male and female control and P. aeruginosa treated mice at different intervals.

Centromeric Dissociation :

The high incidence of precocious separation of chromatids of chromosomes was noted while making the aberration study. Thus with a view to finding out if there was any differential effect in different groups of chromosome, the data of the control and treated specimens were analysed (Table 2). The frequency was highest in the single pair of chromosomes belonging to group V while it was negligible in chromosomes belonging to group III and group IV in the control specimens. On the other hand, in the treated series the occurrence of precocious centromeric separation was seen in chromosomes belonging to all five groups but it was relatively very low in chromosomes belonging to groups I and II. An analysis of the data showed that in the control series, out of 400 metaphases examined in each week, there were 3 in group IV, 5 in group V in the 1st week, 5 in group V in the 2nd week, 4 in group IV and 9 in group V in the 3rd week, 6 in group V in the 4th week, 1 each in group 1, 3, 4 and 9 in group V in the 5th week, 2 in group III, 1 in group IV, 6 in group V in the 6th week and 4 in group V in the 7th week. Thus out of the total 2800 metaphases examined in 7 week control series, there were only 1 in group I, none in group II, 3 in group III, 9 in group IV, 44 in group V making a total of 57 or 2.03% incidence. On the other hand the frequency of precocious separation of chromatids belonging to different groups were significantly high in the treated series. Out of 400 metaphases in each week, it was present in all groups with variable numbers having the maximum number in group V. In 1st week the total was 71 or 17.75% and the increase over control was 15.75%. The same trend was shown in 2nd week also and the total number of chromosome showing precocious separation was 50 or 12.5% and the net increase was 11.25%

In the 3rd week (Table 2) it was found in all the groups except in group I and the total number was 90 or 22.5% and the net increase was 19.25%. Similarly in 4th week the total number was 52 or 13.0% and the net increase was 11.50%. In 5th week the total number was 108 or 27.0% and the net increase was 24.0%. The frequency was highest in 6th week. It was found in all but group II. The total number was 123 or 30.75% and the net increase was 28.50%. In the 7th week it was only found in groups III, IV and V making a total of 45 or 11.25% and the net increase was 10.25%. If the data of 7 weeks were combined, out of 2800 metaphases of the treated series, there were 4 in group I, 5 in group II, 102 in group III, 133 in group IV and 295 in group V making a total of 539 or 19.25% and the net increase over control was 17.21% (Table 2). Further it was evident from the data that the treatment induced centromeric dissociation in chromosomes belonging to all groups but it was very negligible for chromosomes belonging to group I and group II while in group III it was 3.64% against 0.1%, in group IV it was 4.75% against 0.32% and in group V it was 10.53% against 1.75% of the control series respectively. Moreover, if the number of chromosomes belonging to different groups was considered, it was 20 in group III, 6 in group IV and 2 in group V indicating that group V was very vulnerable and the next one was of group IV.

The genetic significance of precocious centromeric dissociation is not known but the differential response in the treated seriesw was highly peculiar and the reason for which was not known. It was noted that for preparation of the chromosome of both control and treated specimens, they were injected with 0.04% colchicine solution 1.5 hr

before taking the tissue. Therefore, one might suspect that it was due to colchicine effect. But it would not explain so much differential response and more specific

Table 2

Compiled Data of Frequency Distribution of Centromeric Dissociation in Five Groups of Chromosomes per 2 males and 2 females determined from 100 Metaphases per Individual of Control and Log-culture of P. aeruginosa treated mice. Control data given in Brackets.

Fix. time	No.of indiv.	Total cells count	Groups of chromosome I	II	III	IV	V	Grand total *	Net incr %
1 wk	4	400	1	1	19	16	34	71	15.75
	(4)	(400)	(-)	(-)	(-)	(3)	(5)	(8)	
2 wk	4	400	1	1	13	15	20	50	11.25
	(4)	(400)	(-)	(-)	(-)	(-)	(5)	(5)	
3 wk	4	400	-	1	32	20	37	90	19.25
	(4)	(400)	(-)	(-)	(-)	(4)	(9)	(13)	
4 wk	4	400	-	1	6	8	27	52	11.50
	(4)	(400)	(-)	(-)	(-)	(-)	(6)	(6)	
5 wk	4	400	-	1	11	26	70	108	24.00
	(4)	(400)	(1)	(-)	(1)	(1)	(9)	(12)	
6 wk	4	400	2	-	18	35	68	123	28.50
	(4)	(400)	(-)	(-)	(2)	(1)	(6)	(9)	
7 wk	4	400	-	-	3	13	29	45	10.25
	(4)	(400)	(-)	(-)	(-)	(-)	(4)	(4)	
Total	28	2800	4	5	102	133	295	539	17.21
	(28)	(2800)	(1)	(-)	(3)	(9)	(44)	(57)	

*Statistical significance of difference $P < 0.001$ in each week.

for chromosomes of group V. It seemed that smaller group of chromosomes had defective centromere because similar differential responses were observed in our laboratory by other workers using different mutagens. The differential sequence of the separation of chromosomes has been recorded by some other workers in bone marrow cells of different materials. In Rattus norvegicus sequential separation of chromosomes in anaphase of bone marrow of colchicine treated specimens was shown that it was highest in No. 1 (largest ST), followed by No. 15-21, 2, 3 and 5-14 while No. 14 (smallest ST) was last to separate (Sarihyan and Singh, 1985). It has also been claimed Farook and Farook, 1985) by analysis of C-banded and non-C-banded mitotic chromosomes that in man No. 2 was earliest and D group was last to separate; in cattle the centromere of XX and XY separate earlier than any of the autosome and again the active X of 2Xs in female separate earlier than the passive X-chromosome; in mouse the Y separates first followed by the rest and so on. It was opined (Farook and Farook, 1985) that the chromosomes with C-heterochromatin separate later than that of without C-heterochromatin which is however, questionable from various observations. Anyhow, the centromeric separation observed in our present study in control and log-culture of Pseudomonas treated mice and same in F_1 and F_2 generation (vide infra) would favour the genetic control though precise mechanism is yet to be studied.

Nonrandom distribution of chromatid breaks in Pseudomonas treated mice :

While scoring the data the occurrence of individual type of aberrations appeared nonrandom. With this in view 74 chromatid breaks observed in 2800 metaphase of the treated

specimens were analysed for their group-wise and region-wise distribution. An analysis of the data (Table 3) showed that in group I, the observed number was 21 against 74 and 11.85 expected numbers respectively. It appeared not so glaring for chromosomes belonging to group II because the observed number was 20 against the expected number of 14.8 calculated numerically and 19.10 calculated

Table 3

Analyses of group-wise and region-wise distribution of chromatid breaks in bone marrow cells of *P. aeruginosa* treated mice in seven weeks.

Region from centro.	Groups of chromosome					Total	
	I	II	III	IV	V	Obs	Exp.
Proximal	7	6	3	1	-	17	24.66
Middle	6	4	10	2	-	22	24.66
Distal	8	10	16	1	-	35	24.66
Total Obs.	21	20	29	4	-	74	
Expected per No.	7.4	14.8	37.0	11.1	3.7		74
Per % length	11.85	19.10	34.61	6.70	1.74		74

per percentage length. Therefore, chromosome belonging to group I and also possibly group II seemed more susceptible to the chromosome breaking property of Pseudomonas. On the otherhand chromosome belonging to groups III, IV and V were somewhat resistant because the observed number was lower than that of expected number calculated as per number or relative percentage length. On the whole it could be generalised that there was differential response and the breaks were nonranddmly distributed in different groups of chromosomes. It is interesting to point out that the analysis of the chromatid breaks in the log culture, cultural filtrate, the bacteria in saline suspension and heat-killed bacterial suspension treated mice induced similar types of nonrandom distribution of chromatid breaks in five groups of chromosomes (Dey, 1981). Therefore, two independent sets of data focussing the same result would confirm that the breaks were nonrandomly distributed between different groups of chromosomes.

The nonrandom distribution of chromatid breaks in different regions of the chromosome (Table 3) also showed that the distal segment was most vulnerable and the proximal region was somewhat resistant as compared to the expected number though data in some groups were insufficient. Similar non-random distribution of breaks in different regions of the affected chromosomes were also seen earlier by Dey (1981).

In mice, the chromatid breaks induced by various chemical and other agents were also found to be nonrandomly distributed between and within chromosomes. Since the mode of distribution of the chromatid breaks between and within chromosome obtained by different collaborators of Manna, he (Manna, 1975) reviewed the results and opined that the

occurrence of the nonrandom distribution was due to some inherent weaker regions in chromosomes which due to some physiochemical stress break often than other regions. This has found support from the discovery of heritable fragile sites in chromosomes in human cancer (see Michelle and Rowley, 1984; Changàti 1985). Rowley (1983) showed that the remarkable concordance between the chromosomal location of human cellular oncogene (C-onc) and the points of breaks involved in certain chromosomal rearrangement associated with various cancer. Thus the nonrandom chromosomal rearrangement was associated with fragile sites of chromosomes. A flagile site is a region of a chromosome which does not take stain. The sites are inherited in the Mandelian fashion. Seventeen fragile sites in human genome have been identified so far. Of these 16 are autosomal and one is on X-chromosome. The X-chromosome fragile site is associated with X-linked mental retardation while such information is lacking for autosomal fragile sites. Weaker region has been seen even in virus chromosome (Stall, 1972) and in other Eukaryotos (see Manna, 1975). Thus the discovery of the nonrandom distribution of chromatid breaks in chromosomes of mice made by Manna and his collaborators in early 1970 (Manna, 1971, 1975; Manna and Das, 1972; Manna and Bardhan, 1973a, b; Manna and Mitra, 1971; Manna and Das, 1973) was found to be correct. Even it was suspected in the study of the X-ray induced breaks in grasshoppers by Manna and Mazumdar (1967) and in Drosophila . Therefore, the nonrandom distribution of chromatid breaks between and within chromosome of Pseudomonas culture treated mice added more evidence in the inherent weaker region of chromosomes of mice as generalized by Manna (1975).

b) Micronucleus Test :

The micronucleus test in bone marrow of mice has been found to be a quicker screening method as a mutagenicity testing protocol, the development of which was mentioned before. Mammals when treated with some mutagens, some micronucleated cells in bone marrow might be seen if a cell has completed at least one mitosis after the treatment. Of late this method has also been found to be useful in evaluating the mutagenic effect in fishes treated with X-rays, chemicals and Pseudomonas (Hoolflmann and DeRaat, 1982; Manna and Biswas, 1986; Manna and Sadhukhan, 1985; Manna et al., 1985; Sadhukhan and Manna, 1984). Besides peripheral erythrocytes this has been studied in the smear of gill and kidney cells of some fishes (lit. cit.). However, MNT has more critically been studied in mammalian model specially in mouse. In mammals the acurate scoring of the micronuclei is rather difficult in myeloblast myelocytes and erythroblasts because of the presence of the original nucleus. While their detection is quite easy in erythrocytes after the explusion of the main nucleus when it could be found in the anucleated young erythrocytes as a minute round chromatin material as an obstrusive structure. The number of micronucleus may vary from one to many in different affected erythrocytes but one should be cautious about the artefacts (Evans, 1976). Two forms of micronucleated erythrocytes could be distinguished (1) the young one termed as polychromatic erythrocytes stains bluish while (ii) the old reddish tinged micronucleated erythrocytes are called as normocytes. Therefore, there are three types of micronucleated cells : (i) Myeloblast when the main nucleus is present, (ii) polychromatid erythrocyte with bluish tinge and (iii) normochromatic erythrocytes with reddish tinge in

the May-Grünwalds Eosin-Methylen blue - Giemsa preparation (vide infra).

Micronuclei are formed from laggards or from asymmetrical exchanges while symmetrical exchanges and other simple type aberrations would not be detectable by MNT which is a draw back in the MNT. However, the advantage are many and the limitations are few. It is generally formed by the extrusion of the acentric fragment from anaphase group of chromosome which formed a round structure in the cytoplasm and would persist until it was made to disappear by the action of cytoplasmic enzyme-nuclease and proteases. Ionizing radiation and odd chemicals belonging to alkalating agents, antimetabolites, drugs, antibiotics etc. were found to be strongly positive for MNT studied by various workers while Manna and his collaborators in addition to X-rays and odd chemicals using bacteria (Goswami and Manna, 1984; Sadhukhan and Manna, unpublished) and some species of Aspergillus (Manna and Kundu, unpublished; Manna and Chakraborty, unpublished). The present author has also used MNT as an additional evidences of mutagenic property of P. aeruginosa which was not carried out before.

Experimental procedure, Results and Comments

About 3 week old mice of both sexes in separate sets were intraperitoneally injected only once with 1 ml. 2 ml and 3 ml dose of the log-culture of P. aeruginosa per 100 gm body weight. However, specimens injected with 2 ml and 3 ml doses did not survive long for which the study was carried out only for the treatment of 1 ml dose by

collecting the bone marrow after 24 hr. The common practice of injecting the same dose again after 24 hr and fixing the material 6 hr after the second injection was avoided as it was fatal. Different set of mice infected with nutrient broth served as control. Bone marrow cells of the control and treated specimens collected in citrate solution and smeared preparations were made on clean grease free slide like blood smear. Since in MNT, the cells are required to complete one mitotic cycle after injection (about 22 hrs are taken for the completion of the cell cycle in mice, (Dewey and Humphrey, 1962) the study was made after 24 hr. From each specimen of 2 males and 2 females injected, 1000 polychromatic and the same number of normochromatic cells with or without micronuclei were recorded. The average of 4 specimens of treated and control series was taken to compare the result determined for comparison.

The separate collection of bone marrow cells in 1% sodium citrate solution of control and treated specimens without injecting colchicine was done following the standard procedure. The suspension of bone marrow cells in 1% sodium citrate solution without fixation was smeared on a grease free clean slides. The smeared slides were allowed to dry in air, then stained for 3 min in May-Gruenwald stain (May-Gruenwald powder 250 gm + methanol pure 100 ml), followed by staining again with diluted May-Gruenwald stain for 2 min. The slide was rinsed in distilled water and finally stained in diluted Giemsa stain (1 part Giemsa and 6 part distilled water) for about 10 min. After staining, the slides were rinsed in distilled water and dried in air, cleared in Xyline for 5 minutes and embeded in DPX.

Observation :

The polychromatic erythrocytes contained normally one per cell but the number and size of micronuclei varied to some extent in different cells (PM 11A a-f). The data (Table 4) showed that the frequency of polychromatic erythrocyte was striking high than that of control. In 1000 polychromatic erythrocytes cell/individual examined there were 127 or 12.7% against 2 or 0.2% micronucleated polychromatic chromatic cells in first treated and control individuals 115 or 11.5% against 1 or 0.1% in 2nd individuals, 137 or 13.7% against 3 or 0.3% in 3rd individuals and 140 or 14.0% against 3 or 0.3% in 4th individuals. Thus out of total 4000 polychromatic cells examined there were 519 or 12.97% polychromatic erythrocytes with micronucleus against 9 or 0.22% in the control series. Therefore the difference in the number of micronucleated polychromatic erythrocyte between treated and control was statistically significant ($P < 0.001$).

It was opined by Schmid (1976) that if the frequency of micronuclei was above 5%, one should be cautious about inclusion of artifacts influencing the frequency. We were aware of the same but could not discard the cells for the simple reason that they were not present in control and chromosome aberration frequency was very high. Even if the number of micronucleated cells having more than one was discarded the frequency would still be significantly higher than control.

Like the micronucleated polychromatic erythrocytes,

the frequency of micronucleated normochromatic erythrocyte was also very high. The nature and the number of micronuclei in the normochromatic erythrocyte was also highly variable (PM 118a-h, Table 4). Morphologically there was not much difference in the micronuclei types between polychromatic and normochromatic erythrocytes except the cytoplasmic background was bluish in the former and reddish tinged in the latter.

The analysis of the data (Table 4) revealed that the frequency of micronucleated normochromatic erythrocytes was 13.10% (175 in 1335 cells) in 1st individual, 14.38% (205 in 1425) in 2nd individual, 12.89% (167 in 1295) in 3rd individual and 6.06% (95 in 1567) in 4th individual and the average of four individual (Table 4) was 11.4% (642 in 5622) normochromatic erythrocytes, while in the control specimens it was 0.3% in 1st individual, none in 2nd individual, 0.19% in 3rd individual, 0.1% in 4th individual and the average was 0.14%. Therefore, the statistical analysis of the data (Table 4) showed that the difference in frequency of micronucleated normochromatic erythrocytes between treated and control was highly significant ($P < 0.001$). The data also revealed that the frequency of micronucleated normochromatic erythrocyte cells was higher than that of polychromatic erythrocyte cells while in the control specimens, it was almost the same.

Comments :

The number and morphology of micronuclei in both micronucleated polychromatic and normochromatic erythrocytes were quite variable and the frequency was also high if the

Explanation of Photomicrographs

PM. 11A

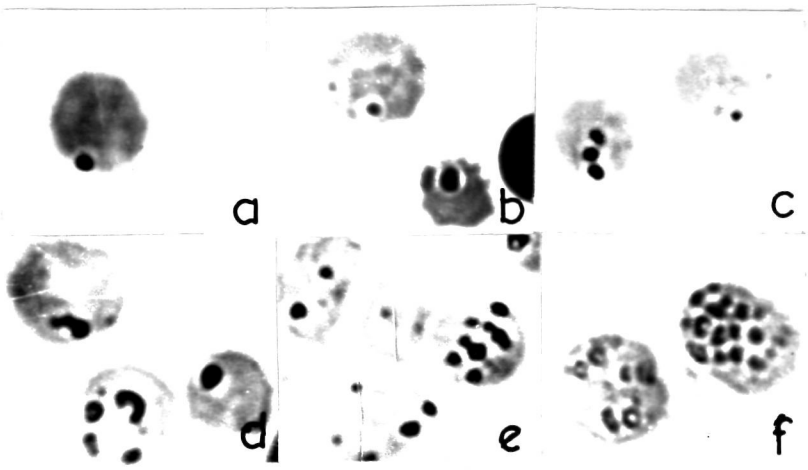
Photomicrographs of polychromatic micronucleated erythrocytes in bone marrow cells of mice treated with log culture of bacterium *P. aeruginosa*.

(a) A cell with one micronucleus.
(b) Two cells each with a micronucleus.
(c) One cell with one and another with 3 micronuclei.
(d) Two cells each with one and another with 4 micronuclei.
(e) Four cells, one with one, one with two, one with 4 and one with seven micronuclei.
(f) Two cells, each with several micronuclei.

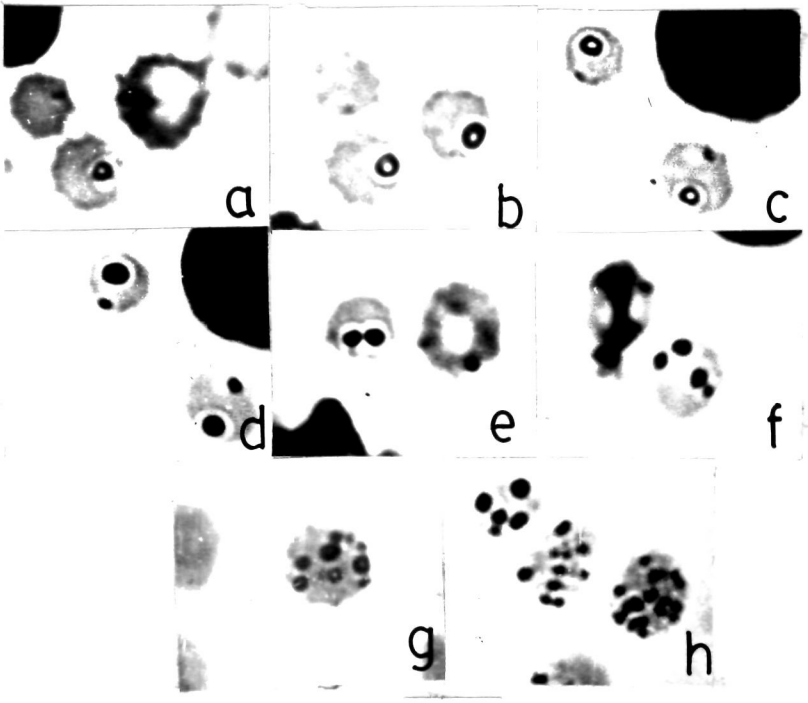
PM 11B

Photomicrographs of normochromatic erythrocytes in bone marrow cells of mice treated with log culture of *P. aeruginosa*.

(a,b,c). Each cell with a micronucleus.
(d) Each cell with two micronuclei.
(e) One cell with two another with 4 micronuclei.
(f) One cell with 4 micronuclei.
(g) One cell with 8 micronuclei.
(h) Three cells with variable number of micronuclei.



11A



11B

same was considered with other chemical treated data (Schmidt, 1976, 1982). It would appear from the present data (Table 4 A and B) that the number in polychromatic erythrocytes with 1 micronucleus was 13.07; with 2, 0.9%; with 3, 0.4% ; with 4, 0.1% and with 5 to 15, 0.75% and that of normochromatic erythrocyte with 1 micronucleus was 8.98%; with 2, 1.1%; with 3, 0.4%; with 4, 0.28% and with 5 to 15, 0.71%.

Therefore, there could be no denial the fact that the frequency of micronculeated erythrocytes induced by the treatment of log-culture of P. aeruginosa was significant high ($P < 0.001$). It was presumed that since the frequency of bone marrow chromosome aberration was also very high (Table 1), the high incidence of micronucleated cells was also expected. The frequency was determined for number of affected cell irrespective of the number of micronuclei they contained. The high frequency could have been partly due to gross affected metaphases which undergoing disintegration by fragmentation and pycnosis. Anyhow on the whole the data supported the clastogenic property of P. aeruginosa in treated mice like that chromosome aberration.

Table 4

Frequency distribution of cells with micronucleus in erythrocytes of Pseudomonas culture treated and control mice (Control data are in brackets)

Ind.	Without MN	No. of cells with micronucleus 1	2	3	4	5-15	Total	Grand total	MNC %
A. Polychromatic erythrocytes									
1	873(998)	110(2)	10(-)	3(-)	2(-)	2(-)	127(2)	1000(1000)	12.7(0.2)
2	885(999)	95(-)	10(1)	5(-)	-(-)	5(-)	115(1)	1000(1000)	11.5(0.1)
3	863(997)	117(2)	3(1)	2(-)	2(-)	13(-)	137(3)	1000(1000)	13.7(0.3)
4	860(997)	111(3)	13(-)	6(-)	-(-)	10(-)	140(3)	1000(1000)	14.0(0.3)
Total	3481(3991)	433(7)	36(2)	16(-)	4(-)	30(-)	519(9)	4000(4000)*	12.97(0.22)
B. Normochromatic erythrocytes									
1.	1160(982)	140(3)	19(-)	4(-)	1(-)	11(-)	175(3)	1335(985)	13.10(0.3)
2	1220(1020)	175(-)	15(-)	7(-)	8(-)	-(-)	205(-)	1425(1020)	14.38(0.0)
3	1128(1040)	131(-)	11(-)	9(-)	2(-)	14(-)	167(2)	1295(1042)	12.89(0.19)
4	1472(994)	55(1)	17(-)	3(-)	5(-)	15(-)	95(1)	1567(995)	6.06(0.1)
To- tal	4980(4036)	501(6)	62(-)	23(-)	16(-)	40(-)	642(6)	5622(4042)*	11.42(0.14)

*Significance control versus treated $P < 0.001$

B. Studies on Male Germinal Cells :

a) Effect on chromosomes at different stages of meiosis :

The study of the effect of a mutagen on the germinal chromosomes has a special significance because it would imply possible hereditary change. However, as the material was not easier for handling, the study of germinal chromosomes has relatively been limited in comparison to that of bone marrow chromosomes or *in vitro* somatic chromosomes. Further among male and female germinal chromosomes the latter have rarely been deployed for the study of effect of ionizing radiations and chemicals (see Brewen and Preston, 1982). Gilliavod and Leonard (1974) found variation in the frequency of structural aberrations in oocytes chromosomes at 24 hour after radiation. The stage sensitivity of developing oocytes was studied by Brewen et al. (1976). Brewen and Preston (1982) listed some 16 chemicals like antibiotics, sulphur dioxide, sodium fluride, caffeine, TEM, MMS etc. which were tested for chromosome breaking property in oocytes.

The effect in male germinal chromosome aberration was studied relatively more by different workers using both ionizing radiation and chemicals. Cattanach (1982) opined that out of different types of translocations, the reciprocal translocation could be considered as an end point induced by ionizing radiation and chemical mutagens in mammalian models. However, the translocation was not associated with the phenotypic effect. It was found to lead to semisterility, reduction in testicular size in male etc. Snell (1933, 1934, 1935) was pioneer to work on mouse

reciprocal translocation. Beside ionizing radiation some radiomimetic chemical like TEM (Cattanach, 1959); EMS (Genereso et al., 1974; Cattanach et al., 1968), TEPA (Epstein, 1975). Mytomycin-C (Adler, 1974) etc. have been tested for induction of reciprocal translocations. Cattanach (1982) tested some 27 chemical mutagens for their response after treatment to various stages of spermatogenesis. Anyhow, so far as the present author was aware the study on the germinal chromosomes aberrations in testes of mice treated with some living mutagens was carried out by Manna and his collaborators using the bacterial species, Staphylococcus aureus (Ghosh and Manna, 1984) and Sarcina lutea (Goswami and Manna, 1984). The present study carried out by the author on the effect of log culture of P. aeruginosa in the testis cells of treated mice at various intervals would remove the lacuna because it was not studied before.

Procedure :

Male mice of about 3 month old were injected intraperitoneally with the log-culture of P. aeruginosa at the rate of 1 ml per 100 gm body weight while another set of male mice injected with the same dose of freshly prepared nutrient broth served as controls. Each specimen was pretreated with colchicine solution one hour before the collection of the tissue. No colchicine injection was given where the tissue was fixed within an hour. Since it takes about 6 weeks for A-type cells of spermatogonia to form mature sperms in mouse (Bateman and Epstein, 1971; Adler, 1982), testes of both control and treated specimens were fixed separately at 18 intervals ranging from 10 min to

7th week (Tables 5-9).

Immediately after removal the testes of control or treated specimens were put separately into 1% sodium citrate solution and the tunica albuginea was removed with the help of two fine needles to make the tubules free into the citrate solution. After about 20 min the tubules were transferred to a fixing tube containing aceto-alcohol mixture (1:3) for a few minute of fixation. Some tubules were then put on a slide smeared with Meyer's albumen smeared slide and added with a small drop of 45% acetic acid. The tissue was squashed between cover glass and the slide with the moderate thumb pressure. The slide was then dried in air for 15 to 20 min and put into a jar of 50.0% alcohol for the detachment of the cover-glass. After the removal of the cover glass, the slide was brought down to distilled water and allowed to dry in air. The slide was stained in diluted Giemsa stain (1:15), followed by air drying and mounted in DPX. All the spermatogonial metaphase, diakinesis and metaphase-II plates to encounter 100 metaphase-I plates per individual were assessed. Thus no uniform number of cells of different stages besides metaphase-I was maintained in different individual.

Spermatogonial metaphase :

The number of spermatogonial metaphases to encounter 100 metaphase-I plates per individual varied widely. In the testes of 3 male individuals examined in each of 18 intervals, the number of spermatogonial metaphases varied between 5 and 189 in treated series and 21 to 237 in control series (Table 5).

The aberrations in spermatogonial metaphases (PM 12-16; Figs. 21-27) consisted of individual types in the form of chromatid break, acentric fragment, translocation like ring and centric fusion etc. and gross types like numerical changes, pycnosis, stickiness etc. More or less the same types of aberrations were encountered both in control and treated series but the palpable difference was the absence of translocation types in control series.

The data were not uniform and in some intervals it was very limited. All types of aberration were combined to have larger sample. In the control series it ranged between 1.83% (1 hr) and 16.21% (120 hrs) and the average of 18 intervals was 8.25% (Table 5). On the other hand, in the treated series the frequency of aberrations ranged more widely between nil (96 hr) and 62.5% (6th week) and the average of all intervals taken together was 26.36% (Table 5). In the treated series the frequency was higher in all but 24 hr, 96 hr and 3rd week and the net increase over control was 1.37% at 10 min, 24.25% at 1 hr, 3.08% at 2 hr, 16.86% at 4 hr, 12.71% at 8 hr, 11.66% at 48 hr, 10.71% at 72 hr, 17.59% at 120 hr, 13.58% at 144 hr, 48.10% at 1st week, 17.33% at 2nd week, 42.31% at 4th week, 24.67% at 5th week, 54.39% at 6th week, 19.09% at 7th week and 17.11% in the average of all intervals taken together. If the data of individual type aberrations were compared, the number of chromatid break, acentric fragment, and translocation, were also always higher in the treated series but they were regular in occurrence which was partly due to the limited data. On the whole the aberration data clearly indicated that the treatment of Pseudomonas induced higher frequency of chromosome aberrations which continued from the beginning

Table 5

Frequency distribution of chromosome aberration in Spermatogonial metaphases of control and log-culture of *P. aeruginosa* treated male mice at different intervals. Control data are in brakcets (3 indiv. used per interval in both control and treated series)

Fix.time	Total cells counted	Individual type aberrations				Gross type aberrations			Grand total	%	Net incr. %
		Break type			Total	Num.change	Others	Total			
		Chrmatid	Fragment	Trans/ Asso.							
10 min	125(139)	2(-)	-(-)	-(-)	2(-)	3(7)	3(-)	6(7)	8(7)	6.40(5.03)	1.37
1 hr	23(109)	-(-)	-(-)	3(-)	3(-)	-(2)	3(-)	3(2)	6(2)	26.08(1.83)	24.25
2 hr	64(138)	-(1)	-(-)	-(-)	-(1)	8(6)	-(6)	8(12)	8(13)	12,50(9.42)	3.08
4 hr	60(127)	-(2)	-(2)	-(-)	-(4)	6(-)	6(-)	12(-)	12(4)	20,00(3.14)	16.86
8 hr	43(180)	-(3)	-(3)	-(-)	-(6)	6(11)	5(6)	11(17)	11(23)	25,58(12,77)	12.71
24 hr	86(55)	-(-)	-(-)	-(-)	-(-)	1(3)	7(3)	8(6)	8(6)	9,30(10,90)	- 1.60
48 hr	54(180)	-(3)	-(3)	-(-)	-(6)	2(3)	7(-)	9(3)	9(9)	16,66(5.00)	11.66
72 hr	57(174)	-(-)	-(-)	-(-)	-(-)	1(6)	9(6)	10(12)	10(12)	17.54(6,83)	10.71
96 hr	5(21)	-(-)	-(-)	-(-)	-(-)	-(-)	-(2)	-(2)	-(2)	0.00(9.52)	- 9.52
120 hr	71(222)	5(-)	3(2)	-(-)	8(2)	10(24)	6(10)	16(34)	24(36)	33.80(16,21)	17.59
144 hr	105(237)	5(1)	7(-)	-(-)	12(1)	3(25)	13(5)	16(30)	28(31)	26,66(13.08)	13.58
1st wk	18(210)	-(2)	-(-)	-(-)	-(2)	6(-)	3(-)	9(-)	9(2)	50.00(1.90)	48.10
2nd wk	18(75)	3(1)	-(2)	-(-)	3(3)	3(4)	-(5)	3(9)	6(12)	33.33(16.00)	17.33
3rd wk	24(178)	-(-)	-(-)	-(-)	-(-)	2(12)	-(5)	2(17)	2(17)	8.33(9.55)	- 1.22
4th wk	36(172)	-(-)	5(-)	2(-)	7(-)	4(14)	8(4)	12(18)	19(18)	52.77(10.46)	42.31
5th wk	169(159)	-(-)	-(-)	-(-)	-(-)	35(3)	12(2)	47(5)	47(5)	27.81(3.14)	24.67
6th wk	96(234)	2(-)	2(-)	3(-)	7(-)	23(17)	30(2)	53(19)	60(19)	62.50(8.11)	54.39
7th wk	30(66)	-(-)	2(-)	-(-)	2(-)	-(2)	6(3)	6(5)	8(5)	26,66(7.57)	19.09
Total	1084(2676)	17(13)	19(12)	8(-)	44(23)	113(139)	118(59)	231(198)	275(221)	25.36(8.25)	17.11

Explanation of Photomicrographs

Photomicrographs (PM) of affected Spermatogonial metaphase plates in mice treated *P. aeruginosa* culture.

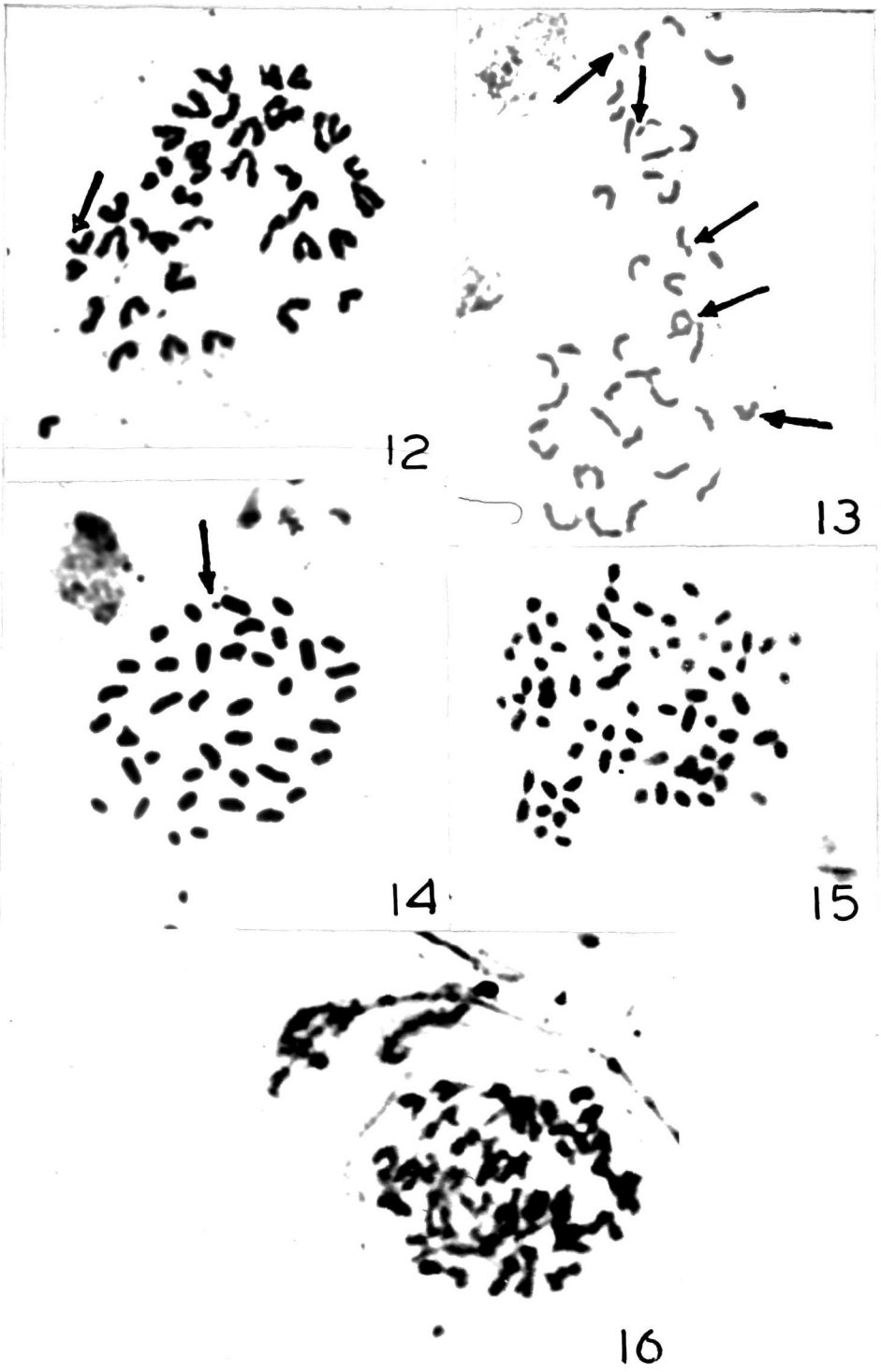
PM 12. A chromosome with part of the chromatid deleted.

PM 13. A chromatid break, a ring chromosome, one fragment and two constrictions.

PM 14. A fragment of unknown origin.

PM 15. A polyploid metaphase with condensed chromosomes.

PM 16. Metaphase with sticky chromosomes.


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Explanation of figures

Camera lucida drawing of a part of spermatogonial metaphase showing different types of aberrations.

Fig. 21. Two chromatid breaks

Fig. 22. One isochromatid break.

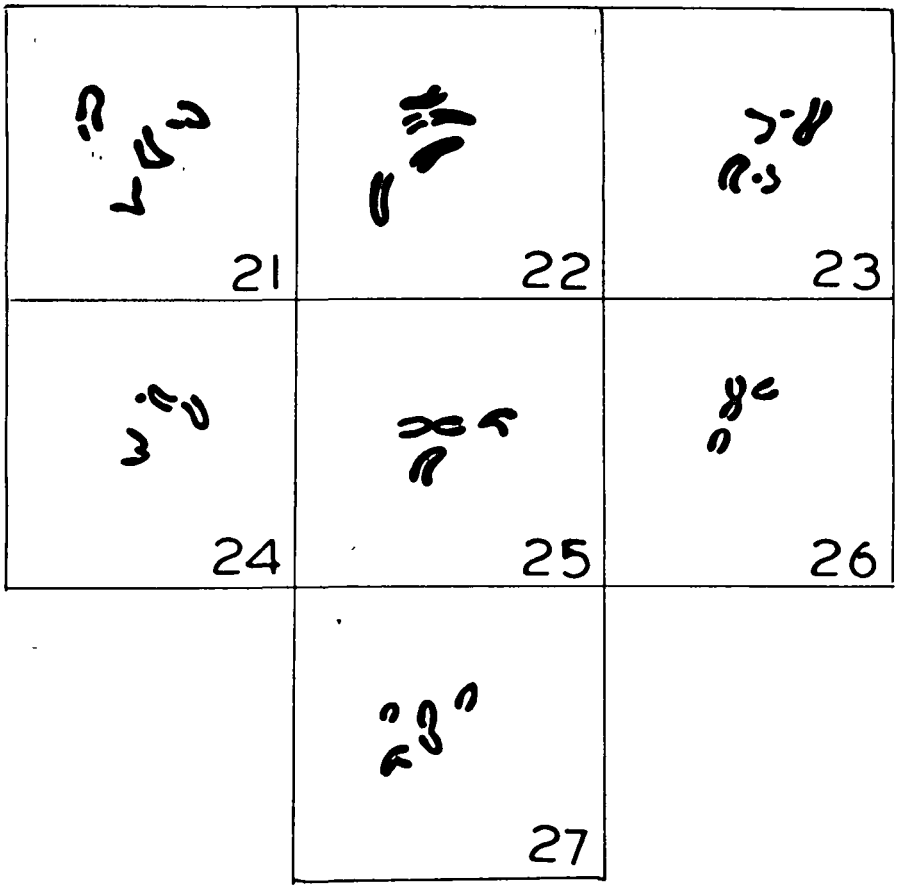
Fig. 23. Two fragments of unknown origin.

Fig. 24. One fragment of unknown origin.

Fig. 25. One centric fusion.

Fig. 26. One centric fusion.

Fig. 27. End to end association between two chromosomes.


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(10 min) to the last (7 week) fixation interval with fluctuated frequency.

Diakinesis :

Like spermatogonial metaphase, the frequency of aberrations in diakinesis cells (Table 6) also ranged widely in 18 intervals between 38 (1st week) and 187 (120 hr) in control series and from 11 (1st week) to 59 (5th week) in treated series. The frequency of diakinesis phases in treated series was relatively low than that of control specimens.

The aberrations (PM 17-23; Figs. 28-33) in diakinesis cells were also individual types consisting of chromatid break, isochromatid break, acentric fragment, multivalent structure, precocious separation of chromosomes of some autosomal bivalents, the X and the Y of the sex pair and the gross types consisted of numerical changes and other types. Among the individual type aberrations the frequency of precocious separation of bivalent and the sex chromosomes was more than other types. In a total of 743 diakinesis cells of 18 intervals there were 27 or 3.63% chromatid break, 11 or 1.48% isochromatid break, 22 or 2.36% acentric fragment, 40 or 5.38% multivalent or bivalent association, 36 or 4.84% precocious separation of autosome in bivalent, 28 or 3.76% precocious separation of X and Y making a total aberrations of 164 or 22.07% individual types. But if the cases of precocious separation were disregarded as true aberration, the frequency of aberration would come down to 100 or 13.45%. However, if the gross type aberrations were added, the total frequency would come to 245 or 32.97%, and

Explanation of Photomicrographs

Photomicrographs (PM) of effected diakynesis cells in male mice injected with the bacterium, *P. aeruginosa* culture.

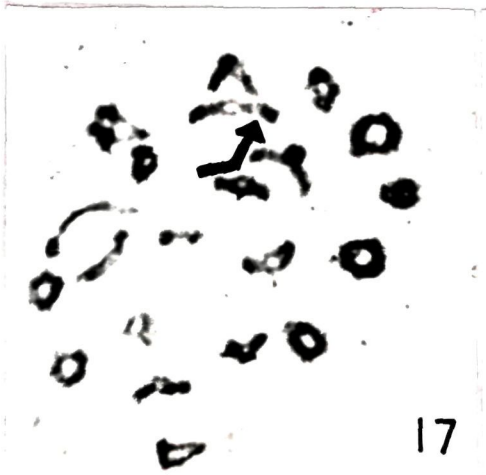
PM 17. A chromosome break and an extra bivalent.

PM 18. A chromosome break and a chromatid break.

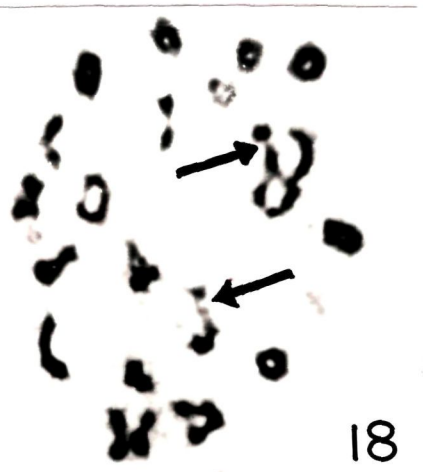
PM 19. A chromosome break, a chromatid break and a multivalent or an association of three bivalents.

PM 20. An extra-element.

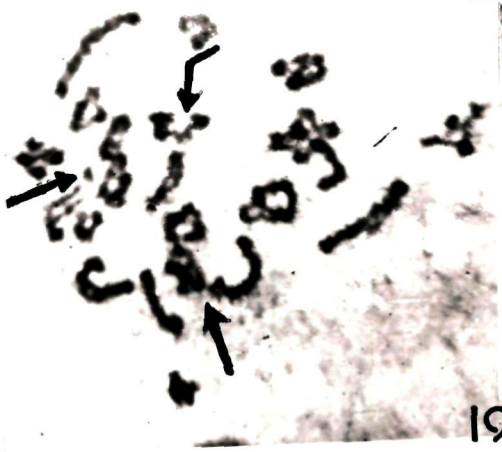
PM 21. An aneuploid cell with 18 bivalents.



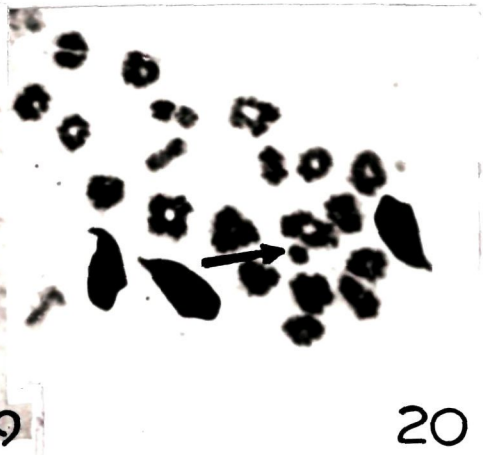
17



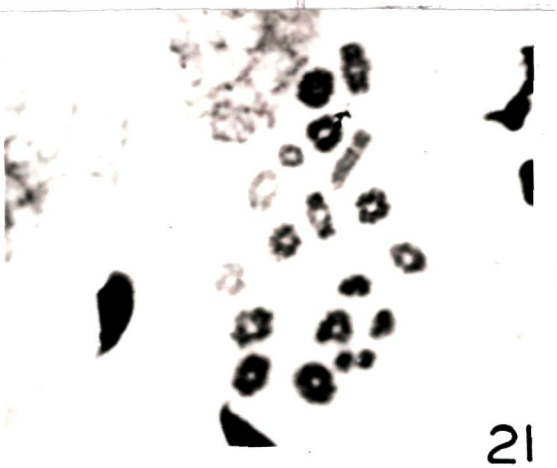
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Explanation of figures

Camera lucida drawing of a part of diakinesis plates showing different types of aberrations

Fig. 28. Chromosome break.

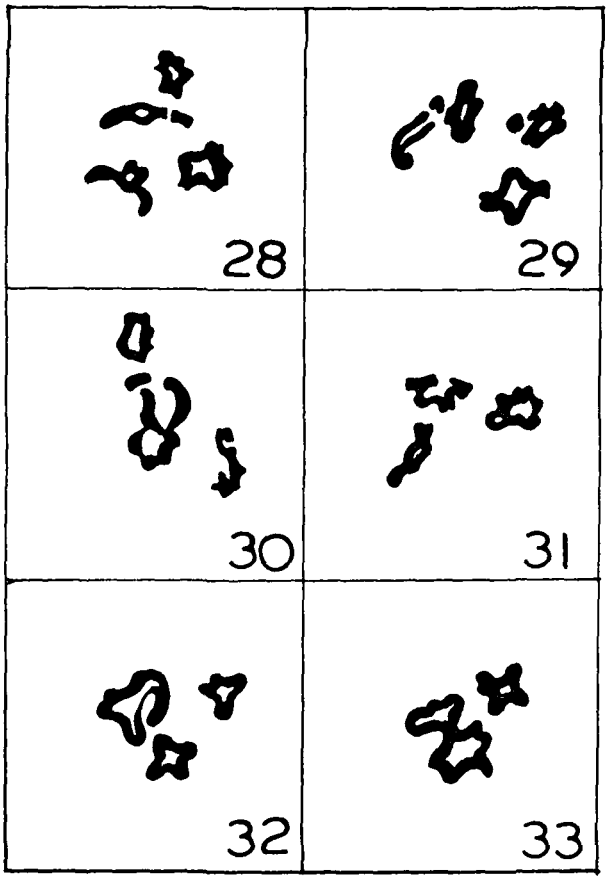
Fig. 29. A chromosome break and a fragment of unknown origin.

Fig. 30. A chromosome break and a chromatid break.

Fig. 31. Chromatid break.

Fig. 32. A multivalent structure

Fig. 33. A quadrivalent or two bivalent association.


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309 or 41.58% without and with precocious separation cases respectively. On the other hand, in the control series (Table 6) out of 1791 diakinesis nuclei examined there were 15 or 0.87% chromatid breaks, 3 or 1.6% acentric fragments, 7 or 0.39% bivalent association, 25 or 1.39% precocious autosomal separation, 11 or 0.61% precocious sex chromosome separation making a total of 64 or 3.24% individual types of aberrations. If the precocious separation cases were excluded the number would come down to 28 or 1.42% which was significantly lower than that of treated specimens. Further if the gross and individual type aberrations were taken together, the total number would be 136 or 7.59% and excluding the precocious separation cases it would be only 100 or 5.58%. Further, the frequency of aberrations in diakinesis nuclei at different intervals, it would show the range between 3.22% (4 hr) and 17.74% (96 hr).

In the treated series the frequency of aberrations ranged between 1.26% (10 min) and 100% (1st week) indicating a very wide range of variation. The net increase in the frequency of aberration in the treated series over control at different interval ranged between 1.96% (1 hr) and 92.11% (1st week) and the average increase was 33.99%. On the whole the data indicated that the treatment of Pseudomonas induced higher frequency of aberrations in chromosomes of diakinesis stage than that of control (Table 6) as it was also found in the spermatogonial metaphase.

Metaphase I :

It was intended to asses 100 metaphase-I plates per individual as a standard but it could not be maintained in

Table 6

Frequency distribution of chromosome aberrations in Diakinesis of control and log-culture of P. aeruginosa treated male mice fixed at different intervals. Control data are in bracket (3 ind. used per interval)

Fixation	No.of cells counted	Individual type aberrations							Gross type aberrations			Grand total	%	Net incr. %
		Break type				Prec.sep.		Total	Nume.change	Other	Total			
		Chroma.	Chromo.	Fragment	Mv/Asso.	Aut.	X & Y							
														a
10 min	71(50)	3(-)	-(-)	-(-)	-(-)	2(-)	1(-)	6(-)	-(-)	2(2)	2(2)	8(2)	11.26(4.00)	7.26
1 hr	59(69)	2(-)	-(-)	2(-)	1(-)	-(3)	2(1)	7(4)	-(2)	1(2)	1(4)	8(8)	13.55(11.59)	1.96
2 hr	49(138)	-(1)	-(-)	-(-)	1(1)	-(1)	-(2)	1(5)	-(1)	5(3)	5(4)	6(9)	12.24(6.52)	5.72
4 hr	48(124)	2(1)	2(-)	1(-)	1(-)	2(-)	1(1)	9(2)	-(-)	3(2)	3(2)	12(4)	25.00(3.22)	21.78
8 hr	50(291)	3(4)	2(2)	2(1)	-(-)	2(1)	-(-)	9(8)	5(2)	20(-)	25(2)	34(10)	68.00(3.42)	64.57
24 hr	30(185)	1(-)	-(-)	-(-)	1(-)	-(-)	-(-)	2(-)	-(-)	4(11)	4(11)	6(11)	20.00(5.94)	14.06
48 hr	18(80)	-(2)	1(-)	1(1)	9(-)	2(-)	2(-)	13(3)	-(-)	3(3)	3(3)	16(6)	88.88(7.50)	81.38
72 hr	92(39)	3(1)	-(-)	-(-)	10(-)	8(-)	6(1)	27(2)	-(-)	12(3)	12(3)	39(5)	42.39(12.82)	29.57
96 hr	25(62)	2(2)	1(1)	1(-)	1(2)	-(2)	-(-)	5(7)	-(1)	-(3)	-(4)	5(11)	20.00(17.74)	2.26
120 hr	35(187)	2(2)	-(-)	3(-)	1(2)	4(1)	-(-)	10(5)	3(3)	2(7)	5(10)	15(15)	42.85(8.02)	34.83
144 hr	24(95)	-(-)	2(-)	-(-)	2(-)	-(5)	-(2)	4(7)	-(1)	14(6)	14(7)	18(14)	75.00(14.73)	60.27
1st wk	11(38)	2(1)	-(-)	1(-)	-(-)	1(1)	1(-)	5(2)	-(-)	6(1)	6(1)	11(3)	100.00(7.89)	92.11
2nd wk	42(90)	3(1)	-(-)	2(-)	1(-)	4(5)	-(2)	10(8)	4(2)	-(-)	4(2)	14(10)	33.33(11.11)	22.22
3rd wk	36(68)	-(-)	1(-)	-(1)	-(-)	1(2)	9(-)	11(3)	6(-)	13(5)	19(5)	30(8)	83.33(11.76)	71.57
4th wk	29(69)	1(-)	-(-)	4(-)	3(1)	4(1)	2(1)	14(3)	-(-)	3(1)	3(1)	17(4)	58.62(5.79)	52.83
5th wk	59(50)	-(-)	-(-)	2(-)	1(-)	3(-)	1(1)	7(1)	4(1)	10(1)	14(2)	21(3)	35.59(6.00)	29.59
6th wk	30(87)	2(-)	2(-)	1(-)	1(1)	1(2)	1(2)	3(3)	3(2)	15(3)	15(5)	25(8)	83.33(9.19)	74.14
7th wk	35(69)	1(-)	-(-)	2(-)	7(-)	2(1)	4(-)	13(1)	-(2)	7(2)	7(4)	23(5)	65.71(7.24)	58.47
Total	743(1791)	27(15)	11(3)	22(3)	40(7)	36(25)	28(11)	164(64)	24(17)	121(55)	145(72)	309(136)	41.58(7.59)	33.99

testes of 3 treated males fixed at 96 hr, 120 hr, 144 hr, 1st week, 5th week, 6th week and 7th week. In control series there was no such difficulty (Table 7). The inadequate occurrence of metaphase-I was very likely due to some effect of the treatment of the bacterial culture as was experienced in Aspermatogonial metaphase and diakinesis stage of the treated specimens.

The types of aberrations (PM 22-27; Figs. 34-43) were more or less similar in nature as found in diakinesis nuclei. These were individual types consisting of chromatid or chromosome break, fragment of unknown origin, translocation/ bivalent association, precocious separation of autosomes in some bivalents and the X and Y in sex pair. These were also gross types showing numerical change, stickiness etc.

In the testes of a total of 54 control specimens examined at 18 different intervals, there were 25 or 0.46% chromatid break, 3 or 0.05% isochromatid break, 12 or 0.22% fragment of unknown origin, 42 or 0.77% multivalent/bivalent association, 133 or 2.46% precocious separation of autosomes, 90 or 1.66% precocious separation of X and Y making a total of 305 or 5.64% individual types of aberrations (Table 7). If the cases of precocious separation of autosomes and sex chromosomes were eliminated, the aberration frequency would come down to 82 or 1.51%. Taking all individual and gross type aberrations together, the average frequency was 413 or 7.64% of which if the precocious separation cases were eliminated, it would be 190 or 3.51%. On the otherhand in the treated series out of 4334 metaphases I, there were 62 or 1.43% chromatid breaks, 18 or 0.41% isochromatid breaks,

Explanation of Photomicrographs

Photomicrographs (PM) of affected metaphase I plates in male mice for the infection of bacterium P. aeruginosa.

PM 22. A chromatid break in an aneuploid metaphase I with 18 bivalents.

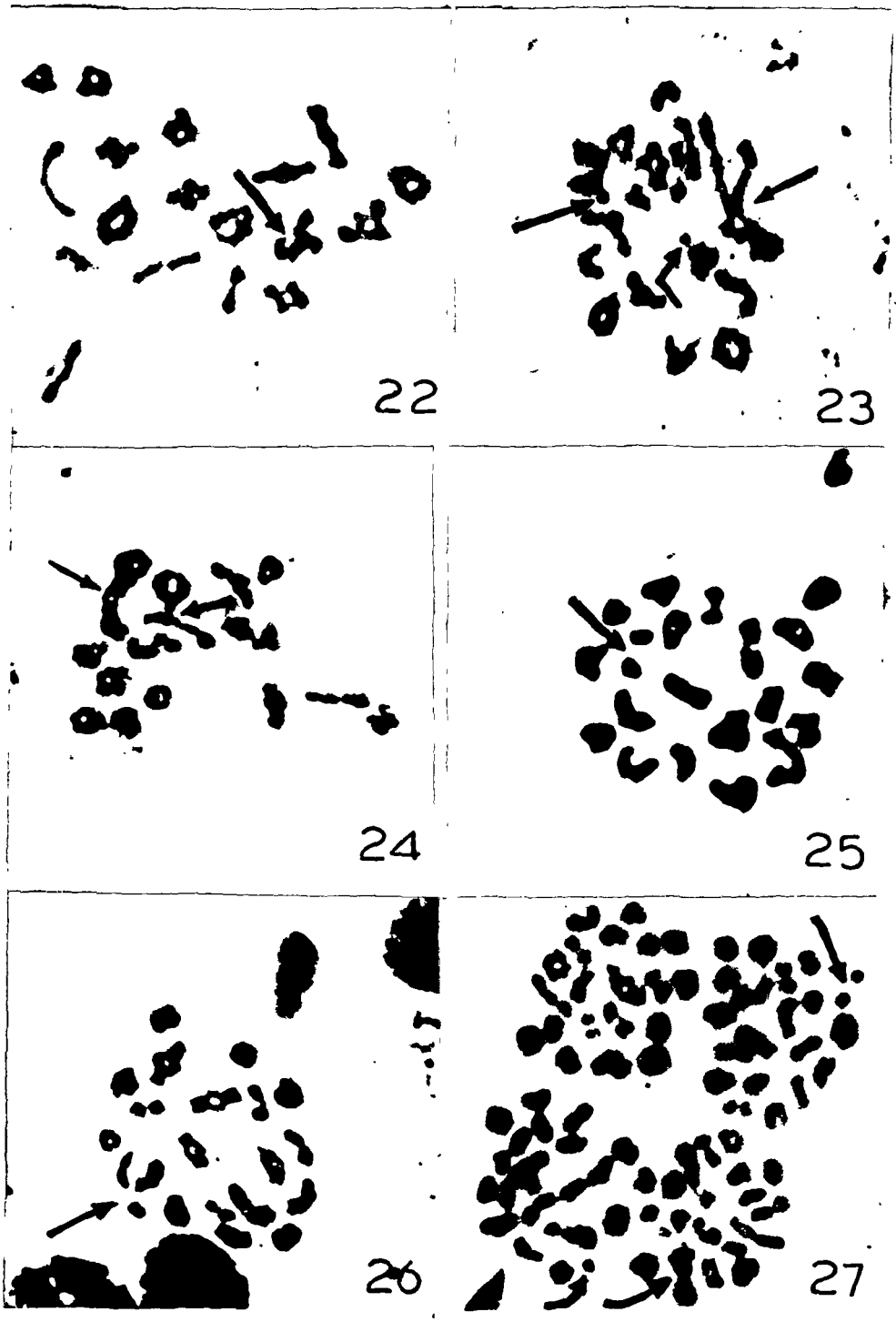
PM 23. A fragment, a constriction and two bivalent association (translocation).

PM 24. A multivalent and some association with aneuploid number of 17 bivalents.

PM 25. Precocious separation of autosomes of bivalent.

PM 26. Precocious separation of the X and Y.

PM 27. Metaphase I plates upper left - 20 bivalents
upper right - 20 bivalents of which one precocious autosomal separation.
Lower left - 21 bivalents with precocious separation of X and Y.
Right - 19 bivalents with a multivalent or bivalent association.


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Explanation of figures

Camera lucida drawing of a part of metaphase I plates showing different types of aberrations.

Figs. 34 and 35. A chromatid break.

Figs. 36-37. A chromosome break.

Fig. 38. A fragment of unknown origin.

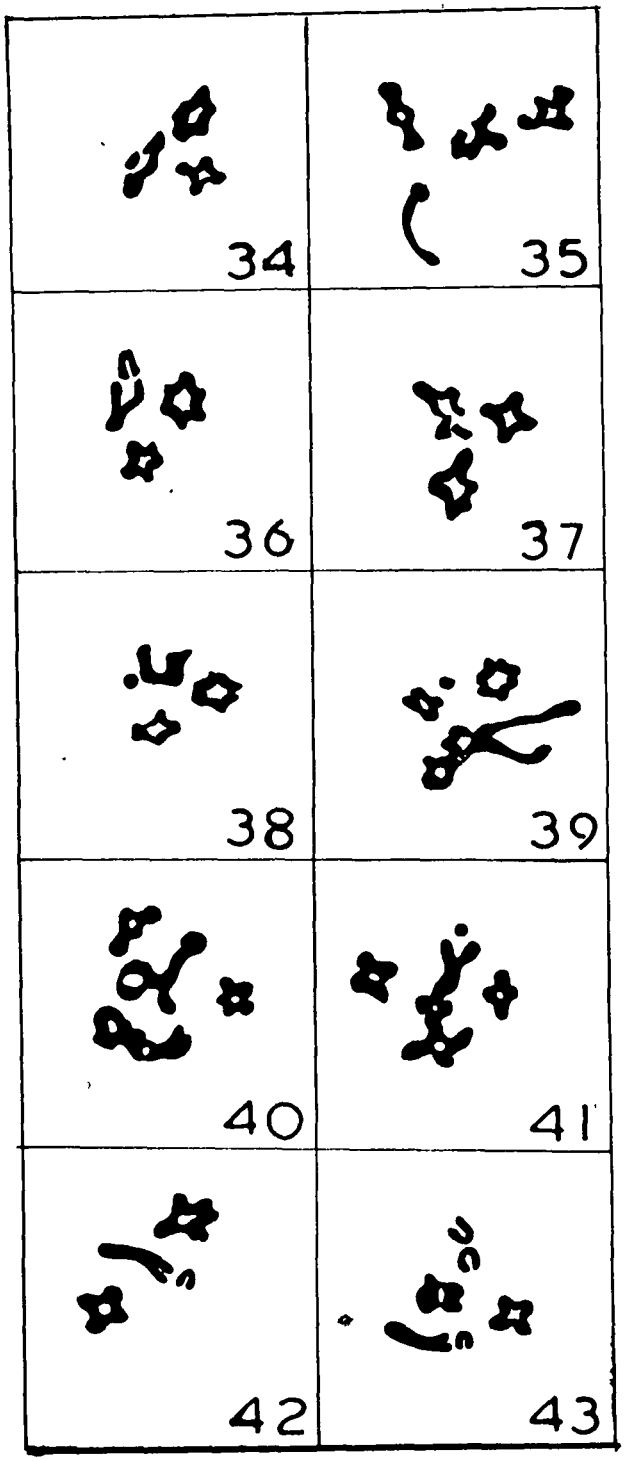
Fig. 39. A fragment and a multivalents structure.

Fig. 40. Two tetravalents or association of two bivalents.

Fig. 41. A chain of three bivalents and a fragment.

Fig. 42. Precocious separation of sex chromosome.

Fig. 43. Precocious separation of autosomes and sex chromosome.


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47 or 1.08% fragment of unknown origin, 174 or 4.01% translocation or association, 265 or 6.11% precocious autosomal separation, 134 or 3.09% precocious separation of X and Y pair making a total of 700 or 16.15% individual type aberrations of which if the precocious separation cases were eliminated, the number of aberrations would come down to 301 or 6.94% which was, however, much higher than that of control specimens. If the gross types and individual types were combined, the number would be 1180 or 27.27% out of 4334 metaphase I plates. If the number of precocious separation cases was disregarded, the total number of aberrations would come down to 781 or 18.02% but it was much higher than that of control indicating thereby that the treatment of Pseudomonas induced much higher frequency of chromosome aberrations in metaphase I like that spermatogonial metaphase and diakinesis stage.

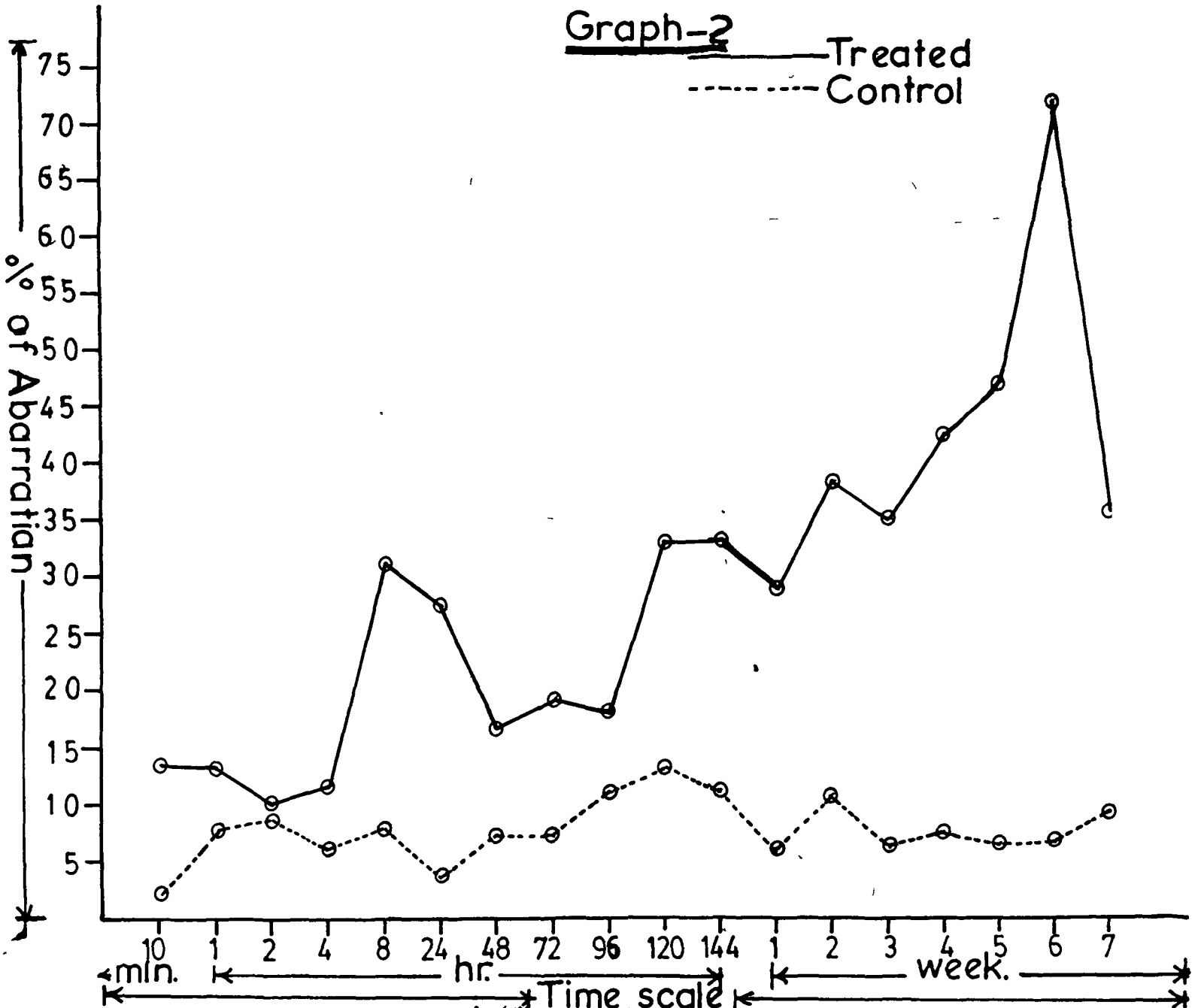
If the frequency of chromosome aberrations at different intervals (Table 7) between the control and treated specimens were compared, it was found to be always higher in the treated series and net increase in the treated was 11.33% at 10 min, 5.34% at 1 hr, 1.34% at 2 hr, 5.66% at 4 hr, 23.34% at 8 hr, 23.67% at 24 hr, 9.66% at 48 hr, 11.67% at 72 hr, 7.0% at 96 hr, 19.84% at 120 hr, 22.14% at 144 hr, 22.75% at 1st week, 27.67% at 2nd week, 28.67% at 3rd week, 35.0% at 4th week, 40.52% at 5th week, 65.93% at 6th week, 26.42% at 7th week and 19.56% in the average of data of all intervals taken together (Table 7). The inclusion of precocious separation of autosomes of sex chromosomes had increased the frequency of aberrations but even their exclusion would no doubt show that the treatment of P. aeruginosa induced higher frequency of aberrations even if

Table 7

Frequency distribution of chromosome aberration in metaphase I of control and log-culture bacterium, P. aeruginosa treated male mice fixed at different intervals. Control data are in brackets (3 indiv/fixation in both control and treated line were used).

Fixation	No.of cells counted	Individual type aberrations: Break type: Chroma.	Chromo.	Fragment	Trnas/ Asso.	Brec. sepa. Autosome	X & Y	Total	Gross type aberrations: Num.change	Others	Total	Grand total	%	Net.incr. %
10 min	300(300)	8(-)	2(-)	2(-)	5(-)	12(3)	7(-)	36(3)	3(-)	3(3)	6(3)	40(6)	13.33(2.00)	11.33
1 hr	300(300)	6(-)	1(-)	7(-)	5(-)	4(7)	14(8)	37(15)	-(3)	2(5)	2(8)	39(23)	13.00(7.66)	5.34
2 hr	300(300)	-(2)	2(-)	3(2)	10(4)	4(7)	3(5)	22(20)	4(6)	4(-)	8(6)	30(26)	10.00(8.66)	1.34
4 hr	300(300)	-(1)	-(-)	4(-)	7(2)	12(6)	11(8)	34(17)	1(1)	-(-)	1(1)	35(18)	11.66(6.00)	5.66
8 hr	300(300)	2(4)	1(-)	3(2)	12(3)	24(5)	8(3)	50(17)	27(6)	16(-)	43(6)	93(23)	31.00(7.66)	23.34
24 hr	300(300)	3(-)	-(-)	2(-)	20(2)	6(-)	3(2)	34(4)	-(-)	48(7)	48(7)	82(11)	27.33(3.66)	23.67
48 hr	300(300)	2(7)	-(1)	-(3)	10(4)	5(4)	6(1)	23(20)	1(-)	26(1)	27(1)	50(21)	16.66(7.00)	9.66
72 hr	300(300)	-(1)	-(-)	-(-)	11(2)	20(7)	18(6)	49(16)	-(-)	8(6)	8(6)	57(22)	19.00(7.33)	11.67
96 hr	*300(300)	2(3)	-(-)	-(1)	4(3)	3(6)	5(5)	14(18)	1(3)	3(12)	4(15)	18(33)	18.00(11.00)	7.00
120 hr	*204(300)	7(1)	-(-)	2(-)	3(4)	23(14)	4(9)	39(28)	24(9)	4(2)	28(11)	67(39)	32.84(13.00)	19.84
144 hr	*175(300)	-(-)	-(-)	-(-)	4(2)	6(12)	-(13)	10(27)	17(4)	31(2)	48(6)	58(33)	33.14(11.00)	22.14
1st wk	* 80(300)	3(1)	-(-)	-(-)	-(3)	2(4)	3(7)	8(15)	1(2)	14(1)	15(3)	23(18)	28.75(6.00)	22.75
2nd wk	300(300)	6(2)	5(-)	4(-)	6(-)	60(19)	2(6)	83(27)	32(5)	-(-)	32(5)	115(32)	38.33(10.66)	27.67
3rd wk	300(300)	2(-)	1(-)	5(1)	36(2)	18(8)	-(3)	62(14)	18(-)	26(5)	43(5)	105(19)	35.00(6.33)	28.67
4th wk	300(300)	6(-)	-(-)	7(-)	12(5)	31(8)	19(2)	75(15)	30(2)	22(5)	52(7)	127(22)	42.33(7.33)	35.00
5th wk	*175(300)	2(-)	2(-)	3(-)	2(2)	12(8)	8(3)	29(13)	21(4)	32(2)	53(6)	82(19)	46.85(6.33)	40.52
6th wk	*135(300)	4(-)	4(-)	2(-)	5(2)	12(10)	15(5)	42(17)	24(2)	32(1)	56(3)	98(20)	72.59(6.66)	65.93
7th wk	*165(300)	9(3)	-(2)	3(3)	22(2)	11(5)	8(4)	53(19)	-(4)	6(5)	6(9)	59(28)	35.75(9.33)	26.42
Total	4334(5400)	62(25)	18(3)	47(12)	174(42)	265(133)	134(90)	700(305)	203(51)	277(57)	480(108)	1180(413)	27.22(7.64)	19.56

* Not possible to collect 300 plates.



Graphical representation of frequency of aberration in metaphase-I (male germinal cells) of control & P. aeruginosa treated mice at different intervals.

that type was excluded at metaphase I as experienced in other stages mentioned before. Moreover, since at metaphase I, the number of plates examined both in control and treated series was the same in most of the intervals, the comparison was more effective and suffered no limitation of data as found in spermatogonial metaphase and diakinesis stage. However, frequency at different intervals did not show a regular mode (Table 7 Graph 2).

Metaphase II

The frequency of metaphase II to encounter 100 metaphase I per individual was lower and in the combined data (Table 8) of three treated individuals, the total number ranged between 16 (1st week) and 233 (72 hr). In the control series it ranged between 30 (96 hr) and 204 (72 hr) among 18 fixation intervals between 10 minute and 7 week. However, the scoring of data in this stage was handicapped for the prevalence of sticky effect in chromosomes and their unfavourable disposition. The aberrations as in other stages were of individual types consisting of (PM 28-32; Figs. 44-55), chromatid break, fragment of unknown origin, constriction, gap etc. and gross types showing numerical change and stickiness, pycnosis etc.

In a total of 1472 metaphase-II plates examined in 54 treated male individuals, there were 131 or 8.90% chromatid breaks, 58 or 3.94% acentric fragments, 23 or 1.56% translocation or association, 13 or 0.88% constriction or gap making a total of 225 or 15.29% individual type aberrations (Table 8). The individual and gross types taken together,

Explanation of Photomicrograph

Photomicrographs of metaphase II showing different forms of chromosome aberrations in log culture of *P. aeruginosa* treated male mice

PM 28. A normal metaphase II plate.

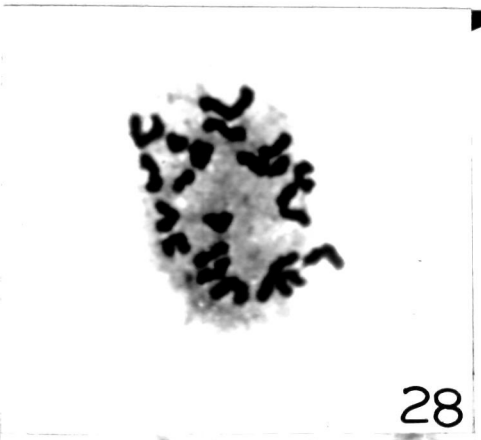
PM 29. Chromosome with chromatid breaks, constriction, gap etc.

PM 30. A fragment and some chromosomes with early centric dissociation.

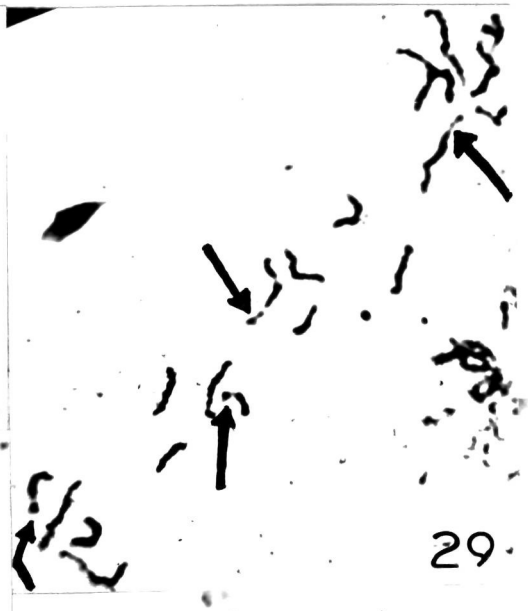
PM 31. Sticky chromosomes with one ring like structure.

PM 32. Two chromatid break and one centric fusion.

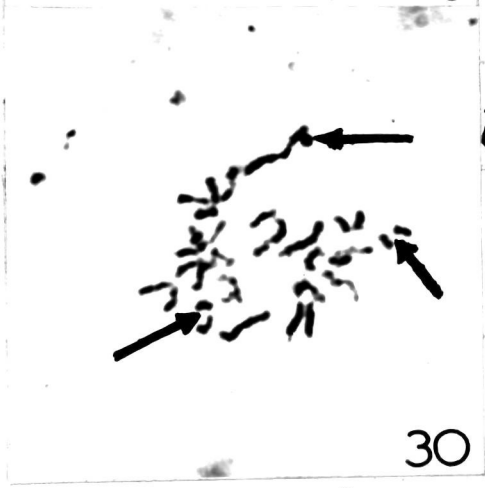
PM 33. One constriction.



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Explanation of Figures

Camera lucida drawing of a part of metaphase II showing different types of aberrations treated with bacterium, Pseudomonas aeruginosa in male mice Mus musculus.

Fig. 44. A chromatid break in the terminal region.

Fig. 45. Two chromatid breaks in two chromosome.

Fig. 46. A chromatid break.

Fig. 47. A chromatid break.

Fig. 48. A fragment and a chromosome with early centric separation.

Fig. 49. A chromatid break and a constriction.

Fig. 50. A centric fusion.

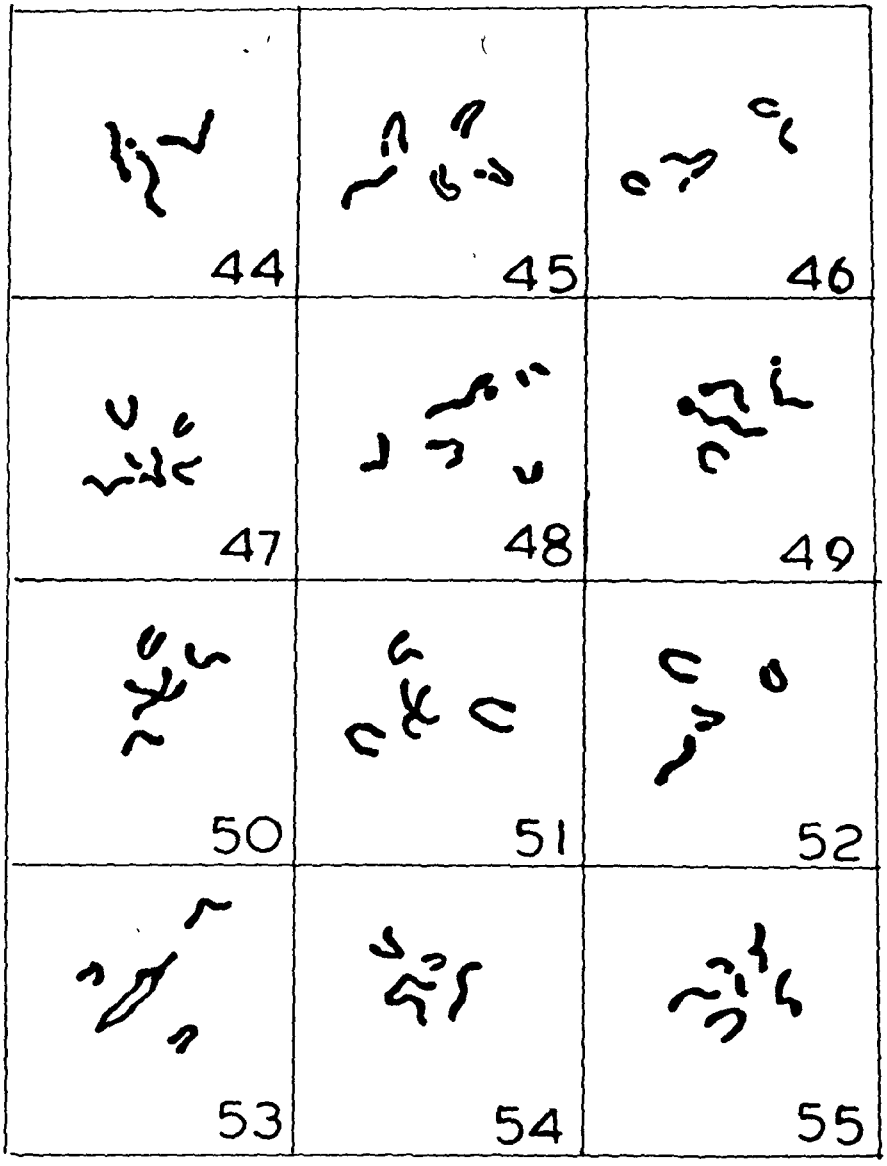
Fig. 51. A centric fusion.

Fig. 52. A constriction.

Fig. 53. A ring-like structure formed due to stickiness.

Fig. 54. End to end association between two chromosomes.

Fig. 55. A chromosome with early centromeric dissociation.


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the total number was 627 or 42.59% while in the same number of control male out of 2433 plates examined, there were 34 or 1.39% chromatid breaks, 14 or 0.57% fragments making a total of 48 or 1.97% individual type aberrations. These with gross types taken together, the number was 342 or 14.06%. Therefore, the net increase in the aberration frequency was 28.53% in the treated series. If the aberration frequency between control and treated series at 18 different intervals were compared, it was always higher in the treated specimens except that 24 hr (Table 8) though the data were not very adequate. The net increase was 15.14% at 10 min, 17.05% at 1 hr, 11.05% at 2 hr, 15.72% at 4 hr, 11.78% at 8 hr, -4.74% at 24 hr, 20.83% at 48 hr, 15.03% at 72 hr, 5.29% at 96 hr, 39.18% at 120 hr, 24.63% at 144 hr, 66.92% at 1st week, 44.23% at 2nd week, 58.25% at 3rd week, 65.92% at 4th week, 55.25% at 5th week, 66.36% at 6th week, 47.99% at 7th week and 28.53% in the average (Table 8).

Comments :

The data of the net increase of aberration frequency in chromosomes at spermatogonial metaphase, diakinesis, metaphase I and metaphase II when compiled (Table 9) were found that there were some amount of differences at different stages though in all cases it was higher than the respective controls. However, the present data suffered some limitation because the equal number of cells could not be studied. Some aberrations included in the data might have no genetic significance and so on. Therefore, we refrained from claiming definitely the stage sensitivity though there was some indication. If the aberration frequency of all meiotic stages were combined the net

increase in the frequency was 14.62% in the treated series (Table 9). In the bone marrow cells, the net increase in aberration frequency was 41.14% (Table 1) indicating that the bone marrow chromosomes were more sensitive than the germinal chromosomes. Differential sensitivity between somatic chromosome and germinal chromosome has been recorded by earlier workers in specimens treated with X-rays. The present study also indicated differential responses in chromosome aberrations in different stages of meiosis and it was highest at diakinesis and lowest in spermatogonial metaphase for the treatment of P. aeruginosa but much more critical study was needed to have a definite claim. On the whole it could be concluded there was effect in chromosomes of both germinal and somatic tissue.

Table 8

Frequency distribution of chromosome aberration in Metaphase II of control and log-culture of P. aeruginosa treated male mice at different intervals. Control data are in brackets (used 3 indv/per interval in both control and treated)

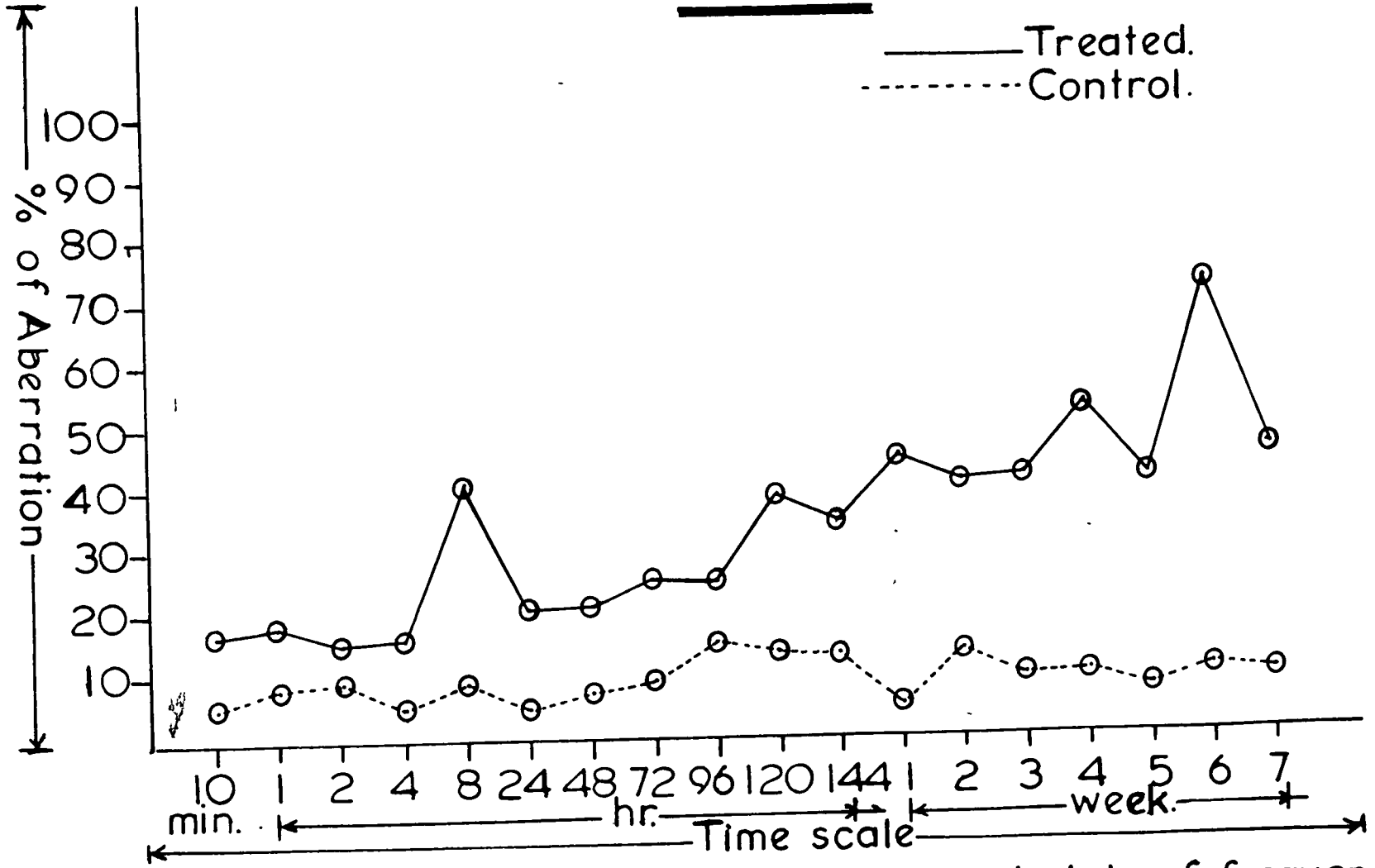
Fix.time	Total cells counted	Individual types: Break type: Chr.br.	Break type: Frag.	Break type: Trans./ Asso.	Phy. type: Cons/Gap	Total	Gross type: N.C.	Gross type: Others	Total	Grand total	%	Nett incr. %
10 min	180(121)	23(2)	8(-)	-(-)	-(-)	31(2)	5(6)	21(12)	26(18)	57(20)	31.66(16.52)	15.14
1 hr	186(85)	14(2)	3(-)	3(-)	2(-)	20(2)	16(2)	25(9)	41(11)	61(13)	32.79(15.29)	17.05
2 hr	143(177)	2(2)	6(2)	3(-)	-(-)	11(4)	6(8)	19(13)	25(21)	36(25)	25.17(14.12)	11.05
4 hr	96(153)	3(-)	-(-)	-(-)	-(-)	3(-)	6(8)	13(3)	19(11)	22(11)	22.90(7.18)	15.72
8 hr	49(160)	2(6)	3(-)	2(-)	-(-)	7(6)	28(7)	5(9)	33(16)	40(22)	81.63(19.75)	11.78
24 hr	57(150)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	3(15)	3(15)	3(15)	5.26(10.00)	- 4.74
48 hr	33(96)	3(6)	1(-)	-(-)	-(-)	4(6)	-(-)	7(6)	7(6)	11(12)	33.33(12.50)	20.83
72 hr	233(204)	10(-)	7(-)	3(-)	2(-)	22(-)	9(6)	36(22)	45(28)	67(28)	28.75(13.72)	15.03
96 hr	17(30)	-(1)	3(1)	-(-)	-(-)	3(2)	1(2)	2(5)	3(7)	6(9)	35.29(30.00)	5.29
120 hr	78(150)	9(3)	7(-)	-(-)	1(-)	17(3)	8(11)	20(13)	28(24)	45(27)	57.69(18.50)	39.18
144 hr	23(200)	-(3)	-(-)	-(-)	-(-)	-(3)	-(8)	9(18)	9(26)	9(29)	39.13(14.50)	24.63
1st wk	16(99)	-(-)	-(-)	1(-)	-(-)	1(-)	2(5)	9(3)	11(8)	12(8)	75.00(8.08)	66.92
2nd wk	71(185)	10(3)	3(3)	3(-)	2(-)	18(6)	14(11)	9(8)	23(19)	41(25)	57.74(13.51)	44.23
3rd wk	37(129)	7(1)	4(3)	3(-)	-(-)	14(4)	8(6)	5(9)	13(15)	27(19)	72.97(14.72)	58.25
4th wk	91(107)	24(3)	3(3)	3(-)	5(-)	35(6)	11(5)	31(9)	42(14)	77(20)	84.61(18.69)	65.92
5th wk	48(74)	7(-)	4(-)	-(-)	-(-)	11(-)	9(4)	15(9)	24(13)	35(13)	72.91(17.56)	55.25
6th wk	44(160)	5(1)	-(-)	-(-)	-(-)	5(1)	4(19)	29(12)	33(31)	38(32)	86.36(20.00)	66.36
7th wk	70(153)	12(1)	6(2)	2(-)	3(-)	23(3)	1(5)	16(6)	17(11)	40(14)	57.14(9.15)	47.99
Grand total	1472(2433)	131(34)	58(14)	23(-)	13(-)	225(48)	128(113)	274(181)	402(294)	627(342)	42.59(14.06)	38.53

Table 9

Compiled data of aberration frequency of all stages of meiosis in Pseudomonas treated and control mice (control data in bracket)

Stage	Total No.of cells	Individual type aberrations							Total	Gross type aberrations			Grand total	%	Net incr %
		Break type					Pre-sep.			N.C.	Others	Total			
		Chr.	Ch.	Frag.	Trans.	Cons.	Aut.	Sex							
Sp.	1087	17	-	19	8	-	-	-	44	113	118	231	275	25.36	17.11
	(2676)	(13)		(12)	(-)	(-)			(23)	(139)	(59)	(198)	(221)	(8.25)	
Diak.	743	27	11	22	40	-	36	28	164	24	121	145	309	41.58	33.99
	(1791)	(15)	(3)	(3)	(7)	(-)	(25)	(11)	(64)	(17)	(55)	(72)	(136)	(7.59)	
Met.I	4334	62	18	47	174	-	265	134	700	203	277	480	1180	27.22	19.56
	(5400)	(25)	(3)	(12)	(42)	(-)	(133)	(90)	(305)	(51)	(57)	(108)	(413)	(7.64)	
Met. II	1472	131	-	58	23	13	-	-	225	128	274	402	627	42.59	28.53
	(2433)	(34)		(14)	(-)	(-)			(48)	(113)	(181)	(291)	(342)	(14.06)	
Grand total	7636	237	29	146	245	13	301	162	1133	468	790	1258	1807	23.66	14.62
	(12300)	(87)	(6)	(41)	(49)	(-)	(158)	(101)	(440)	(320)	(352)	(669)	(1112)	(9.04)	

Graph-3



Graphical representation of the combined data of frequency of aberration in germinal cells of control and P. aeruginosa treated male mice at different intervals.

b) Effect on sperm count :

The genetic significance of sperm depletion test is yet to be established but different mutagen affecting the sperm count was found by some workers in our laboratory using the living mutagens like Staphylococcus aureus (Ghosh, 1985), Sarcina lutea (Goswami, 1985) and spores of Aspergillus fumigatus (Manna and Patel, 1982) and some chemical mutagens like D-Glucose amine hydrochloride (Banerjee, 1982), Chloramphenicol (Roy, 1982) etc. and by (Bateman, 1958) using X-rays. It was found that the litter size was dependent on the sperm density in rabbit (Walton, 1927) or the fertility would be reduced in mice if the epididymal sperm count fell below 10% of the normal count (Searle and Beechy, 1974). With a view to finding out if the treatment of Pseudomonas culture to male mice would induce sperm depletion effect because of the stage sensitivity in spermatogenesis, the present study was conducted.

Procedure :

While fixing the testes of the male specimens of both treated and control series for the study of meiotic chromosome aberration, the right and left epididymes of each male were also taken out separately for the study of the total sperm count presented in this section and the sperm head morphology presented in the next section. The sperm samples were prepared as follows :-

Epididymis of each side of the control and treated male was taken separately into 2 ml of 0.87% normal saline. It was made free from fat, vas deferens and other adhering tissues. The inner content of each epidedymis was teased

out in the solution and the tissue was minced. The material was thoroughly shaken to make free the sperms to suspend in the saline solution. The sperm suspension was then filtered through silken cloth to remove the debris and the filtrate collected into a graduated tube was added with more saline solution to make the volume 8 ml. After the final dilution drops of sperm suspension was put in the chambers of a Neubauer haemocytometer slide. The number of sperms in each of the five chambers of the slide was counted and five such separate readings were taken. The mean number of the five counts in 25 squares was determined which formed the mean number of sperms per side of an individual. In the data average mean of three male individuals at each of 18 intervals has been presented (Table 10).

Result :

The study was conducted at 18 intervals between 10 min and 7th week. The week-wise data (Table 10) between 1st and 7th week would show the stage sensitivity if any of spermatogenesis (Adler, 1982). The data of the right epididymis would show that the average number ranged between 4.26 $\pm$ 0.46 and 16.04 $\pm$ 0.47 in the treated specimens while in the control specimens it ranged between 11.89 $\pm$ 0.34 and 26.72 $\pm$ 0.71 (Table 10). The difference in the average count of sperm in control and treated specimens was statistically significant at different level in most of the fixation intervals (Table 10 and Graph 3).

In the left epididymis, the same situation was also

found. In the treated line the average count ranged between 3.00 ± 0.32 and 20.40 ± 0.16 and in the control line it ranged between 10.03 ± 0.66 and 25.69 ± 0.42. Though the frequency was fluctuating (Graph 4) in different intervals but in the same individual, it was not so wide .

The maximum difference was found in 4th week. It was 13.82 ± 1.51 against 4.33 ± 0.29. Anyhow, the data of the left and right epididymal sperm count were combined to have the larger sample. The average frequency in the combined data of the left and right epididymes ranged between 3.68 ± 0.40 and 17.83 ± 0.45 in the treated specimens and in the control line it ranged between 11.78 ± 0.76 and 25.82 ± 0.48 (Table 10). A comparison of the data between treated and control specimens showed that the difference was statistically significant at various level. Therefore, the present data (Table 10 and Graph 5) showed the significant decrease in the sperm count in all the fixation intervals indicating thereby that the treatment of log-culture of Pseudomonas reduced the sperm production. That the depletion in sperm count could be under genetic control was because there was not only definite depletion effect for the treatment of the Pseudomonas in the parental generation but it was also seen in F_1 and F_2 generation (vide infra).

Sperm head morphology

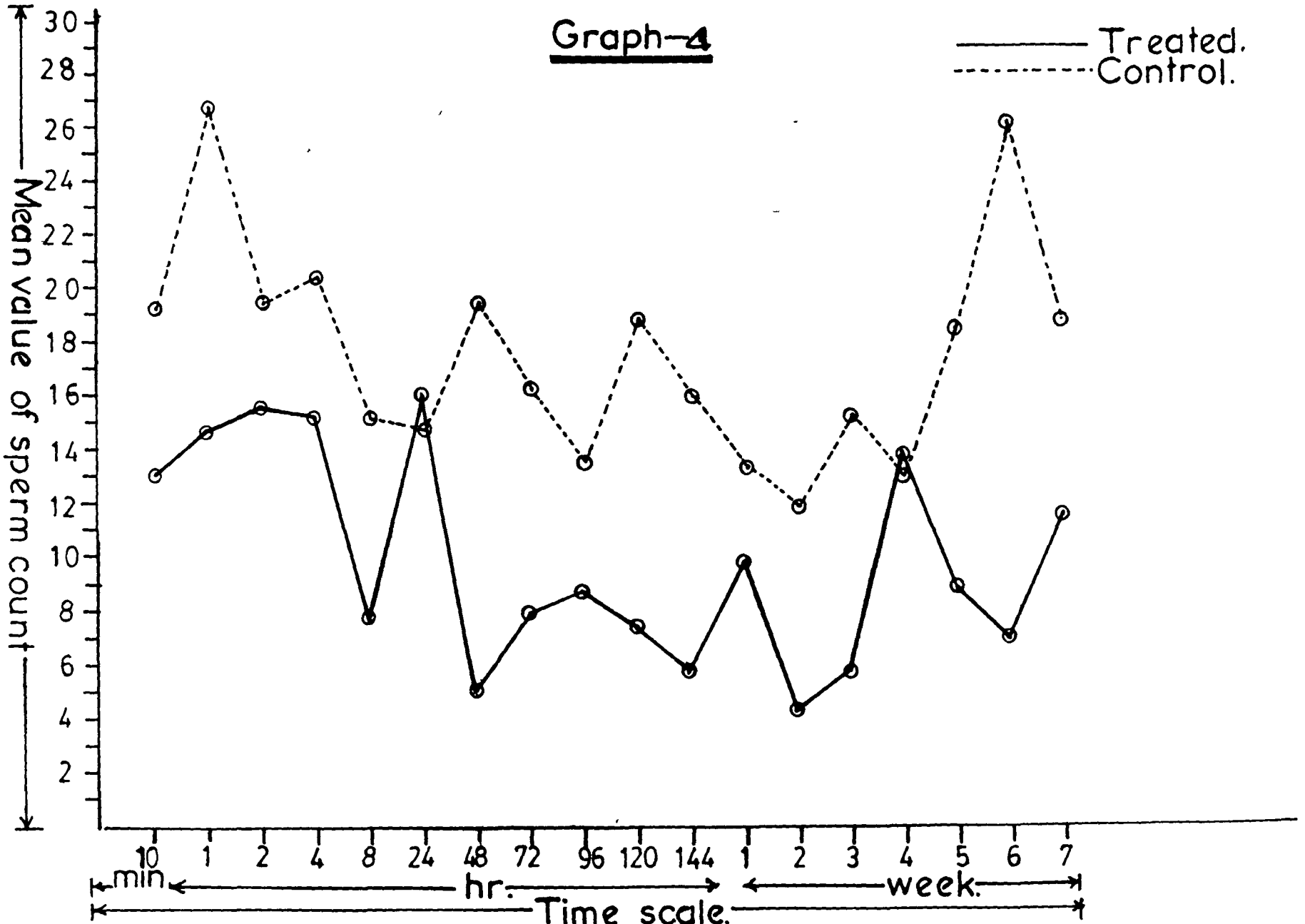
It has been mentioned already that the mutagenicity testing in germinal cells is more laborious but it has special significance. According to some workers the study of the chromosome aberrations is relatively tedious and require special training while the study of sperm count and

Table 10

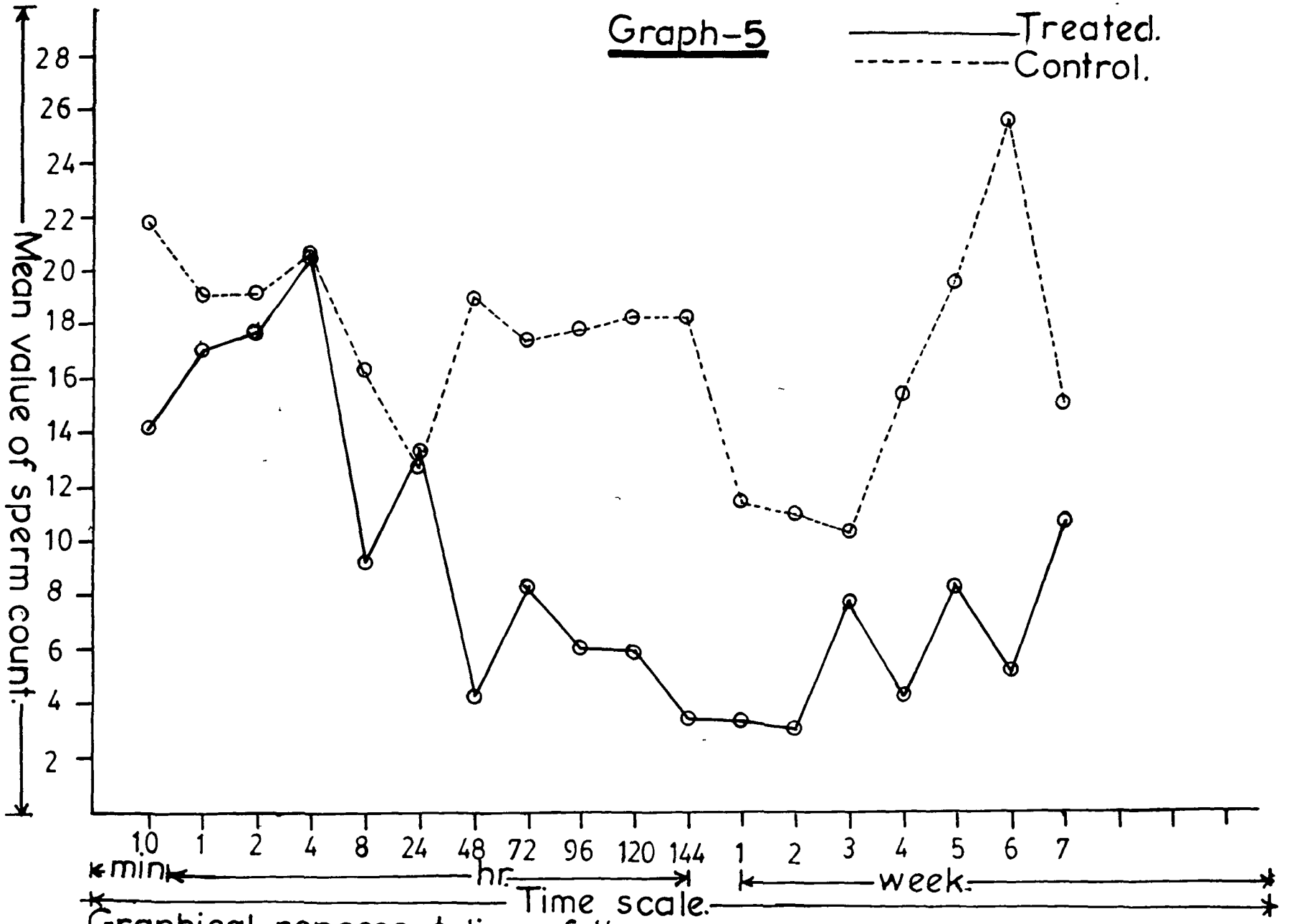
Mean number of sperms in the Epididymes on control and log culture of P. aeruginosa treated male mice. Control data are in brackets (used 3 indv./per interval in both control and treated series)

Fix.time	Right epididymes (Mean value ± S.E.)	Left epididymes (Mean value ± S.E.)	Right and left combined (Mean value ± S.E.)
10 min	13.08 ± 0.34(19.02 ± 0.51)^{b}	14.17 ± 0.41(21.85 ±0.49)^{a}	13.61 ± 0.47(20.41 ± 0.53)^{a}
1 hr	14.70 ± 0.51(26.72 ± 0.71)^{a}	17.06 ± 0.79(19.12 ± 0.35)^{e}	15.88 ± 0.68(22.92 ± 0.71)^{a}
2 hr	15.05 ± 0.36(19.46 ± 0.38)^{b}	17.65 ± 0.41(19.10 ± 0.51)^{e}	16.35 ± 0.43(19.28 ± 0.45)^{d}
4 hr	15.26 ± 0.45(20.36 ± 0.71)^{b}	20.40 ± 0.16(20.68 ± 0.48)^{e}	17.83 ± 0.45(21.35 ± 1.36)^{a}
8 hr	7.72 ± 0.37(15.16 ± 0.76)^{b}	9.21 ± 0.41(16.22 ± 0.80)^{a}	8.42 ± 0.40(15.48 ± 0.78)^{a}
24 hr	16.04 ± 0.47(14.62 ± 0.48)^{e}	13.38 ± 0.74(12.77 ± 0.66)^{e}	16.24 ± 0.47(14.03 ± 0.50)^{b}
48 hr	5.09 ± 0.44(19.41 ± 0.52)^{a}	4.37 ± 0.36(19.00 ± 0.51)^{c}	4.89 ± 0.42(18.26 ± 0.66)^{a}
72 hr	7.98 ± 0.40(16.22 ± 0.71)^{a}	8.30 ± 0.32(17.52 ± 0.40)^{a}	8.14 ± 0.36(13.71 ± 0.83)^{a}
96 hr	8.78 ± 0.57(13.48 ± 0.26)^{a}	6.00 ± 0.48(17.80 ± 0.48)^{a}	7.30 ± 0.54(15.63 ± 0.46)^{c}
120 hr	7.46 ± 0.53(18.78 ± 0.42)^{a}	5.97 ± 0.50(18.34 ± 0.37)^{a}	6.72 ± 0.52(18.63 ± 0.38)^{a}
144 hr	5.85 ± 0.34(15.98 ± 0.42)^{a}	3.45 ± 0.25(18.33 ± 0.25)^{c}	4.61 ± 0.33(16.86 ± 0.39)^{a}
1st wk	9.86 ± 0.43(13.36 ± 0.38)^{b}	3.34 ± 0.57(11.45 ± 0.45)^{a}	6.31 ± 0.58(12.64 ± 0.40)^{b}
2nd wk	4.26 ± 0.46(11.89 ± 0.34)^{a}	3.00 ± 0.32(11.06 ± 0.32)^{a}	3.68 ± 0.40(11.82 ± 0.76)^{e}
3rd wk	5.78 ± 0.58(15.20 ± 0.53)^{a}	7.66 ± 0.96(10.30 ± 0.66)^{e}	5.86 ± 0.68(11.78 ± 0.76)^{a}
4th wk	13.82 ± 1.51(12.97 ± 0.80)^{e}	4.33 ± 0.29(15.45 ± 0.52)^{a}	9.27 ± 1.24(11.81 ± 0.81)^{e}
5th wk	8.88 ± 0.56(18.30 ± 0.97)^{a}	8.21 ± 0.47(19.61 ± 0.95)^{a}	7.64 ± 0.64(18.55 ± 0.96)^{a}
6th wk	7.02 ± 0.61(26.18 ± 0.48)^{c}	5.18 ± 0.28(25.69 ± 0.42)^{c}	6.11 ± 0.49(25.82 ± 0.48)^{c}
7th wk	11.50 ± 0.43(18.73 ± 1.00)^{a}	10.73 ± 0.60(15.13 ± 0.45)^{b}	11.10 ± 0.53(16.33 ± 0.85)^{b}

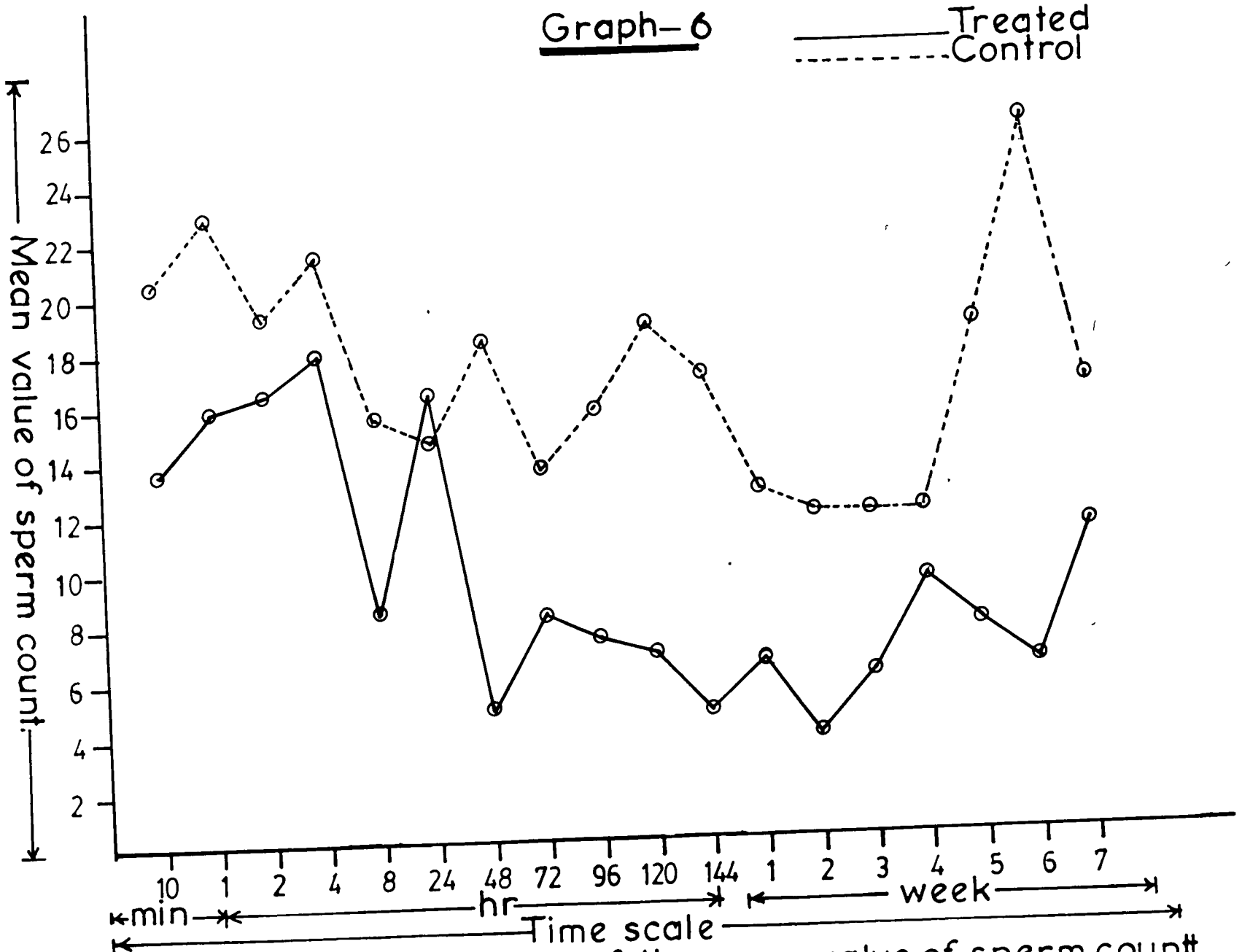
a = $P<0.01$, b = $P<0.02$, c = $P<0.001$, d = $P<0.05$, e = $P>0.05$



Graphical representation of the mean value of sperm count in right epididymis of control and P.aeruginosa treated mice at different intervals.



Graphical representation of the mean value of sperm count in left epididymis of control and P. aeruginosa treated mice at different intervals.



Graphical representation of the mean value of sperm countt in combine (right & left) epididymal sample of both control and P. aeruginosa treated mice at different intervals.

sperm head morphology was simple. The sperm depletion effect for the treatment of the log-culture of P. aeruginosa in mice was positive but its genetic implication was not certain. On the otherhand the genetic involvement for the change in frequency of the sperm head morphology induced by chemical and X-ray was reviewed by Wyrobek (1979). He discussed the problem relating to the morphological factors and genetic factors, and the effect of mutagens in the parent and progeny. He opined in favour of the genetic involvement. A genetic factor affecting sperm morphology in mouse was linked with the X-chromosome (Hugenholtz and Bruce, 1977) beside sex-linked factor, autosomal factors like "T" locus alleles (Bennett, 1975), hopesterile (Johnson and Hunt, 1971), pink-eye sterile (Hunt and Johnson, 1971) etc. were also studied. There was some contradiction (Hillman and Nadijeka, 1978a, b). The genetic factor controlling sperm abnormalities showed recessive pattern of inheritance. The genetic studies have identified about 10 genetic regions affecting sperm morphology which were claimed to be distributed on the X and Y chromosome as well as in autosomes. The role of chromosome abnormalities associated with the sperm head abnormalities in mouse was not very certain (Wyrobek, 1975). Besides the genetic factor, other factors could have influenced on the sperm morphology as the sperms of the 1st ejaculation of a young male mouse had more abnormal head morphology than that of successive one (Krazanowska, 1972). The elevated temperature was also shown to have more abnormal sperm (Van Demark and Free, 1970). It was shown that radiation induced higher frequency of sperm with abnormal head morphology and the maximum effect was noticed at 5th and 6th week after irradiation indicating that early

spermatocytes and late spermatogonia were more sensitive to radiation (Wyrobek, 1977). Similarly, the sperm head abnormalities induced by chemicals have also been reported. Using living mutagens, the elevated frequency of sperm head abnormalities were shown when male mice were treated with the culture of S. aureus, S. lutea and Aspergillus fumigatus etc. in our laboratory.

The present study would also add the effect on sperm head morphology in male mice treated with P. aeruginosa culture.

Method :

The epididymal sperm suspension prepared for the sperm depletion study in the control and Pseudomonas culture treated specimens was also used for the study of the sperm head morphology. Each sample of the control and treated specimens was dropped on separate clean slides. The slides were then rotated to spread the drops uniformly on the surface and dried in air. The sperm smeared slides were then stained in diluted Giemsa (1:15) for one and half hour after which the slides were rinsed in tap water and again dried in air. The slides were then ready for the study of the sperm head morphology. In each specimen of control and treated series, the number of sperms with abnormal head morphology was determined from 600 sperms per individual and the frequency in 18 different intervals between 10 min and 7th week was determined in 3 specimens at each interval.

Results and Comments :

The normal sperm of mouse (PM 34a) has an axe type head with a long tail, which showed various morphological changes (PM 34b-j) more in the treated and few in the normal specimens. The data would show that out of 600 sperms examined per individual, the number with abnormal head morphology was variable among three individuals in a fixation interval (Table 11). The different was not so wide in control series as compared to that of treated specimens (Table 11). Further the average frequency did not differ too much in the control specimens at various intervals while in the treated individuals, the frequency was relatively high with the lapse of time. The increase however, was not very consistent.

If the total number of sperms with abnormal head morphology out of 1800 sperms of three individuals at each interval was considered (Table 12, Graphs 6), in the control line the number ranged between 4 (2nd week) and 60 (7th week) while in the Pseudomonas culture treated individuals, it ranged widely between 22 (10 min) and 504 (6th week) indicating that the treatment induced much higher frequency of sperms with abnormal head morphology. The mean frequency sperms with abnormal head morphology between control and treated line was compared (Table 12). The difference was statistically significant in all the intervals except at 10 min, 8 hr, 144 hr, 3rd wk and 7th wk. If the data for all these intervals were combined, the difference was found also statistically highly significant ($P < 0.001$). Thus it was clearly indicated that the treatment of P. aeruginosa induced higher frequency of sperms with

Explanation of Photomicrographs

Photomicrgaphs showing normal and abnormal sperm head morphology in *P. aeruginosa* treated mice.

PM 34a. Normal sperm head.

PM 34b-j. Different types of abnormal sperm head morphology.

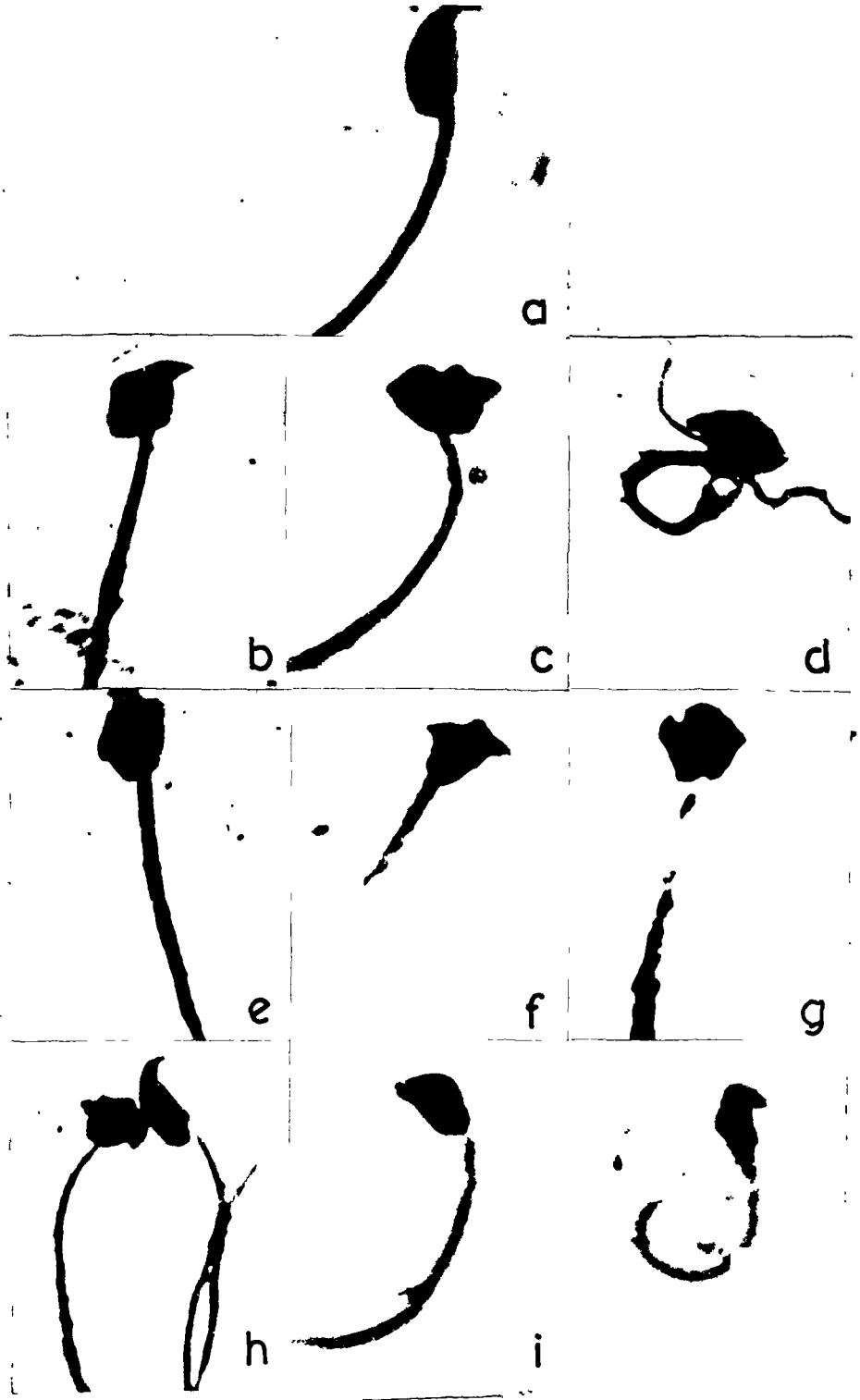

a
b
c
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i

Table 11

Number of sperms with abnormal head in 600 sperms examined in each of 3 specimens at each interval of control and log culture of P. aeruginosa injected male mice.

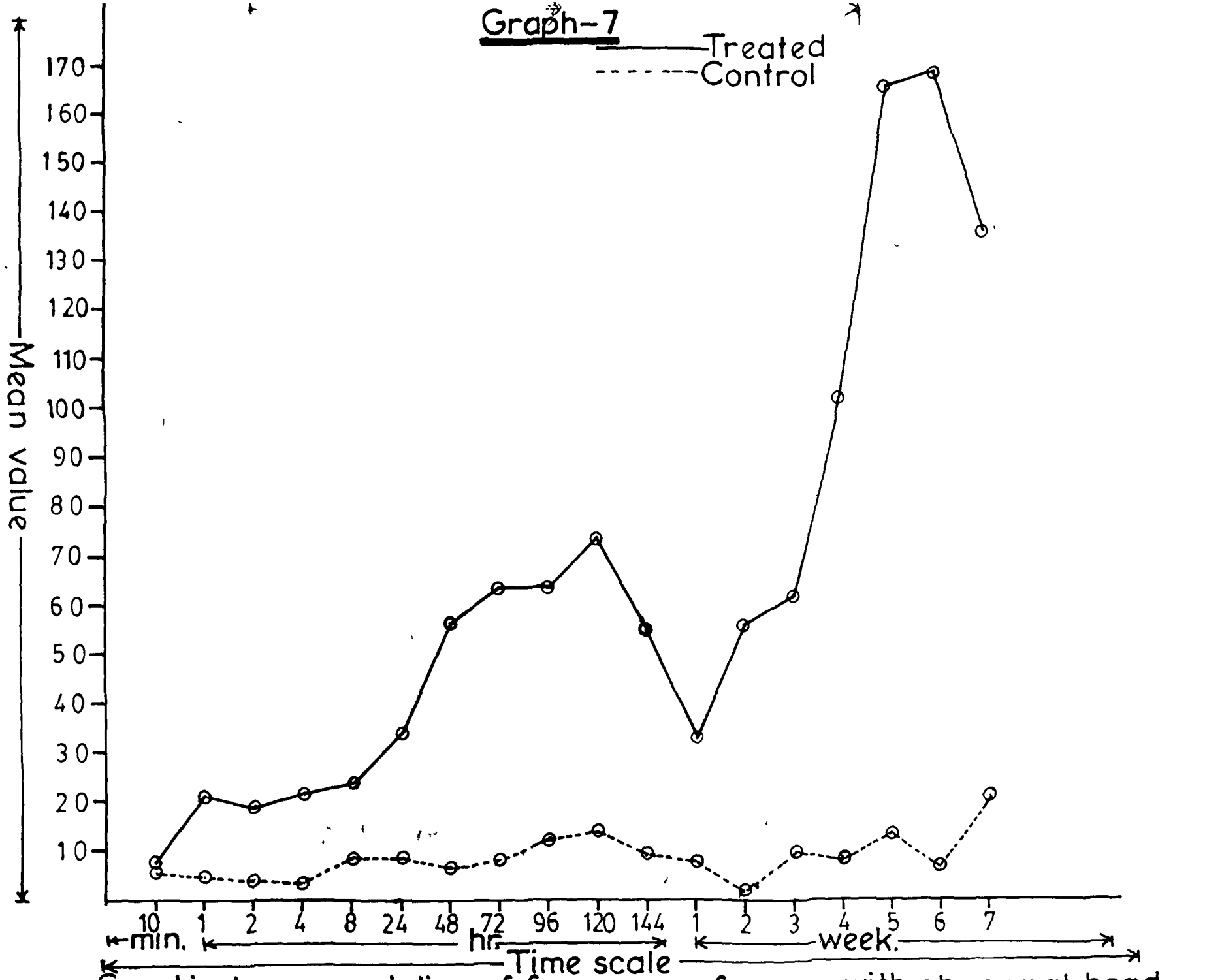
Abnormalities		Abnormalities		Abnormalities	
Treated No.	Control No.	Treated No.	Control No.	Treated No.	Control No.
10 min		48 hr		2nd week	
5	8	53	5	40	0
10	3	59	8	71	2
7	6	56	6	55	2
1 hr		72 hr		3rd week	
19	3	64	6	85	13
23	6	62	10	87	9
21	5	63	8	12	7
2 hr		96 hr		4th week	
18	3	53	18	101	6
20	5	72	6	120	10
19	4	64	12	85	6
4 hr		120 hr		5th week	
17	2	70	9	225	14
20	5	76	18	105	11
28	4	73	14	165	12
8 hr		144 hr		6th week	
16	7	35	7	191	3
31	10	89	11	145	9
24	9	42	9	168	6
24 hr		1st week		7th week	
37	7	43	8	230	20
31	10	22	7	40	20
34	9	33	7	145	20

abnormal head morphology. Further, if the data were considered against the time-table of spermatogenesis and spermiogenesis (Adler, 1982; Oakberg, 1956, 1957), it appeared that all stages e.g. spermatogonia, spermatocytes spermatids and spermatozoa were not equally sensitive to the effect of P. aeruginosa.

Table 12

Frequency of sperm with abnormal head morphology in 1800 sperms of each 3 control and the log culture of *P. aeruginosa* treated mice in Epididymal samples studied at different 18 intervals (control data in brackets)

Fix.	Total No.of abn.sperm head	Mean frequency of abn.sperm head/individual ± S.E.	%	Remarks
10 min	22(17)	7.33 ± 1.44(5.66 ± 1.44)	1.22(0.94)	P> 0.05
1 hr	63(14)	21.00 ± 1.15(4.66 ± 0.87)	3.50(0.77)	P< 0.01
2 hr	57(12)	19.00 ± 0.57(4.00 ± 0.57)	3.16(1.50)	P< 0.01
4 hr	65(13)	21.66 ± 3.27(3.66 ± 0.87)	3.60(0.72)	P< 0.05
8 hr	71(26)	23.66 ± 4.33(8.66 ± 0.87)	3.90(1.44)	P> 0.05
24 hr	102(26)	34.00 ± 1.73(8.66 ± 0.87)	5.66(1.44)	P< 0.01
48 hr	168(21)	56.00 ± 1.73(6.33 ± 0.88)	9.33(1.16)	P< 0.01
72 hr	189(24)	63.00 ± 0.57(8.00 ± 1.15)	10.5 (1.33)	P< 0.001
96 hr	189(36)	63.00 ± 5.50(12.00 ± 3.46)	10.5 (2.00)	P< 0.02
120 hr	219(51)	73.00 ± 1.73(13.66 ± 2.59)	12.16(2.83)	P< 0.01
144 hr	216(27)	55.33 ±16.95(9.00 ± 1.15)	12.00(1.50)	P> 0.05
1st wk	98(22)	32.66 ± 6.06(7.33 ± 0.32)	5.44(1.22)	P< 0.05
2nd wk	166(4)	55.33 ± 8.94(1.33 ± 0.66)	9.22(0.22)	P< 0.02
3rd wk	184(29)	61.33 ±24.67(9.66 ± 1.76)	10.22(1.61)	P> 0.05
4th wk	306(22)	102.00 ±10.11(7.33 ± 1.32)	17.00(1.22)	P< 0.01
5th wk	495(37)	165.00 ±42.06(12.33 ± 0.87)	27.50(2.05)	P< 0.05
6th wk	504(18)	168.00 (±13.27(6.00 ± 1.73)	28.00(1.00)	P< 0.01
7th wk	405(60)	135.00 ±54.84(20.00 ± 0.00)	22.50(3.33)	P> 0.05
Total	3519(459)	64.22 ± 7.33(8.24 ± 0.64)	32.58(4.25)	P< 0.001



Graphical representation of frequency of sperm with abnormal head morphology in control & P. aeruginosa treated mice at different intervals.

C. Histological studies :

It was mentioned in the introductory part that X-ray, chemical agents and living mutagens specially the viruses were found to have not only the cytogenetic effects but also the teratogenic effect studied in various laboratory mammals specially in rats and mice. U.S. food and Drug administration (Tuchmann-Duplessis H., 1972) suggested some protocol for screening the teratogenic drugs. Spyker and Smithberg (1971 and 1972) studied the effect of Methylmercury on prenatal and post-natal development in mice. Fuykta et al. (1979) also made a similar study in mice while Inouye and Murakami (1975) studied the teratogenic effect of orally administered methylmercuric chloride in rats and mice. Harris et al. (1972) studied the effect of the same chemical in golden hamster. On the other hand Fujimoto et al. (1979) studied the protective teratogenic effect of Methylmercuric chloride in mice using Tiopronin. Recently Dennis et al. (1983) evaluated the teratogenic and cytogenetic effect of Methylmercuric chloride. Bal (1984) studied the effect of Methoxychlor (Methoxychlor hydrocarbon pesticide) on reproductive systems of the rat. The teratogenic effect induced by viruses has been studied both in man and mammals by different workers (Greenberg et al., 1957; Holowach et al., 1957; Hill et al., 1958; Manson et al., 1960; Potter, 1961; Corston et al., 1964 etc.). Though the present author concentrated his studies more on the cytogenetic effects caused by the bacterium, P. aeruginosa in treated mice using different testing methods, some attempts have also been made to study the teratogenic effect as well as the histological changes in testis, ovary and uterus etc.

Effect on Testis :

Young male mice were injected with the standard dose of the log-culture of the bacterium, P. aeruginosa and their testes were fixed in Bouin's fixative at the end of the 1st week, 2nd week, 3rd week, 4th week, 5th week, 6th week and 7th week. The testes of nutrient broth injected males fixed at the corresponding intervals served as controls. The histological section of the testis of both control and treated specimens were cut 6 micra thick and the slides were stained with Haematoxilin-Eosin stain and finally mounted in DPX after following the dehydration procedure.

Observations :

Control series :

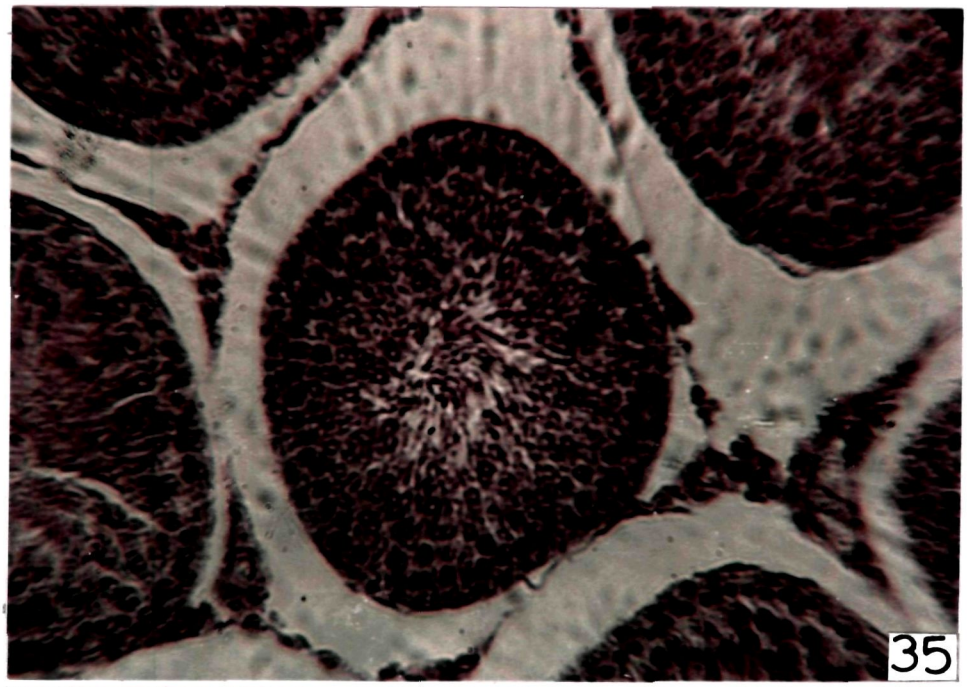
The histological sections of the testis of control specimens fixed at 1st to 7th week did not show much difference from the normal structure (PM 35-36). Thus a general description has been presented. The histological study showed that the epithelium of the seminiferous tubules was regular and continuous having numerous spermatogonial and spermatocytes (PM 35-36) arranged linearly within the wall of the tubules. Within each tubule the lumen occupied considerable area and was filled with spermatozoa. The seminiferous tubules were connected with one another by the cementing material but in some area it was not seen. The matrix in between the seminiferous tubules at the pheripheral part was properly seen and it bounded the seminiferous tubules together. In the central part the binding materials were not clearly detected.

Explanation of Photomicrographs

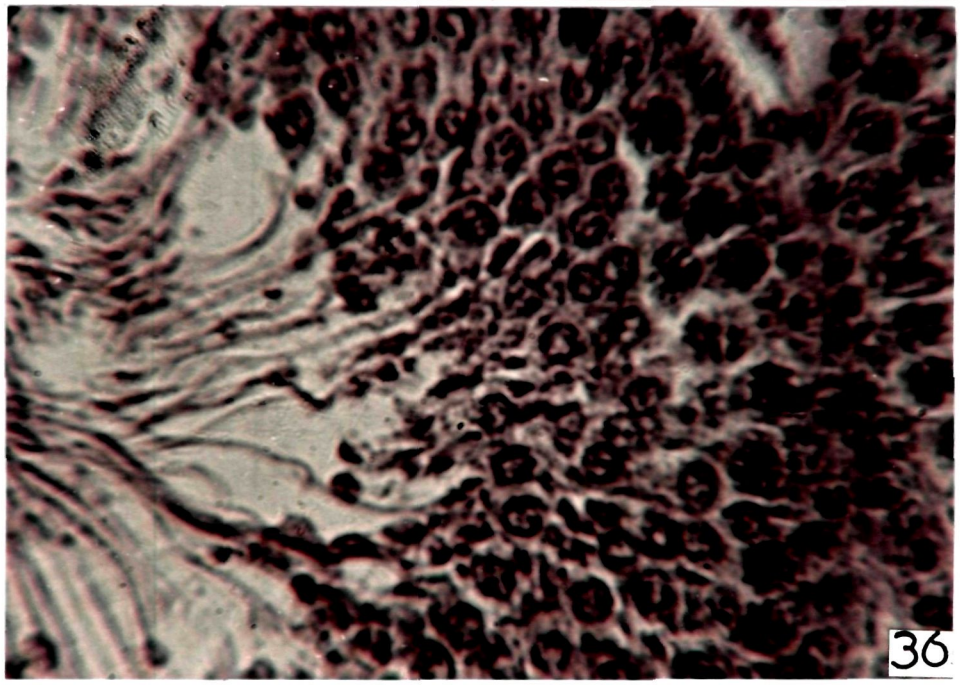
Photomicrograph of T.S. of testis showing different normal structure in nutrient broth (control) treated male mice

PM 35. Normal section of testis (Ca X 280).

PM 36. Magnified view of normal section of testis showing the normal structures of the follicule (Ca X 700).



35



36

Treated series :

The histological sections of testis of treated specimens showed some changes (PM 37-45) as compared to that of the control specimens (PM 35-36).

1st week :

The histological sections of the testis fixed in the 1st week after treatment showed that the epithelium of the seminiferous tubules was wavy and at some places it was discontinuous (PM 37-38). The spermatogonia inside the seminiferous tubules appeared disorganised and not so compact and contained some pycnotic nuclei (PM 39-41). The lumen of the tubules was narrow and in the peripheral part of the testis the seminiferous tubules remained compact and the matrix disintegrated. The central area of the tubule was different from normal and the number of sperms and spermatids in the lumen was scanty. Therefore, the treatment of the log culture of *P. aeruginosa* caused some abnormalities in the testis fixed in the 1st week after the treatment with regard to the orientation and functioning of the seminiferous tubules. The production of sperms appeared depleted and the occurrence of the pycnotic nuclei indicated some degeneration of cells. The general features of the seminiferous tubules appeared different from control. All these indicated the changes at the end of the 1st week after the treatment of the bacterium.

2nd week :

The histological examination of the testis of the treated male fixed at the end of the 2nd week also showed that the seminiferous tubules were thin and wavy (PM 37), the lumen of the tubules was narrow. The tubules were not compact and the cells within the tubule lost organized arrangement (PM 39-42). Many spermatids were with pycnotic nuclei. The tunica albuginea of the testis was not uniformly thick and appeared wavy with some vacuoles (PM 38). Therefore, the effect of the bacterial treatment continued and increased as compared to the section of testis of 1st week.

3rd week :

The effect of the bacterium, P. aeruginosa also continued as revealed from structure of the testis fixed in 3rd week which was indicated by narrow and sometimes blocked the lumen (PM 39) of the seminiferous tubules. The spermatogonia and spermatids were scattered and scanty and the dividing cells were not seen. Many vacuolations were observed within the tubules and within the cell lining (PM 39-41 and 45). Many spermatids showed pycnotic nuclei. Some tubules were empty situated in the periphery and also in the center. The tunica albuginea of the testis showed wavy outline. Therefore, in the 3rd week the effect was more than that of earlier weeks.

4th week :

The histological examination of the testis fixed in the 4th week after treatment revealed that the epithelium of the seminiferous tubules remained undulated with the lumen showing more space and containing dead spermatids and fragmented cells (PM 44). The spermatocyte cells were not compact. The cells in division were very rare and the seminiferous tubules near the epithelium of the testis were disorganised and lumen of the tubules were narrow (PM 42). The tunica albuginea remained undulated. Therefore, in the 4th week, many abnormalities like narrow lumen, absence of dividing cells, occurrence of dead cells indicated clearly that the effect of the bacterium was still there.

5th week :

The histological structure of the testis of treated male fixed in the 5th week after treatment also maintained the abnormalities found in the section of the 4th week showing undulating epithelium of the tubules. The lumen in the tubules of the central part was normal but in the peripheral part, it was still narrow. Spermatids were found within the lumen of the tubules and many of them were pycnotic. Some dividing cells were present. The covering of the testis appeared wavy. Therefore, the section showed some part regaining normal histology, witnessed for the occurrence of the number of spermatids within the lumen. However, the presence of pycnotic cells and narrowness of the lumen at the peripheral side indicated the effect was still there but in low tone.

6th week :

The histological section of the testis of the treated male fixed on 6th week after the treatment showed more regularization and recovery i.e. the effect of the bacterium was reduced. The epithelium of the tubule was regular and normal. The spermatids within the lumen were many. Some dividing cells were present and blood vessels appeared within the matrix. The tubules were compact in appearance. However, the occurrence of lesser number of dividing cells in the tubules and weak pattern of their orientation would indicate that the effects of the bacterium were still persisting to some extent.

7th week :

The histological section of testis of the treated male fixed on 7th week indicated more normal appearance than that of 6th week. This was indicated by the appearance of the epithelium of the tubules mostly normal, lumen large with many spermatids. Spermatogonial and spermatocyte cells arranged in the normal fashion and so on. However, there were many dividing cells but some were with pycnotic nuclei. The tubules of the peripheral area showed abnormalities by their irregular outlines and some cells with vacuoles (PM 43). Therefore, with the lapse of time the effect of the treatment of the bacterial culture was practically removed with little effect left and the tissue structures appeared mostly in normal condition.

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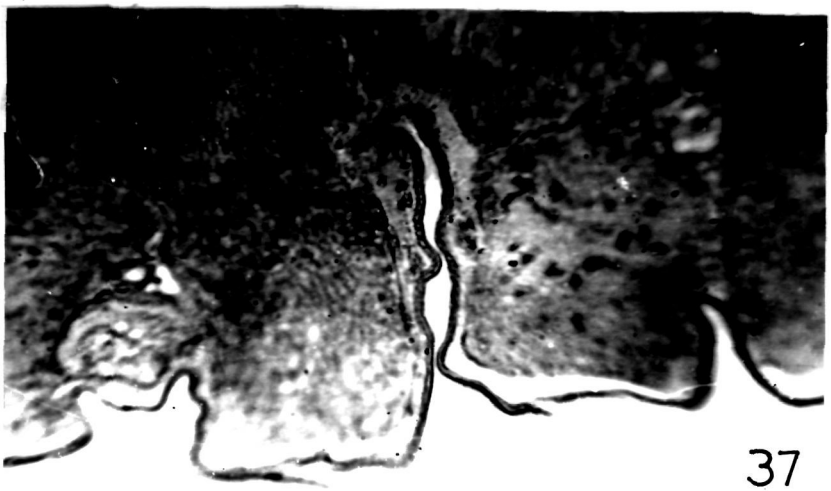
Explanation of Photomicrographs

T.S. of testes showing different abnormalities in log culture of bacterium, P. aeruginosa treated male mice.

PM 37. Epithelium (Tunica albuginea) is thin and wavy (X 70).

PM 38. Epithelium (Tunica albuginea) is not uniform and with some vacuoles (X 70).

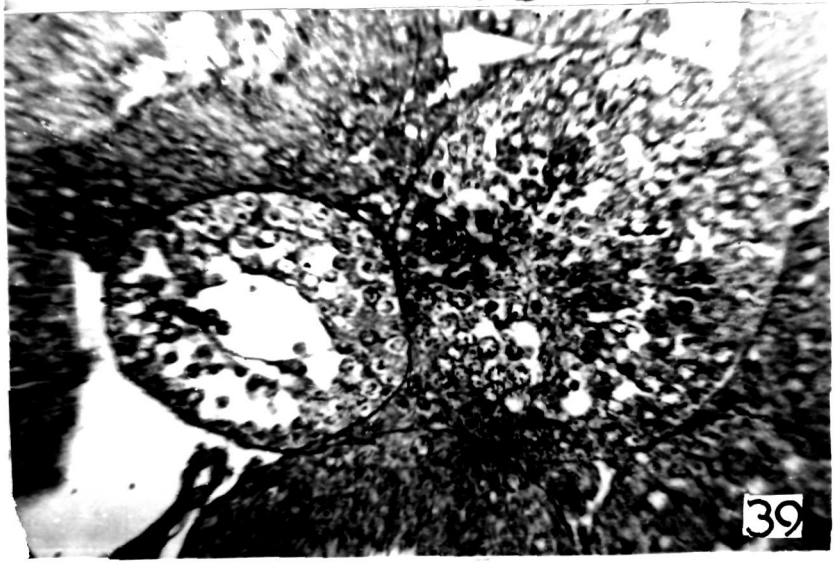
PM 39. One tubules without lumen and some vacuoles. Other tubules with lumen but without spermatozoa and the cells within the tubules are not linearly arranged with many vacuoles (Ca. X 70).



37



38



39

Explanation of Photomicrograph

T.S. of testis showing different abnormalities in log culture of bacterium, P. aeruginosa treated male mice

PM 40. Tubules with many vacuoles and lumen without spermatozoa (X 280.)

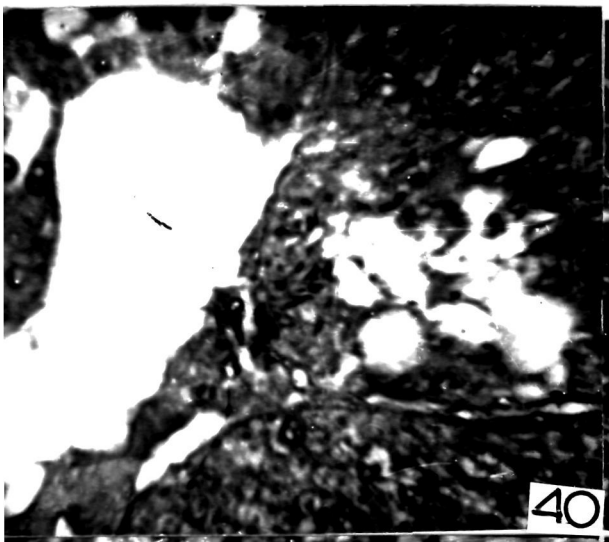
PM 41. Cells within the tubules are not linearly arranged large vacuolation and tubules without spermatozoa.

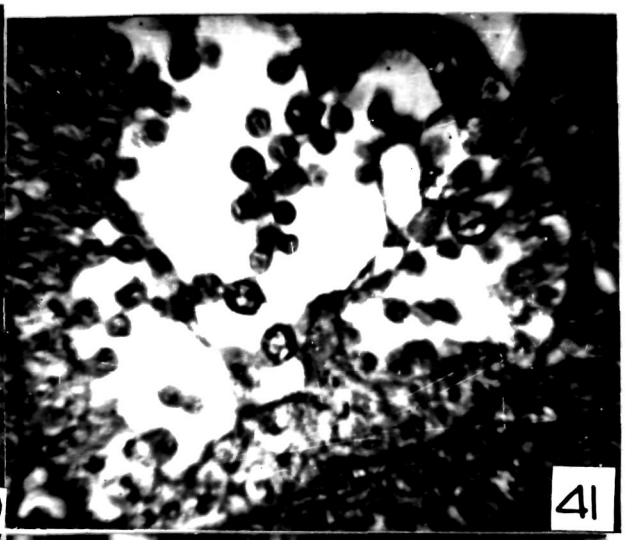
PM 42. Cells of the tubules are disorganised; no dividing cells in the tubules and the lumen of the tubules without spermatozoa (X 700).

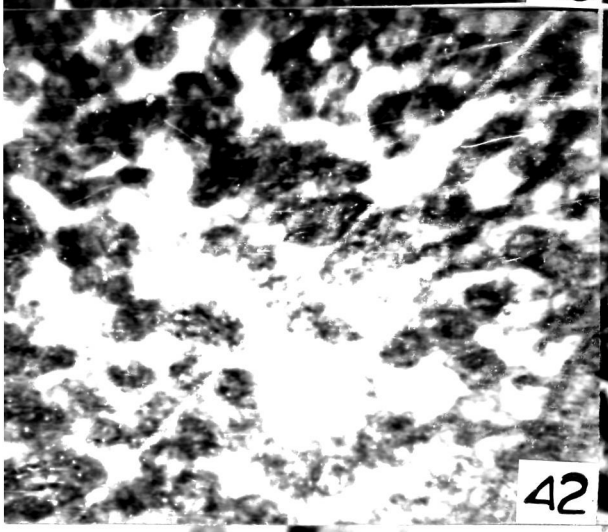
PM 43. No dividing cells within the tubules and some cells are vacuolated. Lumen without spermatozoa (X 700).

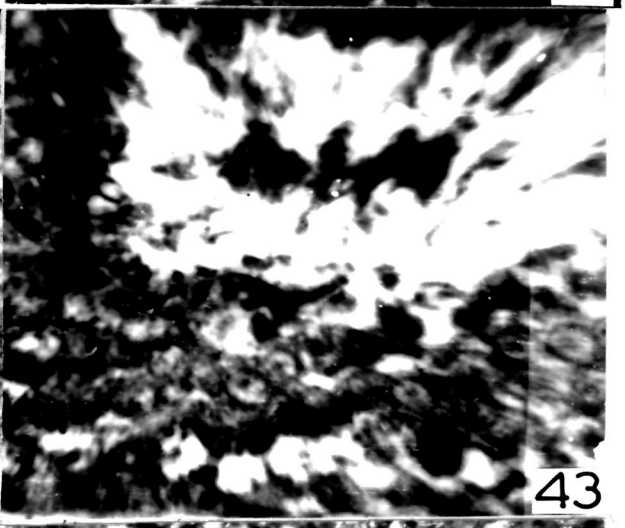
PM 44. No dividing cells within the tubules and large lumen with few spermatozoa.

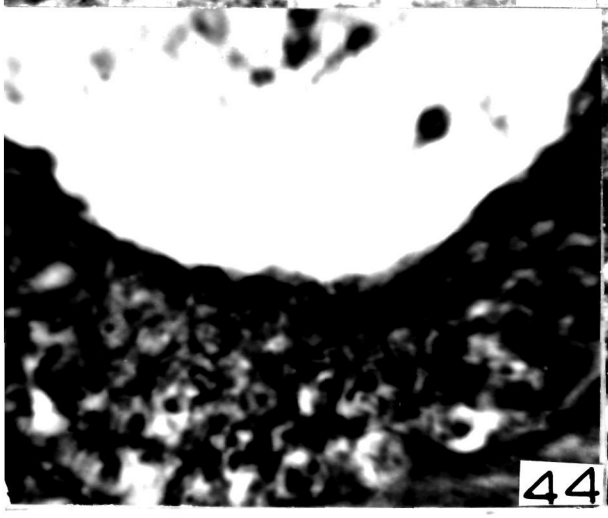
PM 45. No dividing cells within the tubules and many vacuolation within the cell lining.

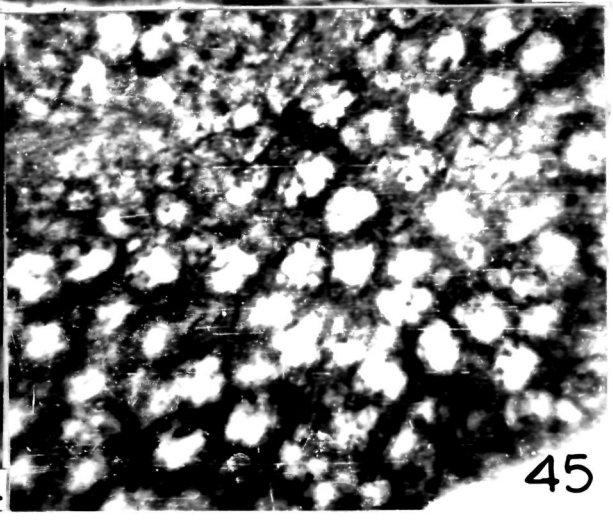

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Remarks :

The agent responsible for bringing the change in the histological sturcture of the testis is yet to be known very definitely but in all probability it could be the toxin liberated by the bacteria. Anyhow, due to the effects of the bacterial agent the luman became narrow, the production of the sperm was depleted, cell division was retarded and some cell death took place. These effects were increased for few weeks after treatment but it then decreased with the lapse of time after treatment. However, similar studies carried out in F_1 and F_2 generation showed some effect (vide infra) which might suggest some form of genetical involvements. The changes found in the structures of the testis in treated males definitely had an effect on the fertility of the males because in our study of the lethal effect we failed to obtain a good number of fertilized females when Pseudomonas treated males were mated to those virgin females in different weeks (vide infra).

Effect on ovary :

Since the teratogenic effect concerns more with the prenatal and post-natal manifestations of some inducing agents, the effect of P. aeruginosa on ovary of treated female mice was also examined histologically.

The young female mice were injected intraperitoneally with the standard dose of the log-culture of P. aeruginosa (1 ml/100 gm body weight) against nutrient broth injected controls. The ovaries of the control and treated specimens were fixed separately in Bouin's solution. The

tissues were processed and 6 micra thick microtome sections were cut and fixed on slides. The sectioned slides were stained in Haematoxiline-Eosin and mounted permanently in DPX after following the standard dehydration procedure.

Observations

Control series :

The histological sections of the ovary of the control mice fixed in different weeks did not show much difference (PM 46) from that of normal individual. The section showed that the ovarian epithelium was continuous, the eggs were at different stages of maturation, the follicular and nurse cells were compact around the ovum. The nucleus was large and prominent, the connective tissue was compact, blood vessels filled with large number of corpuscles. Since in the histological section of the ovary of control specimens fixed in different weeks did not show much difference, their structures have been described only once here.

Treated series :

1st week :

The histological sections of the ovary of treated females fixed at the end of the 1st week after the treatment showed that the ovarian epithelium was continuous with some slightly disrupted area having a number of vacuoles (PM 47). The number of mature ova was not many, some showing disorganised nucleus (PM 47) and the cytoplasm with

Explanation of Photomicrograph

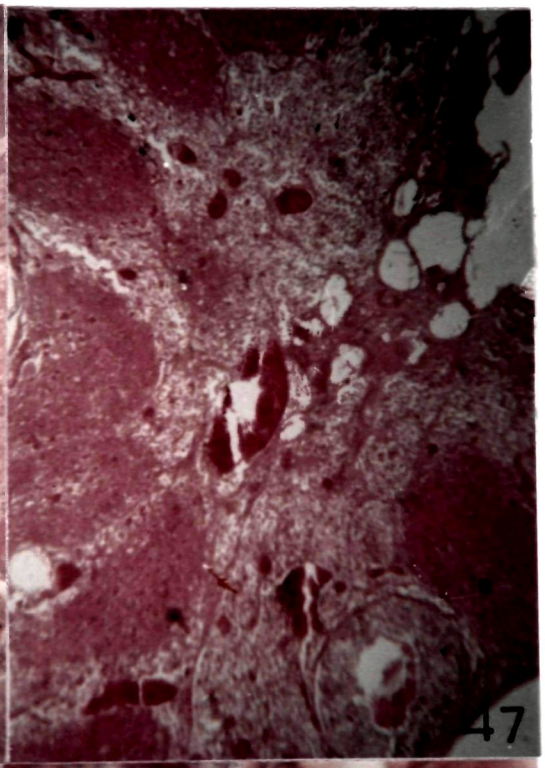
Photomicrograph of T.S. of ovary showing different abnormalities in log culture of *P. aeruginosa* treated female mice.

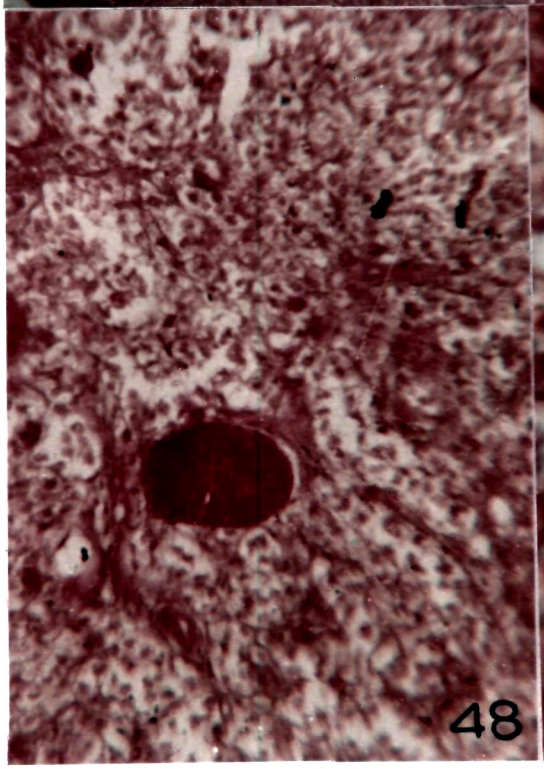
PM 46. A normal section of the ovary (X 70).

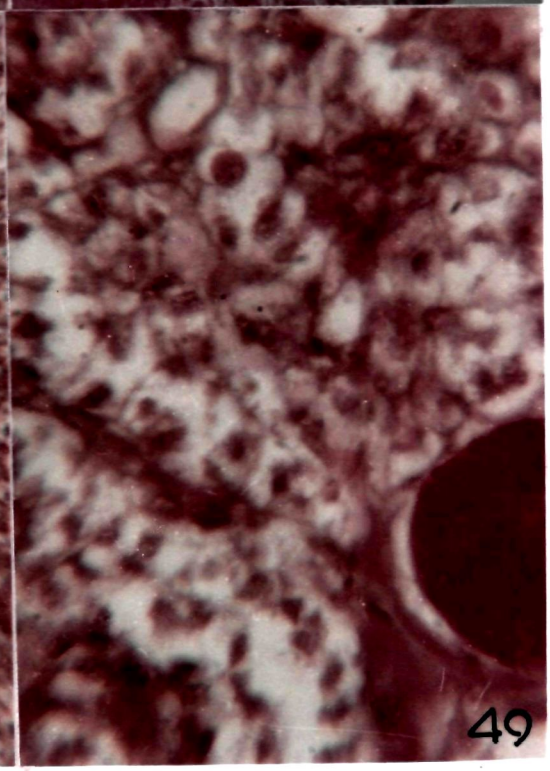
PM 47. Many vacuoles within the ovary (X 70).

PM 48, 49. Degenerating cells with many vacuoles and necrotic cells (X 140 and & 700).


46


47


48


49

vacuolation (PM 48-49 and 51). The nurse cells and the follicular cells showed loose arrangement (PM 58), connective tissue disorganised with a number of vacuoles and blood vessels were not very many. Some cytolysed cells with pycnotic nuclei were seen in the connective tissue, follicular cells and nurse cells. Therefore, the treatment of the bacterial culture had its effect on the ovarian structure within a week as found in the testes of male. In female, the treatment interrupted the maturation of ovum along with the disintegration in different areas of the ovary like connective tissue, blood vessles etc. and there were indications of good number of cell death.

2nd week :

In the 2nd week after the treatment, the ovary of the treated females showed more or less the same types of effect. There were thin epithelium with disrupted areas, vacuolation, less number of mature eggs with reduced diameter, follicular and nurse cells scanty leaving empty space and vacuolation, connective tissue disorganised and appearance of many vacuoles. Some dead and pycnotic cells were found in connective tissue and blood vessels underwent further reduction with less number of corpuscles. Therefore, in the 2nd week after the treatment of the bacterium the ovarian structures showed more pronounced effect than that of 1st week.

3rd week :

The histological sections of the ovary of the Pseudomonas treated female fixed on the 3rd week showed that

the effect was retained. The epithelium was thin and vacuolated in many areas, maturing follicules were not very many, cytoplasm of some of them was vacuolated and granular, the nucleus was disorganised, large perevitelline space appeared within and the follicular cells (PM 48, 49 and 51,52). The nurse cells were not compact (PM 58). The vacuolation was observed in the connective tissue which was loose and disorganized. The blood vessels were less in number. Some were very thin and contained cytolised R.B.C. Therefore, the histological section of the ovary of 3rd week showed that the effect was more related to maturing eggs and the connective tissue which were likely to affect the fertilization.

4th week :

The histological sections of the ovary of Pseudomonas treated females fixed on 4th week revealed that the effect was waning though not completely free. The epithelium appeared more or less like that of control ovary. The number of maturing egg was on the increase but the nucleus of some secondary and maturing ovum was disorganised with large pervitelline space (PM 52-54 and 56-63). The follicular cells and nurse cells around the ovum maintained a disorganised and loose arrangement. The connective tissue enclosed some empty spaces. The blood vessels in the connective tissue showed more or less normal pattern but some of them were broken and cytolised R.B.C. were formed. Therefore, the histological picture revealed that the maximum effect was reached on 3rd week after treatment which was reduced to some extent in the 4th week.

5th week :

The histological section of the ovary of Pseudomonas treated females examined on the 5th week after treatment showed further recovery of the effect of the bacterium which was indicated by the presence of continuous ovarian epithelium, the organised cells, eggs showing maturation though the nucleus of some eggs was showing changes and the perivitelline space was large and prominent (PM 52-54 and 56-62). The follicular and nurse cells around maturing eggs were organised though occasionally empty spaces were still present. The connective itssue showed some vacuolation though mostly it was like that of the control. The blood vessels contained good number of corpuscles therefore, the effect of the bacterial toxin was reduced in comparison to that of 4th week.

6th week :

The histological sections of the ovary of Pseudomonas treated females in the 6th week appeared more like normal except for the occurrence of the large peritivelline space, disorganised nucleus (PM 52-54 and 56-63) and also slightly disorganised nurse and follicular cells. Therefore, the effect of the bacteria was further reduced as compared to that of 5th week.

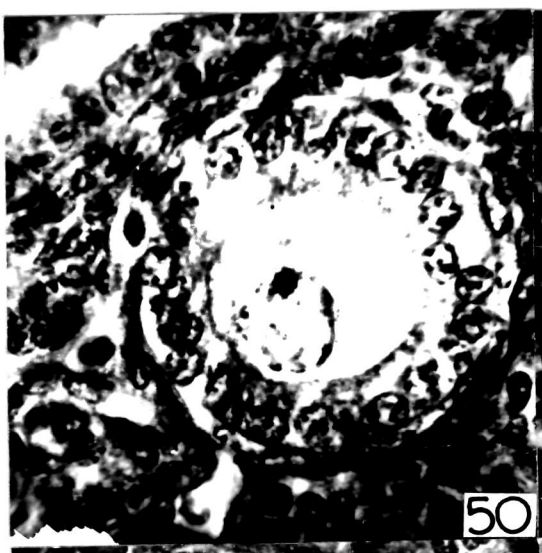
7th week :

The histological section of the ovary of Pseudomonas treated females fixed on 7th week after treatment showed that it was almost similar to that of control but some

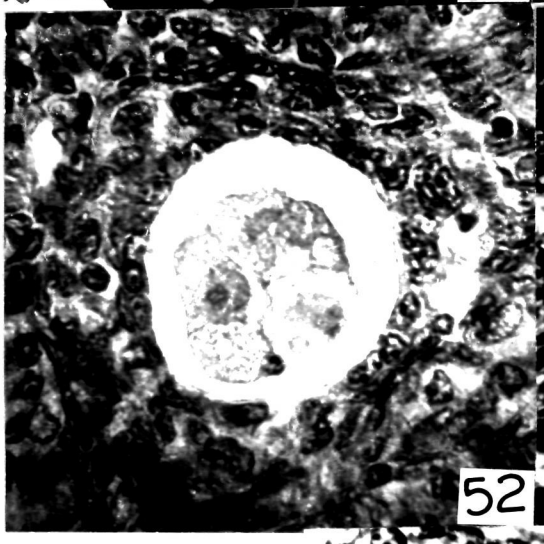
Explanation of Photomicrograph

Photomicrograph of T.S. of ovary showing secondary follicules with different abnormalities in log culture of *P. aeruginosa* treated female.

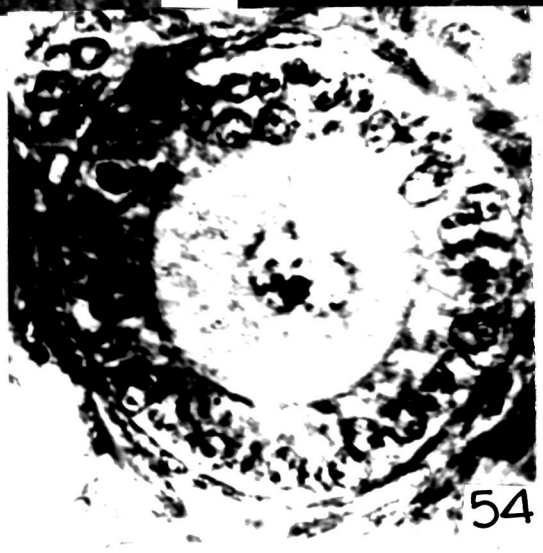
PM 50. A normal secondary follicle.
PM.51. Vacuolation within the cytoplasm.
PM 52. Large perivitelline space with granular cytoplasm.
PM 53. Two nucleoli within a nucleus.
PM 54. Fragmented nucleoli within a nucleus.


50


51


52


53


54

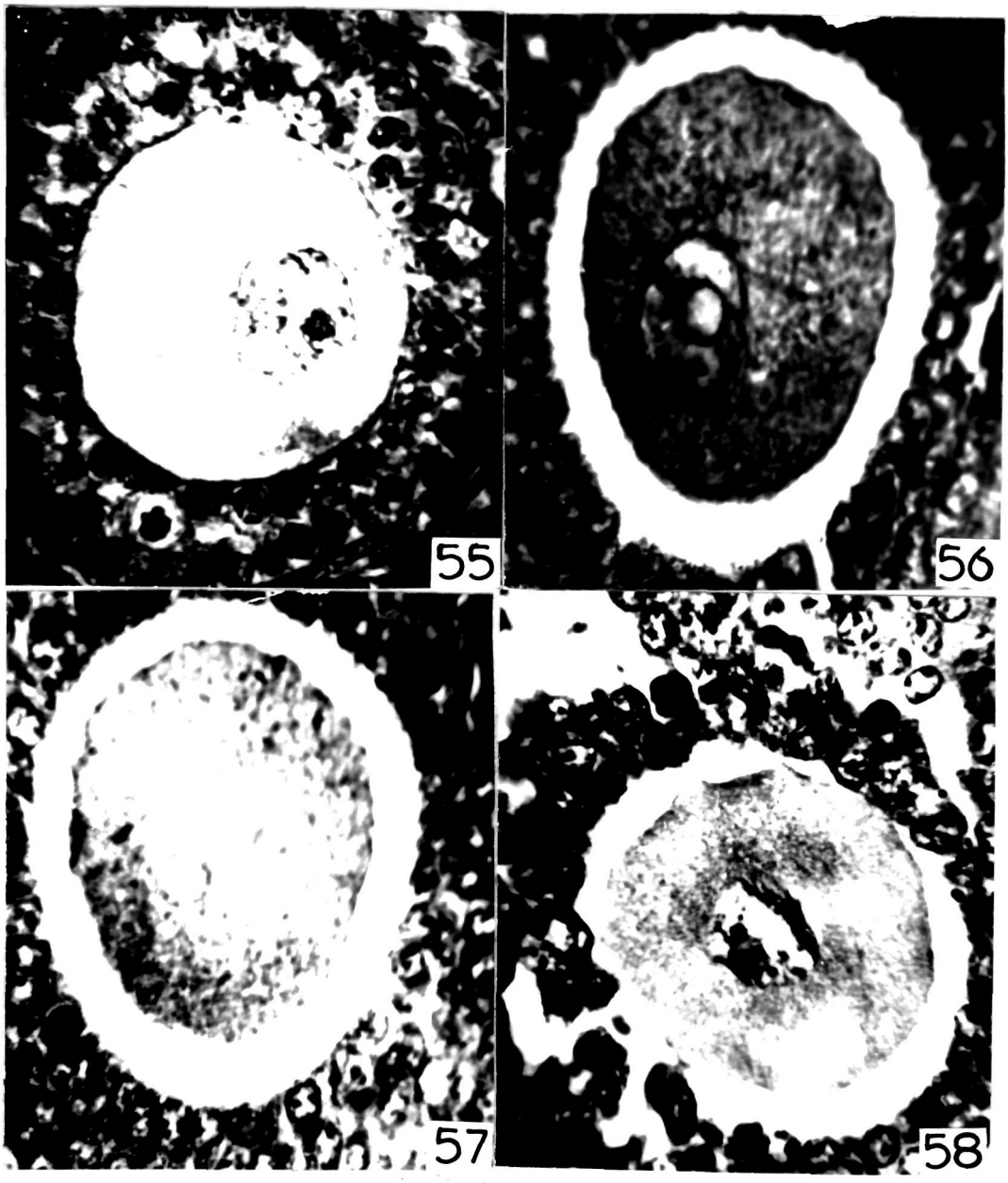
Explanation of Photomicrograph

Photomicrograph of T.S. of ovary showing different types of abnormalities in maturing/graafian follicle in log culture of *P. aeruginosa* treated female mice.

PM 55. Normal maturing follicule (X 700).

PM 56, 57. Graafian follicles showing large perivitelline space (X 700).

PM 58. Maturing follicle showing degenerative nucleolus and disrupted follicular cell lining.


55
56
57
58

Explanation of Photomicrograph

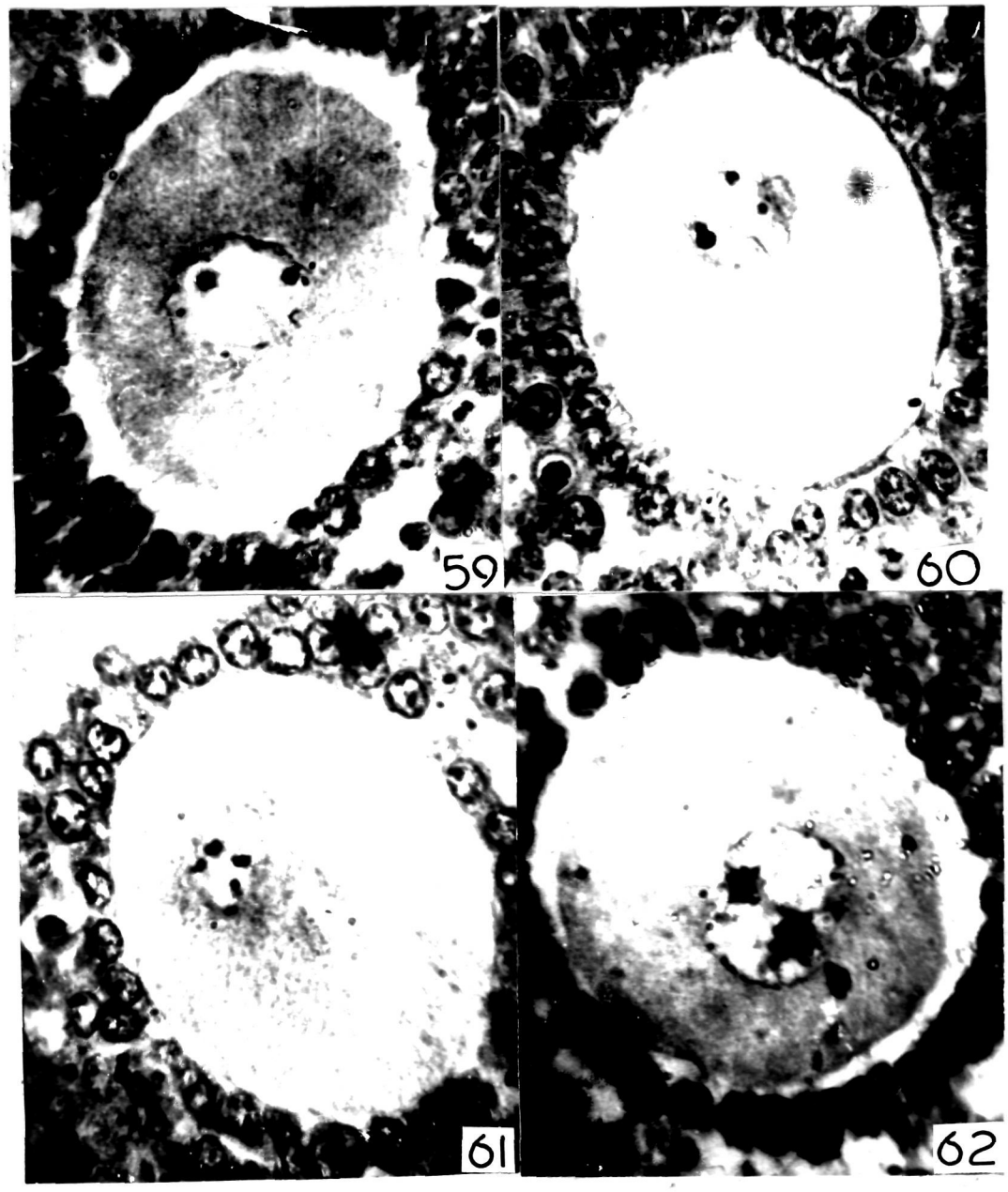
Photomicrographs of T.S. of ovary showing different types of abnormalities in maturing or Graafian follicles in log-culture of bacterium, P. aeruginosa treated female mice.

PM 59. A graafian follicle showing two prominent micro-nucleoli (X 700).

PM 60. A Graafian follicle showing three prominent micro-nucleoli (X 700).

PM 61. A Graafian follicle showing four prominent micro-nuceloli (X 700).

PM 62. A Graafian follicle showing two large nucleoli within a nucleus.


59
60
61
62

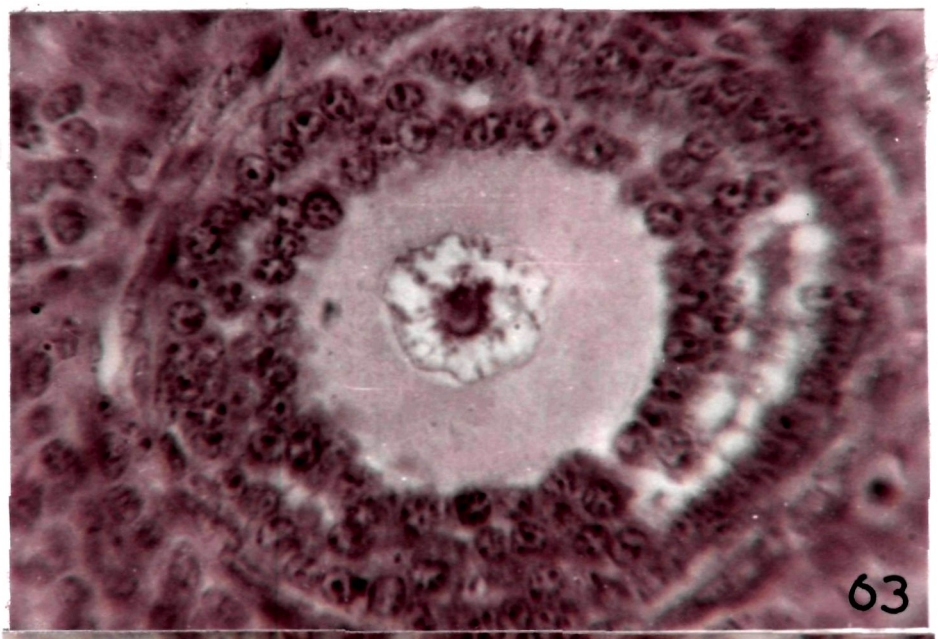
Explanation of Photomicrograph

Photomicrograph of T.S. of ovary showing different types of abnormalities in maturing follicles in log-culture of bacterium, P. aeruginosa treated female mice.

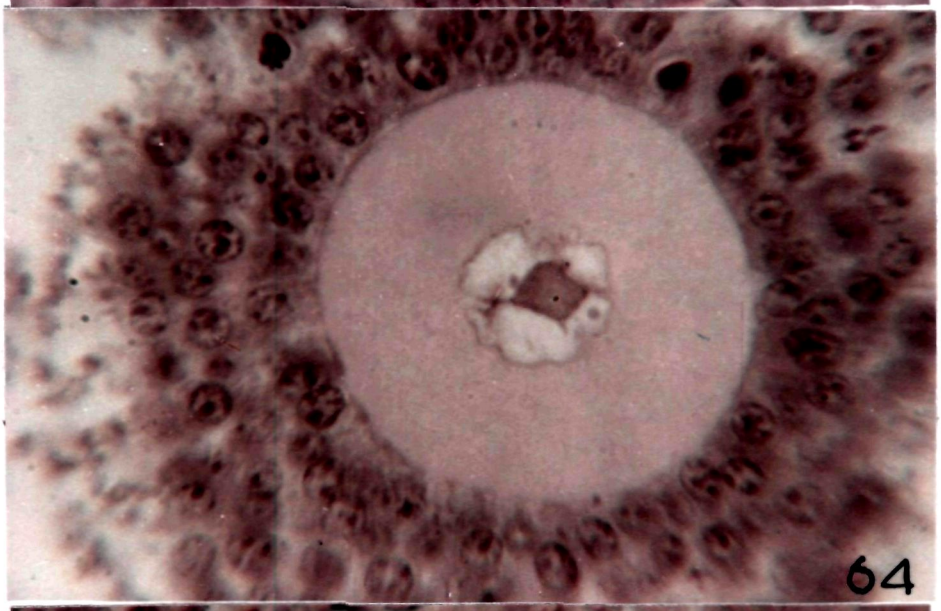
PM 63. Maturing follicle showing degenerative nucleolus.

PM 64. Graafian follicle showing a large nucleolus and micronucleolus.

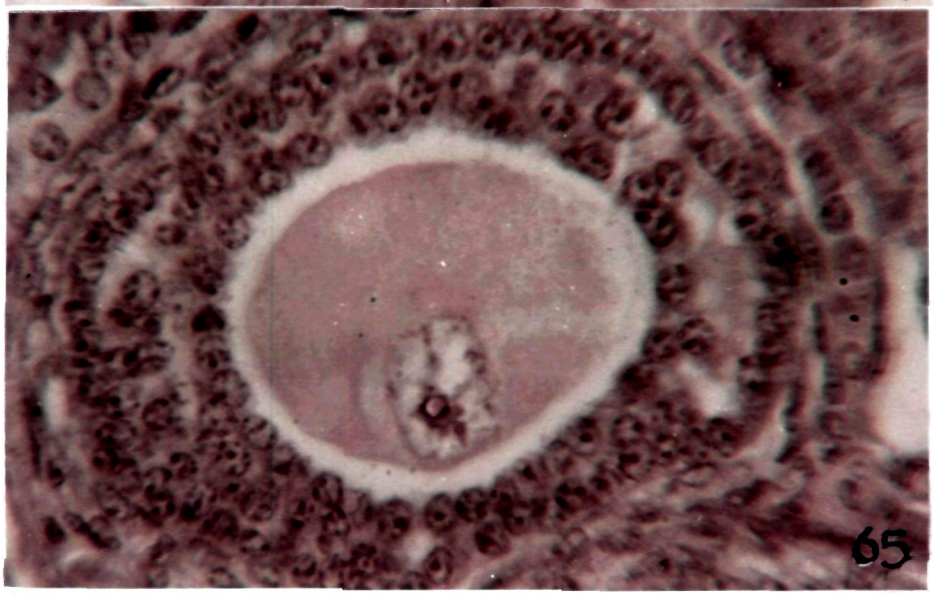
PM 65. A secondary follicle showing degenerative nucleolus, With a large perivitelline space.



63



64



65

effect was seen to be restricted within the nucleus and perivilelline space in some eggs. Therefore, on the 7th week after treatment of the bacterial agent had practically no effect except in some nuclei.

15th week :

With a view to finding out the duration of the histological changes after the treatment of the bacterium, Pseudomonas, the ovary of some treated females was fixed at 15th week after the treatment, sectioned and stained. The effect in 15th week was very negligible as on 7th week. The histological structure of the ovary of the treated female in 15th week was very much like that of control.

Remarks :

The examination of the sections of ovary of the nutrient broth showed no effect on the histological structure while the treatment of log-culture of P. aeruginosa had some effect beginning from 1st week which reached the maximum in 3rd week after the treatment beyond which the effect dwindled gradually. By the 7th week it was practically normal except in nucleus and the perivilelline space. The causative factor in bringing changes to the histological structure of the ovary in treated females was not precisely known. It could possibly the bacterial toxin. The response was to some extent similar to that of testis of treated males indicating the peak effect reached after treatment at about 3 to 4 week and then it declined gradually. The occurrence of some abnormalities found in the section of ovaries of females of F_1 and F_2 generation in treated lines

would perhaps suggest that bacterial product was genotoxic and not only the toxic one.

Effect on uterus :

The histological changes have been considered as a protocol for testing mutagenic property of an agent (Tuchmann-Duplessis, H., 1972). Recently Bal (1984) showed the histological changes in the reproductive system of rat after treating them with the pesticide, Methoxychlor. In our study the reproductive potentiality in the treated specimens was greatly reduced. We not only studied the histological changes in the primary glands like testis and ovary presented before but also the changes in the uterus, since this structure is important in mammalian reproduction and development of embryos.

Method :

While fixing the ovary, the uterus of the same female which was injected with nutrient broth as control and log-culture of Pseudomonas as treated one was removed separately from 1st week to 7th week. The uterus was fixed in Bouin's solution and transverse sections were cut at 6 micra thick and the sections were stained in Haematoxiline-Eosin stain and mounted in DPX.

Observations :

Control :

The section of the uterus of the nutrient broth

injected female as control did not show much difference with that of normal in the tissue fixed at different weeks. The general histological structure showed that lumen was small, regular and the epithelium was continuous with no villi projected into the lumen. The connective tissue was compact with large number of blood vessels; fibroblasts were many and compact towards the muscular zone. The muscular coat was thick and continuous (PM 66,67).

Treated series :

1st week :

The histological section of uterus of P. aeruginosa treated female showed that the lumen was narrow, the number of villi increased and some of them branched into a number of sub-villi (PM 68), the uterine epithelium was vacuolated, the connective tissue was weak with a number of vacuoles, the number of fibroblasts and blood vessels was also decreased and a number of vacuoles was observed in the connective tissue area (PM 70, 72). The muscular coat showing muscular bundles displaced (PM 73). Therefore, like the ovary, the uterine structures also showed histological changes for the treatment of the bacterium even on the 1st week.

2nd week :

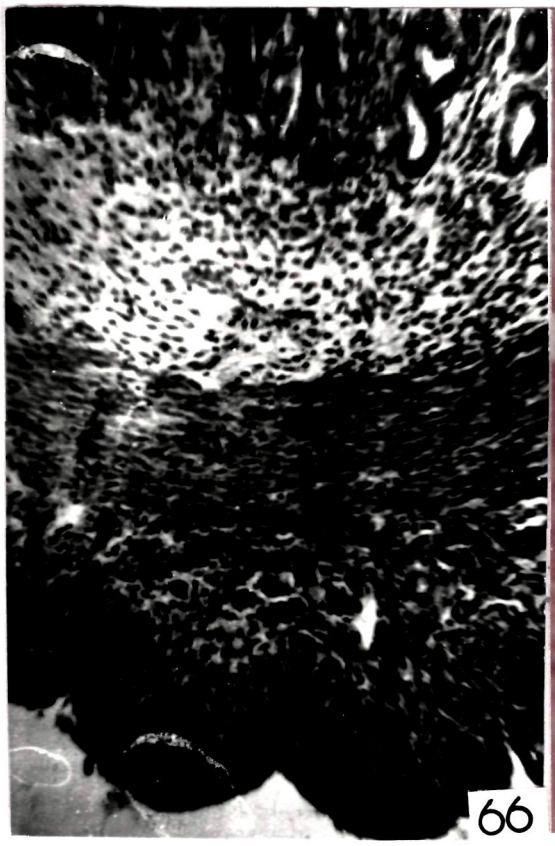
The histological section of the uterus in 2nd week after treatment showed that the uterine epithelium was thin, villi were further vacuolated (PM 69), the number of blood vessels and the connective tissue was further disorganised showing vacuolation (PM 73),the muscular coat though appeared normal like

Explanation of Photomicrograph

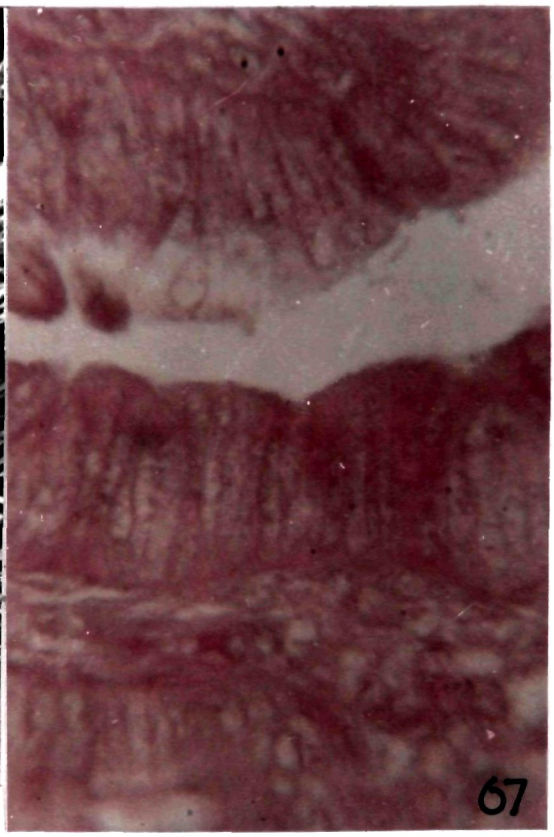
T.S. of uterus showing normal structure of the uterus in control females.

PM 66. Normal structure of uterus (X 280).

PM 67. Lumen of uterus showing normal structure of the epithelial layer (X 700).



66



67

that of 1st week but the tissue was disorganised and lost the compactness (PM:72). Therefore, the bacterial product had its effect more in the 2nd week which was on the increase.

3rd week

The histological sections of the uterus after 3rd week of treatment showed that the effect in some structures was on the increase. The thin villi projected into lumen and the number of vacuoles in the uterine epithelium were decreased. On the other hand the connective tissue was continuous, the number of blood vessels was increased, but blood vessels and the organisation of muscular layer were not normal (PM:71). Therefore, the effect of the bacteria in the uterus was of mixed nature, increased in some structures and reduced in others.

4th week :

The histological section of the uterus of the treated females fixed in the 4th week continued to show some mixed effects as the villi were reducing the passage of the lumen of the uterus, the epithelium was continuous. The number of vacuoles was decreased but the number of blood vessels was increased, the connective tissue showing some vacuolation, the muscular layer appeared more or less normal. Therefore, from the 4th week some structures were reverting to normal appearance and some structure showed the continued effect.

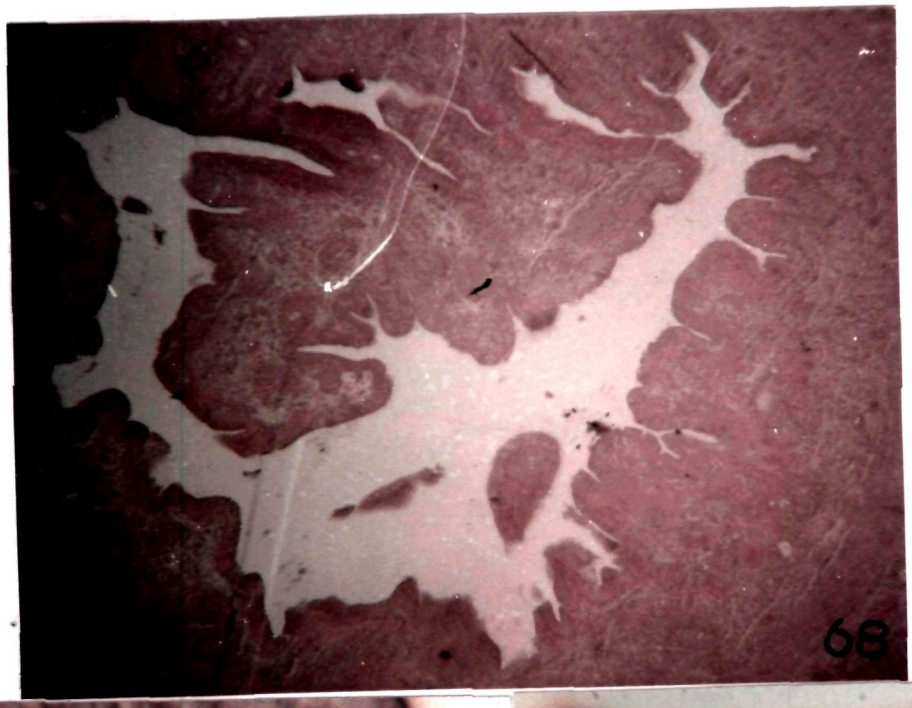
Explanation of Photomicrographs

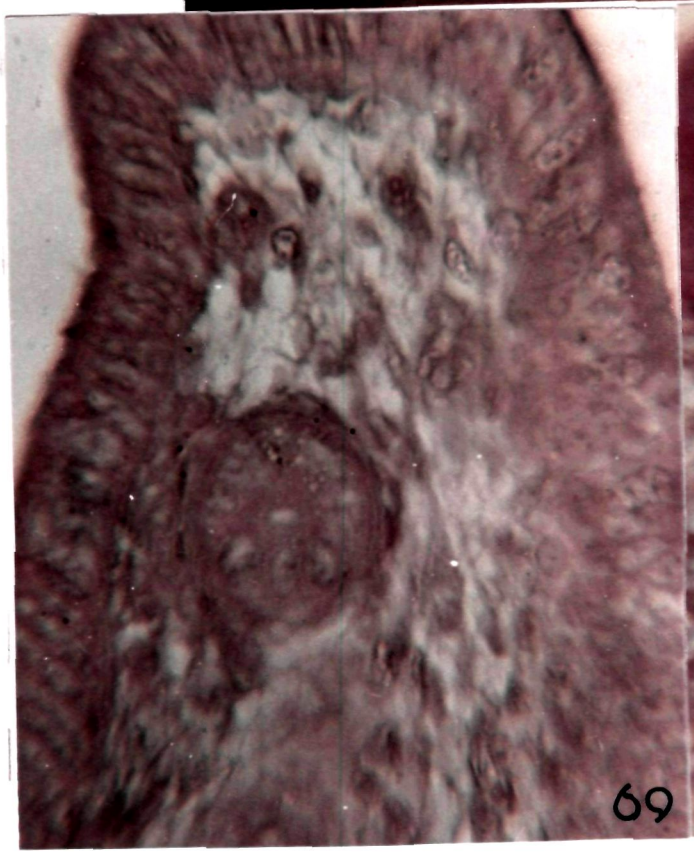
T.S. of uterus showing different abnormalities in log culture of *P. aeruginosa* treated female mice

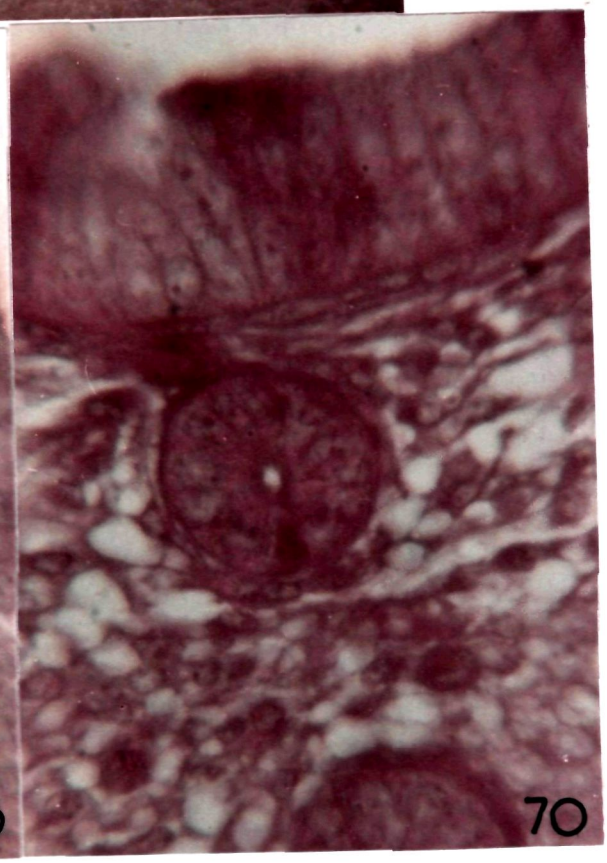
PM 68. Lumen showing highly wavy structure of the epithelium (X 70).

PM 69. A villi of a lumen showing vacuolation and cells discentigration (X 700).

PM 70. Many vacuolation within the endometrium.


68


69


70

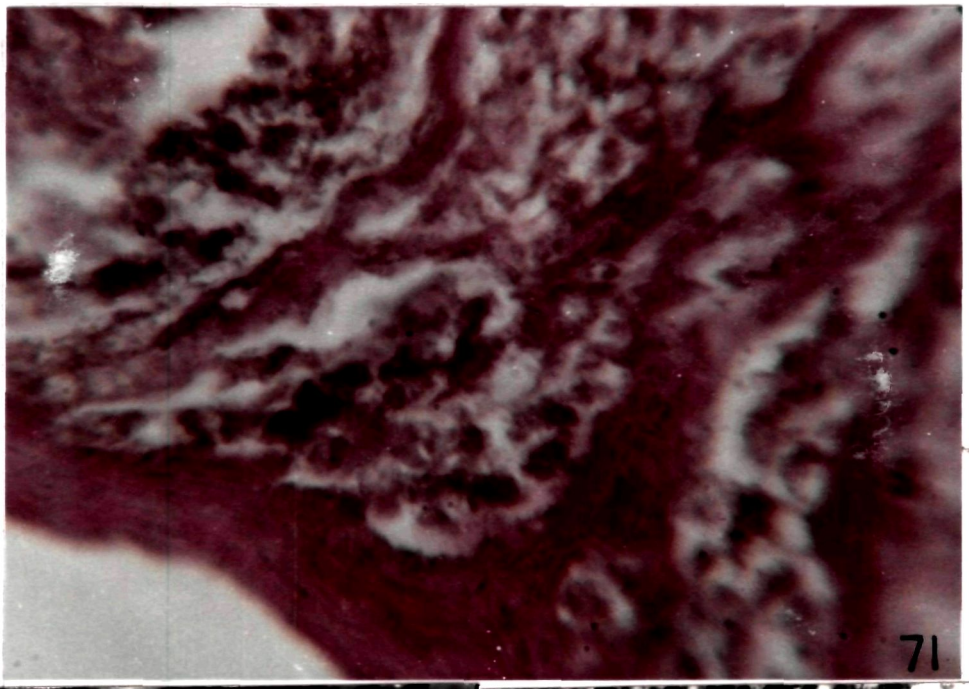
Explanation of Photomicrograph

T.S. of uterus showing different abnormalities in log culture of *P. aeruginosa* treated female.

PM 71. Disorganized cellular arrangement and necrotic cells in perimetrium layer.

PM 72. Large vacuolation within the endometrium and perimetrium layer.

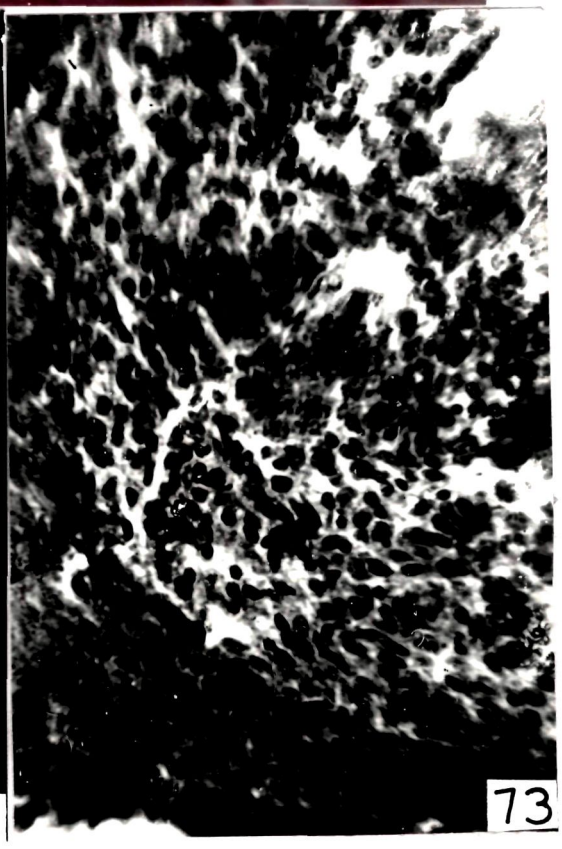
PM 73. Many necrotic cells with disorganized cellular layer in perimetrium.



71



72



73

5th and 6th weeks :

The examination of the histological section of uterus of the treated specimens of 5th and 6th week did not show much difference from that of 4th week. The tendency of the structures recovering to normal pattern was continued.

7th week :

The histological section of uterus in 7th week showed very little difference from that of control as the uterine lumen became narrow, the villi were many with good number of blood vessels, while the muscular layer remained a little disorganised.

15th week :

With a view to finding out even if the slightest effect was continued for a long time, the histological sections of the uterus of the females after 15 week of treatment were examined which appeared very much like normal indicated by the number of villi decreased, vacuoles were reduced, connective tissue and blood vessels appearing normal and the muscular layer was continuous.

Remarks :

The present study showed that bacterial culture had detrimental effect on the uterus causing changes in the epithelium as the target. The cells were vacuolated and the

epithelia were projected the lumen along with certain other changes but this effect did not last long. By the fifth week most of the structures appeared normal. Therefore, the log culture of *Pseudomonas* appeared to be toxic if not genotoxic to testis, ovary and uterus.

General comments on Chapter I :

The chromosome breaking effect of the log culture, isolated bacteria suspended in saline, and the log culture filtrate of *P. aeruginosa* in bone marrow cells of treated mice fixed at various intervals were shown earlier from our laboratory (Dey, 1980). The present author therefore, not only checked if the results were repeatable by examining the bone marrow chromosome aberrations of mice treated with the log culture of *P. aeruginosa* against nutrient broth injected control but also extended the study which confirmed the earlier observation with additional data. The present study added that the effect could continue for a long time. The other purpose of examining the bone marrow chromosome aberrations in treated specimens from 1st week to 7th week was to see if the treated males which were used in mating programme for lethal test (*vide infra*) had aberrations in somatic cell while mating.

The clastogenic effect of the bacterium was further substantiated by the MNT which showed positive effect discussed before. The MNT showed that the effect was relatively severe than what was generally reported by other workers using chemical mutagens and X-rays. These seemed to be some instances when the erythrocytes contained several

micronuclei, they were formed directly by the breaking down of the complement into pieces. In such cases the size and shape of the micronuclei were somewhat different. Needless to mention that most of the cells the normal type micronucleus.

The chromosome breaking effect was also studied in chromosomes of spermatogonial metaphase, diakinesis, metaphase I and metaphase II in materilas fixed at 18 different intervals from 10 min and 7th week which showed higher frequency of chromosome aberrations than that of control and the difference was statistically significant. Therefore, the clastogenic effect was found not only in the somatic cell but also in the chromosomes of the males germinal cells at different intervals. The occurrence of the germinal chromosome aberration found in the present study also implied that the effect could be transmitted to the next generation.

The effect in the germinal cells was found positive not only in chromosomes but also in the gametes produced due to the effect in the testes and epididymes. The study of the sperm depletion in epididymal sperm suspension examined in between 10 min and seven weeks after the treatment in comparison to that of control revealed that the numbers of sperms was reduced. Among different stages according to time table of spermatogenesis and spermiogenesis, the spermatogonium was found most sensitive though the effect in other stages was also not insignificant.

The study of the frequency of sperm with abnormal head morphology in 18 intervals also revealed that the

frequency was higher in treated males than that of control. The treatment thus may have caused the production of higher number of sperm with abnormal head morphology. If these had the chance of fertilization, there was a possibility for the production of abnormal embryos. However, many of them very likely would loose the potentiality of fertilization. It was also mentioned that various others mutagens both chemical and living ones, besides the effect of P. aeruginosa presented here had the similar effect.

The study of the histological changes in testis, ovary and uterus of specimens treated with log culture of Pseudomonas also supported the genotoxic effect of the bacterium. All these evidences favoured reasonably the bacterium, P. aeruginosa which has been further confirmed by the generation study presented in the following chapters.

CHAPTER II

Cytogenetic Assays in First Filial (F_1) Generation

A. Lethal test :

a) At 15 day of gestation and at the end of the weaning period of the mother :

Lethal test in rodents specially in mice by evaluating the pre- and post-implanatation loss was advocated by two pioneer workers, Hertwick (1935) and Snell (1935) (in the same year). This was further elaborated and extended by several workers using X-ray and chemical agents (Epstein and Shafner, 1968; Rohrborn, 1970; Bateman and Epstein, 1971; Manna and Roy, 1979) and living mutagens (Manna, 1980, 1982; Manna and Patel, 1985; Ghosh, 1985). Among the two methods of evaluation *viz.* pre-implantation loss and post-implantation loss, the latter has been used more often because it was easier. Rohrborn (1970) and others have described the method of post-implantation loss during late gestation which was followed by the present author to evaluate the lethal effect during gestation induced for the treatment of the log culture of *P. aeruginosa* in male parent mated to virgin females (*vide infra*). The author has also followed the method of Soares (1972) to determine the post-implantation loss at the end of the weaning period after parturition of the mating pregnant females.

Most of the workers considered the loss of the

implants due to 'dominant lethal effect' while Cattanach (1982) opined it due to chromosomal mutation. The present author designated the methods as the 'lethal test' instead of 'dominant lethal test' because the effect was found here and elsewhere (Manna and Patel, 1985) in F_2 generation after mating F_1 individuals of the treated line. This was also pointed out by Manna (1982, 1984).

Method :

About 3 month old male mice were intraperitoneally injected with the log culture of P. aeruginosa at the rate of 1 ml/100 gm body weight. They were then allowed to mate in a cage putting one treated male per three normal virgin females. The same male was used consecutively from 1st week to 7th week to mate with different sets of normal virgin females to cover the time-period of differentiated spermatogonia to the formation of mature sperms and transferred to the epididymes (see Adler, 1982). In practice the treated male was kept with three normal virgin females for about a week and the females were examined every day for the appearance of vaginal plug to know the effective fertilization. After the 1st week, the same male was transferred to another set of 3 normal virgin females. In this way the same male was allowed to mate for 7 weeks to different set of females. It was planned that if the 3 females were pregnant, 2 of them would be sacrificed at the 15 day of gestation and the 3rd one would be allowed to complete the weaning period after parturition when she was sacrificed to determine the lethal effect if any following the method of Soares (1972). However, the plan could not be adhered to because of tremendous infertility developed in the treated series (Table 13) which imposed a great

limitation of the data of the present study. Only the data of lethal effect in females which were pregnant both in treated and control series have been presented and the dearth of data could not be avoided in spite of our sincere efforts of using 7 treated males to mate with 21 normal virgin females in each week for 7 weeks *viz.* a total of 147 females were deployed.

The determination of the number of dead implants and the living implants was made by exposing the uterus of the pregnant females at 15 day of gestation in which the dead implants were known from the scar mark left by atrophied embryo against conspicuously grown living implants. For determining the number of living and dead implants at the end of weaning period after parturition the pregnant mother was sacrificed and the uterus was exposed by giving a longitudinal incision. The pinkish well vascularised scar mark represented the living implant while somewhat whitish less vascular scar mark represented the dead implant (Soares, 1972). The mutation rate (lethal hit) per gamate was determined by the post-implantation study according to the following formula.

Percentage of dominant lethals =

$$1 - \frac{\text{living implants per pregnant female in treated group}}{\text{living implants per pregnant female in control group}} \times 100$$

$$\text{Mutagenic index} = \frac{\text{No. of dead implants}}{\text{Total No. of implants}} \times 100.$$

parturition which yielded a total of 28 implants but none was dead. In the 5th week 4 males were mated to 12 females of which only 2 were prêgnant. Both of them were sacrificed during gestation which yielded only 11 implants but none was dead. In the 6th week also 4 males mated to 12 females which yielded 2 pregnant females. They were sacrificed during gestation which yielded 14 implants and onew was dead. At the 7th week, 4 control males were mated to 12 virgin females which yielded also 2 pregnant females. They were sacrificed at late gestation. They had a total of 16 implants but none was dead (Table 13). The mating data of the control series showed that the successful mating was very limited unlike expected normal mating. Therefore, it was not known if the nutrient broth had any effect of causing partial sterility. Anyhow, the data of treated series have been compared with the control series to see if there was a higher lethal effect induced for the treatment of P. aeruginosa.

Treated Seires :

In the first week, treated series 7 males injected with log culture of P. aeruginosa were allowed to mate with 21 virgin females in different sets but had only 1 pregnant female which was sacrificed after parturition because the F_1 individuals were to be used for the study of F_2 generation. The pregnant female had a total of 5 implants, 3 living and 2 dead. The same 7 treated males were mated to 21 virgin females in the 2nd week. This yielded to 4 pregnant females of which 2 were sacrificed during gestation and 2 after parturition. There were 24 implants of which 18 were living and 6 were dead. In the 3rd week the same 7 treated males were mated to different set of 21 females which yielded only

3 pregnant females of which 2 were sacrificed on 15th day of gestation and 1 after parturition. There were a total of 22 implants of which 17 were alive and 5 were dead. In the 4th week, the mating of the same 7 treated males to new sets of 21 virgin females resulted 4 pregnant females of which 2 were sacrificed on 15th day of gestation and 2 after parturition. They yielded a total of 31 implants of which 21 were alive and 9 were dead. However, the fertility factor was seriously affected after 4th week because the mating of the same 7 treated males to 21 females did not yield a single pregnant female. The same was the case in 6th week mating (Table 13). On the other hand in the 7th week, the mating of the same 7 treated males with 21 virgin females yielded only 1 pregnant female which was sacrificed during gestation. There were 8 implants, all alive and none was dead.

The present data (Table 13) showed that the number of implants in different pregnant mothers obtained in 7 week mating programme ranged between 5 and 11 in the control series while it ranged between 4 and 9 in the treated series. Secondly the number of dead implants was nil to 1 in the control series while it ranged between nil to 7 in the treated series (Table 13). Thirdly the average number of implants was 6.5 in the control series against 6.9 in treated series indicating not much difference (Table 14).

It was mentioned already, that in spite of deploying a good number of females and males (3 to 7 sets) in the mating programme, the data were limited because of the higher infertility developed possibly for the treatment with the log culture in the treated series

Table 131

Data of lethal test at 15th day gestation and after birth in different mother mated to control and log culture P. aeruginosa treated male parents in different week (Per experiment 1 male and 3 females have been used)

Week	No.of fem.allowed for meeting	No.of preg. fem.	No.of mother	At	Total No. & impl.	No.of L.I.	No.of D.I.
				Control			
			1	Ges	7	7	-
	9	3	2	Ges	9	9	-
			3	A.B.	5	5	-
Total	9	3	3		21	21	-
1st				Treated			
	21	1	1	A.B.	5	3	2
Total	21	1	1		5	3	2
2nd				Control			
			1	Ges	5	5	-
			2	Ges	6	6	-
	12	4	3	A.B.	6	6	-
			4	A.B.	7	6	1
Total	12	4	4		24	23	1

Table 13 (Contd.)

Week	No.of fem. allowed for meeting	No.of preg. fem.	No.of mother	At	Total No. & impl.	No.of L.I.	No.of D.I.
			Treated				
2nd							
			1	Ges	8	5	3
			2	Ges	6	5	1
	21	4	3	A.B.	6	5	1
			4	A.B.	4	3	1
Total	21	4	4		24	18	6
			Control				
3rd			1	Ges	5	5	-
	12	3	2	Ges	7	7	-
			3	A.B.	5	5	-
Total	12	3	3		17	17	-
			Treated				
			1	Ges	9	7	2
	21	3	2	Ges	8	7	1
			3	A.B.	5	3	2
Total	21	3	3		22	17	5
			Control				
4th			1	Ges	7	7	-
			2	Ges	8	8	-
	12	4	3	A.B.	6	6	-
			4	A.B.	7	7	-
Total	12	4	4		28	28	-

Table 13 (Contd.)

Week	No.of fem. allowed for mating	No.of preg. fem.	No.of mother	At	Total No. & impl.	No.of D.I.	No.of D.I.
4th			Treated				
	21	4	1	Ges	9	7	2
			2	Ges	9	9	-
			3	A.B.	4	4	-
			4	A.B.	9	2	7
Total	21	4	4		31	22	9
			Control				
5th	12	2	1	Ges	6	6	-
			2	Ges	5	5	-
Total	12	2	2		11	11	-
			Treated				
	21	Not a single female was impregnated					
6th			Control				
	12	2	1	Ges	7	7	-
			2	Ges	7	6	1
Total	12	2	2		14	13	1
			Treated				
	21	Not a single female was impregnated					
7th			Control				
	12	2	1	Ges	11	11	-
			2	Ges	5	5	-
Total	12	2	2		16	16	-
			Treated				
	21	1	1	Ges	8	8	-
Total	21	1	1		8	8	-

and to some extent the injection of nutrient broth in the control series. Thus there were 30.56% fertile females in the control series and 8.84% in the treated series. If the injection of nutrient broth had affected the fertility in comparison to that of normal was not verified critically but checking of data of the breeding stock of normal colony did not show such indication. Anyhow, the testing of the progeny of the pregnant mother of the control and treated series showed on the whole the significantly higher frequency of lethality in the treated series when the mutagenic index of the control and treated series was compared in those weeks where some data were available (Table 14). Though the lethal data during gestation and after parturition were analysed separately, it had little value because the data were very limited. Therefore, data of the both gestation and after parturition were combined. In the 1st week mating (Table 14), 3 mothers of the control series had no dead implant, while 2 among 5 implants in a mother of the treated series were dead making a very high frequency of 40% mutagenic index and the induced lethality was 57%. In the 2nd week mating, out of 24 implants 1 was dead yielding the mutagenic index of 4.17% in the control against 6 dead in a total of 24 implants of the treated series rendering the mutagenic index of 25%. In the 3rd week, the mutagenic index was nil in control and 22.73% in treated series; in the 4th week also the mutagenic index was nil in the control series against 29.09% in the treated series (Table 14). However, there was no question of comparing the data of 5th and 6th week as no pregnant female was found in the treated series. In control series, there was no dead implants in 5th week, 7.14% in the 6th week and absent in 7th week. If the data of all the weeks were combined to have a larger sample, there would be 22 dead in a total of

Compiled data of lethal tests of different mothers mated with con
Control data are in brackets.

Mother mated with male in	Time	No.of mother tested	Total No of impl.
1st wk	Ges	-(2)	-(16)
	A.B.	1(1)	5(5)
Total	Ges+AB	1(3)	5(21)
2nd wk	Ges	2(2)	14(11)
	AB	2(2)	10(13)
Total	Ges+ZB	4(4)	24(24)
3rd wk	Ges	2(2)	17(12)
	AB	1(1)	5(5)
Total	Ges+AB	3(3)	22(17)
4thwk	Ges	2(2)	18(15)
	AB	2(2)	13(13)
Total	Ges+AB	4(4)	31(28)
5th wk	Ges	-(2)	-(11)
	AB	-(-)	-(-)
Total	Ges+AB	-(2)	-(11)
6th wk	Ges	-(2)	-(14)
	AB	-(-)	-(-)
Total	Ges+AB	-(2)	-(14)
7th wk	Ges	1(2)	8(16)
	AB	-(-)	-(-)
Total	Ges+AB	1(2)	8(16)
Grand total		13(20)	90(131)

90 implants making the mutagenic index of 24.44% in the treated series while in the control series only 2 were dead among 131 implants making the mutagenic index of 1.53%. The data thus showed clearly the high mutagenic potentiality of the log culture of P. aeruginosa to the treated male parents. According to the time scale of spermatogenesis and spermiogenesis, the mature sperms in the epididymes and the spermatids were highly sensitive to the mutagenic effect while it was not certain for the spermatocyte and differentiated spermatogonia because no data were available. The frequency of induced lethality was very high in the compiled data. It was 57% in 1st week, 21% in 2nd week, 21% in 4th week and 19% in all the weeks, taking together ($P < 0.05$). Therefore, the treatment of the log culture of P. aeruginosa to mating male parents induced high frequency of lethal effects as compared to that of control but the limitation of the data might make the result questionable. However, the study of the chromosome aberrations in somatic and germinal cells, sperm count, sperm head morphology, etc. carried out in F_1 generation would support the mutagenic potentiality of the log culture of P. aeruginosa in mice (vide infra).

B. Somatic Chromosome Aberration in F_1 Generation :

Qualitative :

With a view to finding out if the F_1 living implants of the control and treated series mothers were chromosomally normal or not, they were studied using different cytogenetic methods. For embryos only the somatic chromosomes from the liver were assessed. On the other hand, 3 month old F_1 individuals were used for the study of somatic chromosome aberrations in bone marrow cells of both sexes and meiotic chromosome aberration in testes, sperm count and sperm head morphology in epididymes of males. Besides them some special study was made to detect the teratogenic effect if any in 15 day old F_1 fetuses and in organs of 3 months old F_1 individuals.

Since the types of chromosome aberrations in the embryonic liver cells and that of bone marrow chromosome of F_1 adults were not basically different, some representative types have been cited (PM 74-85; Figs. 56-75). The aberration types were more or less the same in F_1 embryos and 3 month old adult as found in the bone marrow cells of the treated specimens. They were put under two main categories as individual types and gross types. The individual types consisted of chromatid break (PM 74-75, 77; Figs. 56-67), acentric fragment (PM 76, 78-79, 83; Fig. 68), centric fusion (PM 74, 78, 80-81; Figs. 70-71), ring chromosome (PM 76, 82; Fig. 69) etc. However, all the types were not found in all individuals and they were relatively very few or absent in the control line.

Explanation of Photomicrographs

Photomicrographs of some affected metaphase in liver /bone marrow cells of F_1 individuals produced after mating of normal females to Pseudomonas culture treated males.

PM 74. A centric fusion and two group I chromosomes each with a chromatid break in the middle region.

PM 75. A chromatid break in the proximal region of a group II chromosome.

PM 76. A ring chromosome and a fragment.

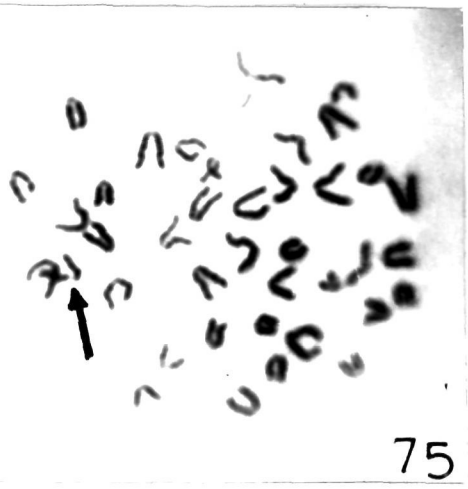
PM 77. A terminal chromatid break in a group III chromosome.

PM 78. A centric fusion, a centromeric dissociation and a fragment.

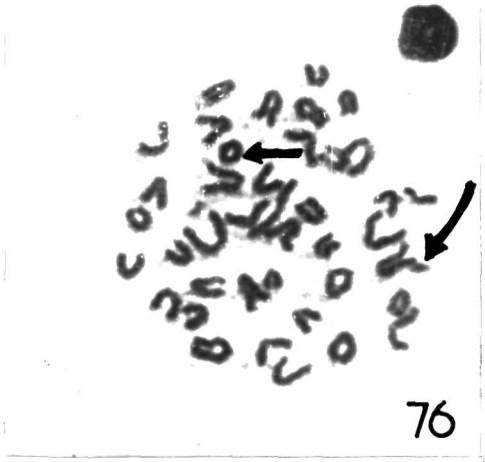
PM 79. An acentric fragment and some sticky chromosomes.



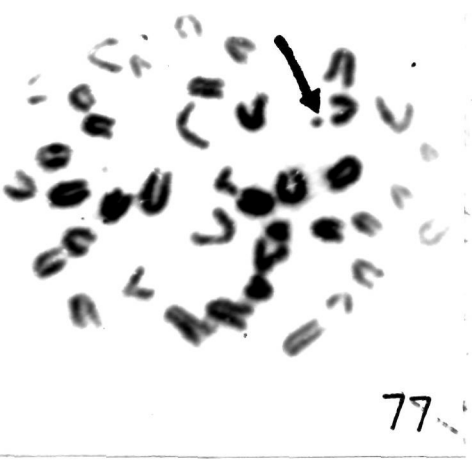
74



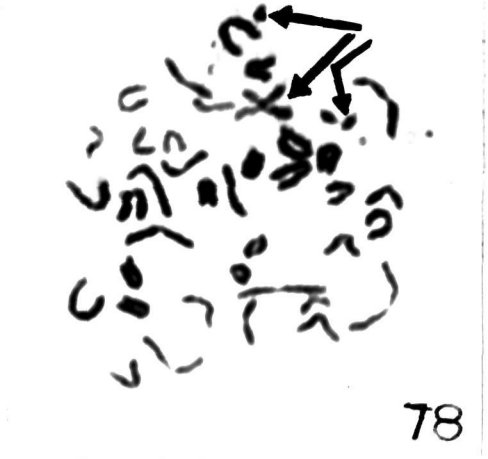
75



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Explanation of Photomicrographs

Photomicrographs of some affected metaphases in liver/ bone marrow cells of F_1 individual produced after mating of normal females to Pseudomonas culture treated males.

PM 80. Plate showing two centric fusions and condensed chromosomes.

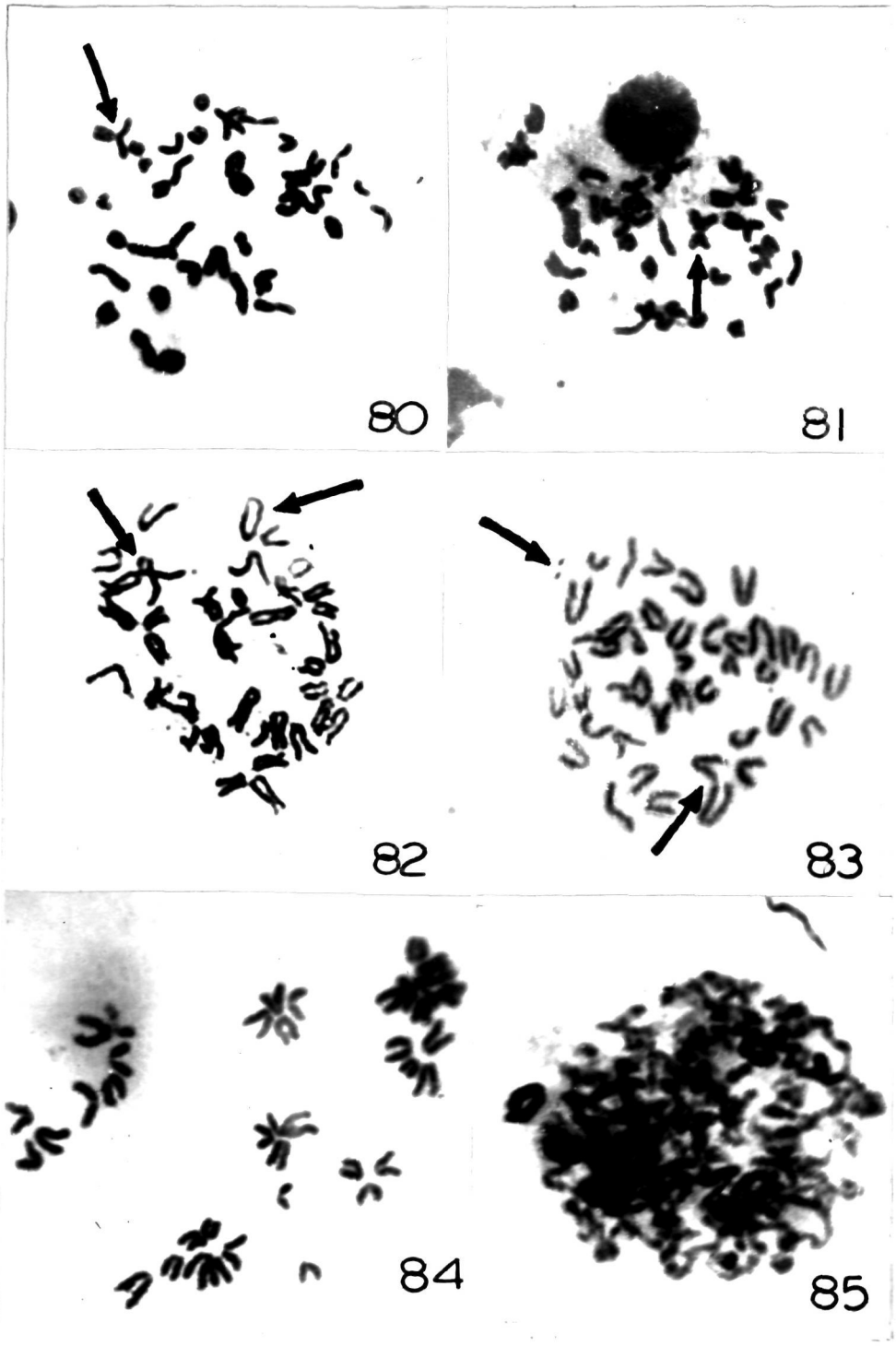
PM 81. A centric fusion and some other showing the same tendency.

PM 82. A ring chromosome and a centric fusion.

PM 83. Terminal chromatid association of two chromosomes and on extra-element with centromeric dissociation.

PM 84. Aster metaphase with association of groups of chromosomes.

PM 85. Plate showing the pulverization.


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Explanation of figures

Camera lucida drawing of a part of metaphases plate of liver/bone marrow showing different types of aberrations.

Fig. 56. A chromatid break in the proximal region of a group I chromosome.

Fig. 57. A chromatid break in the middle region of a group I chromosome.

Fig. 58. A chromatid break in the distal region of a group I chromosome

Fig. 59. A chromatid break in the proximal region of a group II chromosome.

Fig. 60. A chromatid break in the middle region of a group II chromosome.

Fig. 61. A chromatid break in the distal region of a group II chromosome.

Fig. 62. A chromatid break in the proximal region of a group III chromosome.

Fig. 63. A chromatid break in the middle region of a group III chromosome

Fig. 64. A isochromatid break in the distal region of a group III chromosome

Fig. 65. A chromatid break in the proximal region of a group IV chromosome.

Fig. 66. A chromatid break in the middle region of a group IV chromosome.

Fig. 67. A chromatid break in the distal region of a group IV chromosome.

Fig. 68. A fragment of unknown origin.

Fig. 69. A ring chromosome.

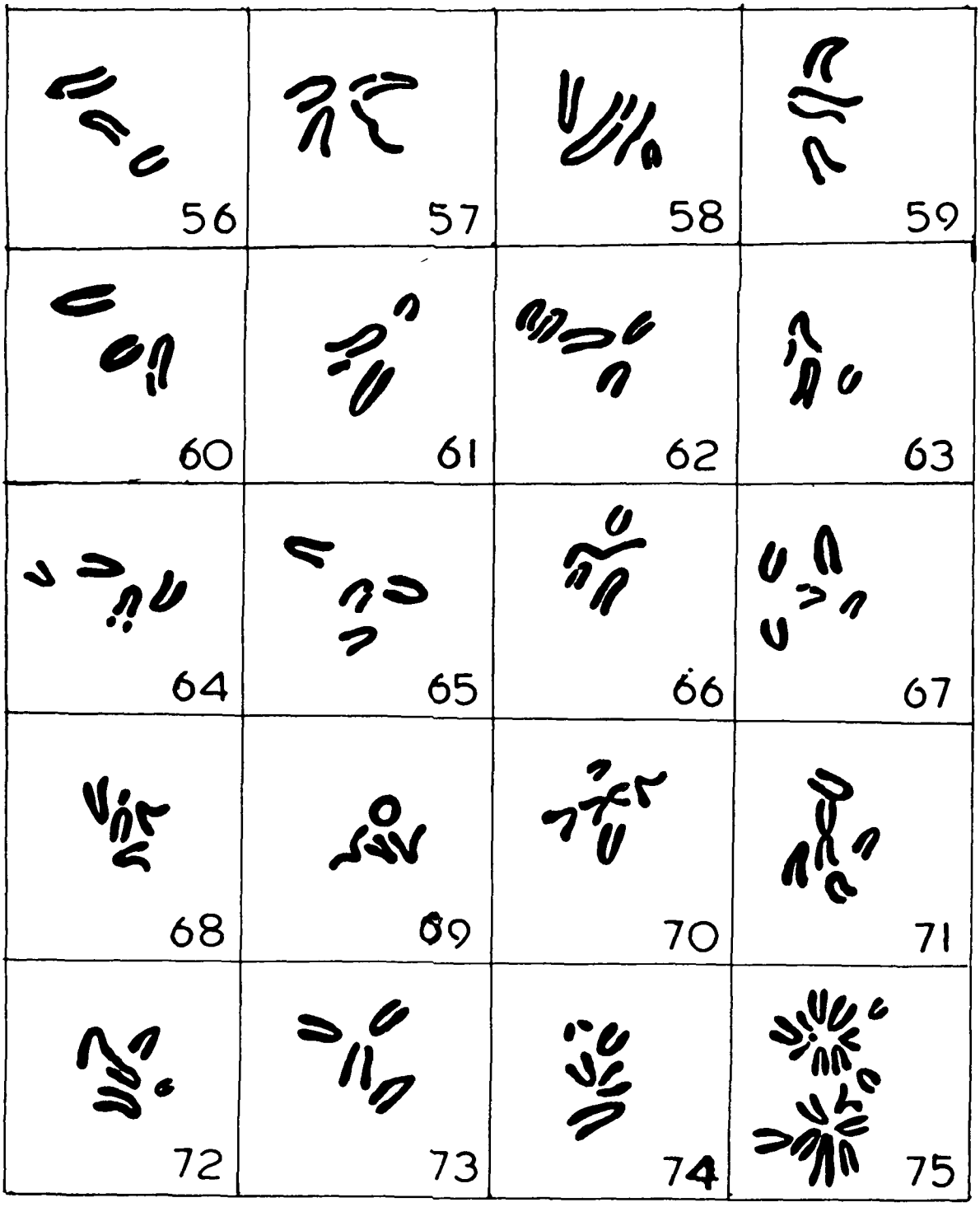
Figs. 70-71. A centric fusion between two chromosome.

Fig. 72. End to end association between two chromosomes.

Fig. 73. Centric dissociation of a group III

Fig. 74. Centric dissociation of a group V.

Fig. 75. A meter metaphase plate.


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Quantitative

a) In liver of 15 day old embryos :

While the pregnant mothers of both control and treated series were sacrificed at 15 day of gestation to determine the number of living and dead implants by exposing the uterus of each individual, the living implants immediately on removal were put into 1% sodium citrate solution. The liver of each individual was dissected out and the tissue was minced and the cells were brought into citrate suspension by repeated flushing with a pasteur pipette. The cell suspension was incubated for 15 minutes at 37°C. It was then centrifuged and the supernatant fluid was discarded. The residual cells were fixed in freshly prepared aceto-alcohol (1:3) mixture for 15-20 min and again centrifuged. The supernatant the fixation was discarded and residual cells were resuspended in aceto-alcohol. The flame dry preparation followed by Giemsa staining was made as described earlier for the preparation of the bone marrow cells. The frequency of different types of aberrations was determined in 100 plates per individual (Table 15).

F_1 control line :-

1st week :

All the 7 embryos obtained from a control line pregnant mother had some form of aberrations, the number of which ranged between 5 and 18 (Table 15). The frequency of individual type aberrations was generally higher than that

of gross type. Out of total 700 metaphases examined in 7 embryos of the 1st mother, there were 7 chromatid breaks, 1 acentric fragment, 13 translocations or associations and 15 centromeric dissociations making a total of 36 individual types and there were also 27 gross type aberrations. The average frequency was 9.0% taking both types together.

In the 2nd mother, out of 9 living embryos, tissues of two were lost while processing. Therefore, in livers of 7 embryos, 1 had no aberration and in the remaining 6, the number ranged between 1 and 7. The data were enhanced for the inclusion of cases of centromeric dissociation. Otherwise the number would range meagrely between nil to 3 in different individuals. Out of the total 700 metaphases examined there were 2 chromatid breaks, 1 acentric fragment, 14 centromeric dissociations. Taking all type aberrations together the total was 23 of 3.28% of which if the frequency of centromeric dissociations was disregarded it would come down to about 1.3%. Out of 14 embryos examined, 6 were males with the aberration number ranging between 2 and 11 and the average frequency was 6.50% and 8 females with the aberration number ranging between nil and 9 and the average frequency was 5.87% indicating not much difference in two sexes (Table 22).

2nd week :

In a 2nd week pregnant mother out of 5 embryos the tissue of 1 was lost during processing. In the remaining 4, the number of aberrations ranged between 5 and 12. In a total of 300 metaphases, the frequency of individual type

aberration was 14 or 4.6% and the average was 31 or 7.75% taking individual and gross types together.

In the 2nd mother, the aberration number in 6 embryos ranged between 6 and 8. In a total of 600 metaphases, the frequency of individual type aberration was 24 or 4.0% and all types taking together was 40 or 6.66% (Table 15).

3rd week :

In a 3rd week pregnant mother 5 embryos had the aberration number ranging between 5 and 11 including a number of centromeric dissociation cases. Out of the total 500 metaphases there were 20 of 4.0% individual type and 32 or 6.4% taking both individual and gross types together.

In the 2nd mother, out of 7 embryos the tissue of two embryos were lost while processing. In the remaining 5 the number of aberrations ranged between 2 and 5. In a total of 500 metaphases, 8 or 1.6% were individual type and 19 or 3.8% taking all types of aberrations together (Table 15).

4th week :

In a 4th week pregnant mother out of 7 embryos, one had no aberration and other 6 had the aberration number ranging between 3 and 6. In a total of 700 metaphases, there were 13 or 1.8% individual types and 27 or 3.8% taking both gross and individual types together.

In the 2nd mother, out of 8 embryos examined, 1 had no aberration while in 7 others, the number of aberrations ranged between 1 and 11. In a total of 800 metaphases there were 24 or 3.0% individual types and 45 or 5.6% taking both individual and gross types together (Table 15).

5th week :

In a 5th week pregnant mother, 6 embryos had number of aberration ranging between 2 and 5. In a total of 600 metaphases, there were 14 or 2.3% individual types and 24 or 4.0% taking both individual and gross type together.

In the 2nd mother, 5 embryos had the number of aberrations ranging between 2 and 9. In a total of 500 metaphases, 18 or 3.6% were individual type and 29 or 5.8% taking both individual and gross types together (Table 15).

6th week :

In a 6th week pregnant mother out of 7 embryos 1 had no aberration while 6 others had the aberration number ranging between 1 and 9. In a total of 700 metaphases, there were 16 or 2.3% individual type and 29 or 4.14% taking both individual and gross types together.

In 6 embryos of another pregnant mother, the aberration number ranged between 6 and 9. In a total of 600 metaphases, there were 29 or 4.83% individual types and 43 or 7.16% taking both individual and gross types together (Table 15).

7th week :

In a 7th week pregnant mother, out of 11 embryos the tissue of 1 was lost during processing. In the liver of remaining 10 embryos the number of aberrations ranged between 1 and 6. In a total of 1000 metaphases, the frequency of individual type aberration was 39 or 3.9% and the individual and gross types taking together was 47 or 4.7%.

In 5 embryos of another mother, the number of aberrations ranged between 2 and 9 and in a total of 500 metaphases the frequency of individual type was 5 or 1.0% and the individual and gross type taking together was 29 or 4.8% (Table 15).

Treated series

1st week :

Unfortunately no embryo was obtained from females which were allowed to mate with treated males in the first week (Table 15).

2nd week :

The 5 F_1 living embryos obtained in a pregnant mother of 2nd week mating, the number of aberration ranged widely between 2 and 26. In a total of 500 metaphases there were 20 or 4.0% individual types and 67 or 13.4% gross and individual types taken together.

In 5 embryos of another mother, the aberration number ranged between 5 and 24. In a total of 500 metaphases, there were 27 or 5.4% individual types and 72 or 14.4% gross and individual types taken together. Therefore, the average frequency in embryos of two mothers was not very different (Table 15).

3rd week :

In 7 embryos of a 3rd week pregnant mother the aberration number ranged between 16 and 38 and out of 700 metaphases of 7 embryos there were 49 or 7.0% individual types and 142 or 20.28% both individual and gross types aberrations taken together.

In another mother, out of 7 embryos obtained, the liver tissue of 1 embryos was lost during processing and in the remaining 6, the aberration number ranged, between 2 and 15. Out of 600 metaphases examined in 6 embryos, there were 34 or 5.6% individual types and 54 or 9.0% both individual and gross types taken together (Table 15).

4th week :

Out of 7 embryos of a 4th week pregnant mother, the tissue of 1 embryo was lost during processing. In the remaining 6 embryos, the chromosome aberration number ranged between 3 and 59. Thus out of the total 600 metaphases, there were 112 or 18.6% individual types and 224 or 37.33% taking individual and gross types together.

In a 2nd mother, 8 embryos were obtained in which

the aberration number in liver ranged between 2 and 30 in different individuals. In a total of 700 metaphase, there were 37 or 4.1% individual type and 130 or 14.4% both individual and gross type taken together.

5th and 6th week :

Unfortunately like 1st week mating, no female was pregnant in 5th and 6th week mating. Therefore, there was no question of scoring the aberration data in embryos of the treated series in these two weeks (Table 15).

7th week :

In the 7th week mating programme, no lethal effect was found unlike that of 2nd, 3rd and 4th week mating mentioned before (vide supra). However, it was very interesting to see in the present study that though no lethal effect was found but the cytological examination of the liver cells of 8 embryos in a 7th week pregnant mother, the number of chromosome aberrations ranged between 21 and 44. In a total of 800 metaphases there were 95 or 11.8% individual types and 230 or 28.75% both gross and individual types taken together (Table 15).

Comments :

The analysis of the chromosome aberration data in the liver cells of different embryos in pregnant mothers of the control series for seven week matings would reveal that

Table 15

Frequency distribution of chromosome aberrations assessed in 100 plates of liver cells per embryo of 15 day old mothers mated to P. aeruginosa treated and control male parents at different weeks

Fix. week	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. Change	Others	Total		
			Chr.	Frag.	Tran. Ass.	Cons & gap	Cent. disso.						
							Mother I						
							Control						
1st wk	1	M	1	-	4	-	-	5	6	-	6	11	11.0
	2	M	-	-	-	-	3	3	4	-	4	7	7.0
	3	M	-	1	2	-	4	7	1	-	1	8	8.0
	4	F	3	-	-	-	1	4	12	2	14	18	18.0
	5	F	-	-	3	-	2	5	-	-	-	5	5.0
	6	F	1	-	2	-	1	4	1	-	1	5	5.0
	7	F	2	-	2	-	4	8	1	-	1	9	9.0
Total			7	1	13	-	15	36	25	2	27	63	9.0
							Mother II						
							Control						
	8	M	2	-	-	-	4	6	-	1	1	7	7.0
	9	M	-	-	-	-	-	-	1	1	2	2	2.0
	10	M	-	-	-	-	3	3	1	-	1	4	4.0
	11	F	-	-	-	-	1	1	-	-	-	1	1.0
	12	F	-	1	-	-	3	4	-	-	-	4	4.0
	13	F	-	-	-	-	-	-	-	-	-	-	-
	14	F	-	-	-	-	3	3	2	-	2	5	5.0
Total			2	1	-	-	14	17	4	2	6	23	3.28

*In treated line not a single female was impregnant.

Table 15 (Contd.)

Fix. week	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans. Asso.	Gaps & cons.	Cent. diss.						
						Mothee II		Control					
2nd wk	1	M	-	-	-	1	1	2	1	2	3	5	5.00
	2	F	2	1	1	1	-	5	5	2	7	12	12.00
	3	F	1	-	1	-	3	5	2	1	3	8	8.00
	4	F	-	-	1	1	-	2	3	1	4	6	6.00
Total			3	1	3	3	4	14	11	6	17	31	7.75
								Treated					
2nd wk	1	M	-	-	-	2	5	7	9	-	9	16	16.00
	2	M	-	-	-	-	-	-	2	-	2	2	2.00
	3	F	3	1	-	1	-	5	20	1	21	26	26.00
	4	F	2	1	1	-	1	5	5	2	7	12	12.00
	5	F	-	-	1	-	2	3	6	2	8	11	11.00
Total			5	2	2	3	8	20	42	5	47	67	13.40

Table 15 (Contd.)

Fix. week	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans. Asso.	Gaps & cons.	Cent. diss.						
						Mother II							
						Control							
	1	M	-	-	-	-	-	-	6	-	6	6	6.00
	2	M	-	-	1	1	-	2	4	1	5	7	7.00
	3	M	-	-	-	-	7	7	1	-	1	8	8.00
	4	F	1	-	1	1	2	5	-	1	1	6	6.00
	5	F	1	-	-	1	3	5	-	2	2	7	7.00
	6	F	1	-	-	1	3	5	1	-	1	6	6.00
Total			3	-	2	4	15	24	12	4	16	40	6.66
2hd wk						Treated							
	1	M	3	1	-	1	4	9	12	3	15	24	24.00
	2	M	-	-	-	6	9	15	5	3	8	23	23.00
	3	M	-	-	-	-	-	-	8	-	8	8	8.00
	4	M	-	-	-	-	-	-	4	1	5	5	5.00
	5	F	-	-	3	-	-	3	9	-	9	12	12.00
Total			3	1	3	7	13	27	38	7	45	72	14.40

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Tran. Asso.	Gaps & cons.	Cent. disso.						
								Mother I					
								Control					
	1	M	-	-	-	-	1	1	2	-	2	3	3.00
	2	M	-	-	1	-	4	5	3	-	3	8	8.00
	3	M	-	-	-	-	2	2	3	-	3	5	5.00
	4	F	-	-	-	-	3	3	2	-	2	5	5.00
	5	F	1	1	-	-	7	9	2	-	2	11	11.00
Total			1	1	1	-	17	20	12	-	12	32	6.40
3rd wk								Treated					
	1	M	2	1	3	-	-	6	10	2	12	18	18.00
	2	M	5	-	3	1	-	9	5	5	10	19	19.00
	3	M	3	-	-	-	-	3	6	3	9	12	12.00
	4	M	2	-	2	1	-	5	7	4	11	16	16.00
	5	F	3	-	-	-	-	3	8	9	17	20	20.00
	6	F	11	4	-	1	-	16	2	20	22	38	38.00
	7	F	3	-	-	2	2	7	10	2	12	19	19.00
Total			29	5	8	5	2	49	48	45	93	142	20.28

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type: Cht.	Frag.	Tran. Asso.	Phy.type: Gaps & cons.	Cent. disso.	Total	Gross type aberration: Num. cha.	Others	Total	Grand total	%
								Mother II					
								Control					
	1	M	-	-	-	-	3	3	2	-	2	5	5.00
	2	M	1	-	-	-	1	2	3	-	3	5	5.00
	3	M	-	-	-	-	2	2	1	-	1	3	3.00
	4	F	-	-	-	-	1	1	3	-	3	4	4.00
	5	F	-	-	-	-	-	-	2	-	2	2	2.00
Total			1	-	-	-	7	8	11	-	11	19	3.80
3rd wk								Treated					
	1	M	-	-	-	-	1	1	2	4	6	7	7.00
	2	M	3	1	3	-	1	8	5	2	7	15	15.00
	3	F	-	-	1	-	6	7	2	-	2	9	9.00
	4	F	-	-	-	-	-	-	-	2	2	2	2.00
	5	F	4	-	4	-	2	10	1	1	2	12	12.00
	6	F	-	-	6	-	2	8	-	1	1	9	9.00
Total			7	1	14	-	12	34	10	10	20	54	9.00

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Tran. Asso.	Caps & cons.	Cent. disso.						
							Mother I Control						
	1	M	-	-	-	-	-	-	3	-	3	3	3.00
	2	M	-	1	-	-	1	2	1	2	3	5	5.00
	3	M	-	-	1	1	2	4	2	-	2	6	6.00
	4	M	-	-	-	-	2	2	-	2	2	4	4.00
	5	F	-	-	1	-	1	1	3	-	3	4	4.00
	6	F	1	-	-	1	2	4	-	1	1	5	5.00
	7	F	-	-	-	-	-	-	-	-	-	-	-
	Total		1	1	2	2	8	13	9	5	14	27	3.85
4th wk							Treated						
	1	M	1	-	4	-	18	23	15	8	23	46	16.00
	2	M	2	-	4	2	18	26	12	6	18	44	44.00
	3	M	7	3	6	3	10	29	2	5	7	36	36.00
	4	F	-	-	2	1	15	18	14	4	18	36	36.00
	5	F	1	-	3	1	9	14	42	3	45	59	59.00
	6	F	-	-	2	-	-	2	-	1	1	3	3.00
	Total		11	3	21	7	70	112	85	27	112	224	37.33

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type Chr.	Frag.	Tran. Asso.	Phy. type Gaps & cons.	Cent. disso.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
						Mother II							
						Control							
	1	M	-	-	-	-	1	1	-	-	-	1	1.00
	2	M	-	-	-	-	-	-	-	-	-	-	-
	3	M	2	-	-	-	3	5	3	1	4	9	9.00
	4	M	-	-	-	1	4	5	2	3	5	10	10.00
	5	F	1	-	1	-	3	5	-	1	1	6	6.00
	6	F	-	1	-	1	1	3	2	1	3	6	6.00
	7	F	-	-	-	-	-	-	-	2	2	2	2.00
	8	F	-	1	-	-	4	5	4	2	6	11	11.00
	Total		3	2	1	2	16	24	11	10	21	45	5.62
4th wk							Treated						
	1	M	-	-	4	-	-	4	5	-	5	9	9.00
	2	M	-	-	2	-	-	2	8	4	12	14	14.00
	3	M	-	-	5	1	-	6	19	5	24	30	30.00
	4	M	-	1	9	-	-	10	1	6	7	17	17.00
	5	F	-	-	4	-	-	4	7	2	9	13	13.00
	6	F	-	-	-	-	-	-	5	-	5	5	5.00
	7	F	1	1	3	-	-	5	14	1	15	20	20.00
	8	F	-	-	-	-	-	-	2	-	2	2	2.00
	9	F	-	-	1	-	5	6	9	5	14	20	20.00
	Total		1	2	28	1	15	37	70	23	93	130	14.44

Table 15 (Contd.)

Fix. week	Ind. No.	Sex	Individual type aberrations						Gross type aberrati.			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Tran. Asso.	Gaps & cons.	Cent. disso.						
						Mother I Control							
	1	M	1	-	-	-	-	1	1	1	2	3	3.00
	2	M	-	-	-	1	2	3	2	-	2	5	5.00
	3	M	-	-	-	-	-	-	1	1	2	2	2.00
	4	F	-	-	-	-	2	2	3	-	3	5	5.00
	5	F	-	-	-	-	3	3	1	-	1	4	4.00
	6	F	-	-	-	-	5	5	-	-	-	5	5.00
Total			1	-	-	1	12	14	8	2	10	24	4.00
5th wk						Mother II Control							
	7	M	-	-	-	2	3	5	-	2	2	7	7.00
	8	M	-	1	1	-	-	2	-	-	-	2	2.00
	9	M	-	2	-	-	1	3	-	2	2	5	5.00
	10	F	1	-	-	1	2	4	1	1	2	6	6.00
	11	F	-	-	-	-	4	4	-	5	5	9	9.00
Total			1	3	1	3	10	18	1	10	11	29	5.80

* In treated line not a single female was impregnant

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type Chr.	Frag.	Tran. Asso.	Phy. type Gaps & cons.	Cent. diss.	Total	Gross type aberrations: Num. Cha.	Others	Total	Grand total	%
						Mother I							
						Control							
	1	M	2	-	-	-	-	2	-	1	1	3	3.00
	2	M	1	-	-	1	1	3	-	2	2	5	5.00
	3	M	-	-	-	-	-	-	1	-	1	1	1.00
	4	F	-	-	-	-	-	-	-	-	-	-	-
	5	F	-	-	-	-	-	-	2	2	4	4	4.00
	6	F	3	-	-	-	4	7	1	1	2	9	9.00
	7	F	-	1	1	-	2	4	1	2	3	7	7.00
Total			6	1	1	1	7	16	5	8	13	29	4.14
6th wk						Mother II							
						Control							
	8	M	-	-	-	1	5	6	-	-	-	7	7.00
	9	M	2	1	-	-	-	3	3	1	4	7	7.00
	10	M	-	-	-	1	2	3	2	1	3	6	6.00
	11	F	-	2	-	-	5	7	2	-	2	9	9.00
	12	F	1	-	1	1	2	5	-	2	2	7	7.00
	13	F	-	-	-	2	3	5	1	2	3	8	8.00
Total			3	3	1	5	17	29	8	6	14	43	7.16

* In treated line, not a single female was impregnant.

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type: Chr.	Frag.	Tran. Asso.	Phy. type: Gaps & cons.	Cent. disso.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
							Mother I Control						
	1	M	4	-	-	1	-	5	-	-	-	5	5.00
	2	M	3	-	-	-	2	5	-	-	-	5	5.00
	3	M	-	-	1	-	1	2	3	-	3	5	5.00
	4	M	-	-	-	4	-	4	2	-	2	6	6.00
	5	F	2	1	1	1	-	5	-	1	1	6	6.00
	6	F	2	-	-	-	2	4	-	-	-	4	4.00
	7	F	2	-	1	1	2	6	-	-	-	6	6.00
	8	F	1	2	1	-	-	4	1	-	1	5	5.00
	9	F	2	-	-	-	1	3	-	1	1	4	4.00
	10	F	1	-	-	-	-	1	-	-	-	1	1.00
7th wk	Total		17	3	4	7	8	39	6	2	8	47	4.70
							Treated						
	1	M	4	2	2	3	7	16	17	-	17	33	33.00
	2	M	1	1	4	2	1	9	17	-	17	26	26.00
	3	M	4	2	-	2	6	14	12	5	17	31	31.00
	4	M	5	-	1	2	1	9	13	-	13	22	22.00
	5	M	2	-	2	-	-	4	10	9	19	23	23.00
	6	F	13	1	4	3	5	26	18	-	18	44	44.00
	7	F	4	-	4	3	-	11	19	-	19	30	30.00
	8	F	1	-	1	-	4	6	12	3	15	21	21.00
	Total		34	6	18	15	24	95	118	17	135	230	28.75

Table 15 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Tran. Asso.	Gaps & cons.	Cent. disso.						
						Mother II							
						Control							
7th wk	11	M	-	1	-	-	-	1	-	1	1	2	2.00
	12	M	2	-	1	-	-	3	1	-	1	4	4.00
	13	F	-	-	-	-	-	-	2	3	5	5	5.00
	14	F	1	-	-	-	-	1	-	3	3	4	4.00
	15	F	-	-	-	-	-	-	6	3	9	9	9.00
Total			3	1	1	-	-	5	9	10	19	24	4.80

*In treated line, not a single female was impregnant.

there were some embryos without any chromosomal aberration while in some cases if the cases of centromeric dissociation were eliminated assuming their doubtful genetic significance, the frequency of individual type aberrations would be reduced considerably. In the liver of some embryos even the individual type aberration was not found at all e.g. in embryo No. 8 of 2nd mother of 1st week (Table 15). Therefore, the occurrence of only very few gross type aberration in some embryos might be considered as they were free from real aberration of genetic significance. On the whole, the frequency of aberrations observed in the embryos of control series was not strikingly high in comparison to that of treated series. Secondly, the average frequency of aberration in the embryos of 2 mothers sacrificed in each week did not differ very widely and maximum difference was seen in 1st week. While it was very much different in treated series.

The frequency of aberration in different embryos of the treated series mothers in different weeks was quite variable and relatively much higher than that of control. The average frequency of aberrations in embryos of two mothers did not vary much in 2nd week mating but in 3rd and 4th week it was widely variable.

If the data of the chromosome aberrations in the liver of different embryos usually of 2 mothers in each week was combined (Table 16) it would be found that the average frequency in the control series was 6.14% in 1st week of which 53 of 3.7% were individual type and that included 29 or 2.0% centromeric dissociation cases. In the 2nd week, the average was 7.1% of which 38 or 3.8% were

individual types which included 19 or 1.9% cases of centromeric dissociation. In the 3rd week, the average frequency was 5.1% of which 28 or 2.8% were individual types and that included 24 or 2.4% centromeric dissociation cases. In the 4th week, the average frequency was 4.8% of which 37 or 2.46% was individual types including 24 or 1.6% centromeric dissociation cases. In the 5th week, the average was 4.81% of which 32 or 2.9% were individual types including 22 or 2.0% centromeric dissociation cases. In the 6th week, the average frequency was 5.53% of which 45 or 3.4% was individual types including 24 or 1.8% centromeric dissociation cases and in the 7th week, the average frequency was 4.73% of which 44 or 2.9% were individual types including 8 or 0.5% centromeric dissociation cases (Table 16). Therefore, the frequency of individual type aberration was very low when the centromeric dissociation cases were eliminated. Further, the average aberration frequency in the liver cells of different week control series did not vary much. In the combined data of all weeks, the average frequency was 5.4% of which 277 or 3.14% was individual type including 150 or 1.7% centromeric dissociation cases. Further, if the cases of gaps and constrictions were eliminated, the true break type aberrations of genetic significance like chromatid break, fragment of unknown origin and translocation or association would be 100 in 8800 metaphases i.e. 1.1%. Even true translocation was practically absent and they were mostly association. Therefore, it seemed that some clastogenic factors might have contained in the nutrient broth medium which could be responsible for transmitting the effect in the next generation.

Treated series :

The liver cells of embryos of mothers which successfully mated to the treated males in 2nd week, 3rd week, 4th week and 7th week had higher frequency of chromosome aberrations than that of controls while no data were available in the remaining weeks possibly due to sterility developed for the treatment of the bacterial log culture. In two pregnant mothers of 2nd week mating with the treated male, the average frequency of aberrations was 13.9% of which 4.5% were individual types including 2.1% centromeric dissociation cases. In two 3rd week mating mothers of the treated line, the average aberration frequency was 15.07% of which 6.3% were individual types including 1.1% centromeric dissociation cases. In the 4th week, the average aberration frequency was 23.6% of which 9.9% were individual types including 5.0% centromeric dissociation cases and in the 7th week, the average aberration frequency was 28.75% of which 11.87% were individual type including 1.8% centromeric dissociation cases. If the aberration data in the liver cells of all embryos of pregnant mothers of different weeks in the treated series were combined, the average aberration frequency was 19.97% of which 8.13% were individual types including 2.91% centromeric dissociation cases (Table 16).

The data further showed that when the individual types and gross type aberrations were compared between control and treated series, it was found to be significantly high in the treated series indicating that the mutagenic factor must be present even in the living embryos.

Table 16

Compiled data of chromosome aberration of F_1 embryos at 15 days gastation in mothers mated from 1st to 7th weeks with control and the bacterium P. aeruginosa treated male. Control data are in brackets.

Fix.	No.of mothers	No.of embry.	No.of meta.	Individual type aberr.					Gross type aberr.			Grand total	%
				Break type			Phy. type	Total	Nu. ch.	Others	Total		
				Chr.	Frag.	Trans/ Asso.	Cons. & gaps	Cent. diss.					
1st wk	*	*	*	*	*	*	*	*	*	*	*	*	*
	(2)	(14)	(1400)	(9)	(2)	(13)	(-)	(29)	(53)	(29)	(4)	(33)	(86)	(6.14)
2nd wk	2	10	1000	8	3	5	10	21	47	80	12	92	139	13.90
	(2)	(10)	(1000)	(6)	(1)	(5)	(7)	(19)	(38)	(23)	(10)	(33)	(71)	(7.10
3rd wk	2	13	1300	36	6	22	5	14	83	58	55	133	196	15.07
	(2)	(10)	(1000)	(2)	(1)	(1)	(-)	(24)	(28)	(23)	(-)	(23)	(51)	(5.10)
4th wk	2	15	1500	12	5	49	8	75	149	155	50	205	354	23.60
	(2)	(15)	(1500)	(4)	(3)	(3)	(4)	(24)	(37)	(20)	(15)	(35)	(72)	(4.80)
5th wk	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	(2)	(11)	(1100)	(2)	(3)	(1)	(4)	(22)	(32)	(9)	(12)	(21)	(53)	(4.81)
6th wk	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	(2)	(13)	(1300)	(9)	(4)	(2)	(6)	(24)	(45)	(13)	(14)	(27)	(72)	(5.53)
7th wk	1	8	800	34	6	18	15	24	95	118	17	135	230	28.75
	(2)	(15)	(1500)	(20)	(4)	(5)	(7)	(48)	(44)	(15)	(12)	(27)	(71)	(4.73)
Grand total	7	46	4600	90	20	94	38	134	374	411	134	545	919	19.97
	(14)	(88)	(8800)	(52)	(18)	(30)	(28)	(150)	(277)	(132)	(67)	(199)	(476)	(5.40)

*No pregnant.

The frequency of centromeric dissociation cases in five groups of chromosomes in 1st to 7 week treated and control series embryos was analysed (Table 17). However, a full comparison was not possible since no F_1 embryos of the 1st, 5th and 6th week treated series were available. Anyhow, in the parental generation, the treatment of log culture yielded the frequency of centromeric dissociations an overall increase than that of control (Table 2) of which chromosome belonging to IV and V were specially more involved. The highest involvement was found in group V. An analysis of the data of frequency distribution of centromeric dissociation cases in different groups of chromosomes in the liver cells of F_1 embryos of 15 day gestation showed the chromosomes specially belonging to groups IV and V had more involvements of which the maximum was found in group V. But the frequency unlike parental generation was little erratic. It was relatively more in treated series examined of 2nd, 4th and 7th week mating but it was reverse in 3rd week and in the average it was lower in the treated series than that of control. However, since the data of 1st week, 5th week and 6th week were not available, the observation had a limitation.

Comments :

Since similar type of result for the occurrence of much higher frequency of chromosome aberrations in F_1 and F_2 generations in mating programme of males treated with add mutagens with normal virgin females using antibiotic (Roy, 1982), chemosterilinat - D-glucose amine hydrochloride (Banerjee, 1982), living mutagens S. aereus

(Ghosh, 1985) and S. lutea (Goswami, 1985), fungus - spores of Aspergillus (Patel, 1982) was found in our laboratory. Manna (1984) after reviewing the different results advocated that there could be some locus or loci in chromosome which in normal states maintains the structural integrity of chromosomes but if mutated the control may be lost. It was further stated that the chromosomal mutation might lead to the production of lethal effect when it was of vital nature otherwise it might produce general type aberration in the living implants in F_1 and F_2 general as found in the present study. Therefore, it seemed that the chromosomal mutation was responsible for the lethal effect on the developing embryos as well as inducing chromosome aberration in living embryos of F_1 generation.

With a view to finding out if the chromatid breaks were nonrandomly distributed between and within chromosomes in F_1 generation, a total of 91 chromatid breaks obtained in the liver cells of F_1 embryos of treated series were analysed (Table 18). The analysis revealed that chromosomes belonging to groups I and II were relatively more susceptible while those of group III to group V were somewhat resistant when the observed and expected numbers were compared in five groups of chromosomes (Table 18). However, the difference in some groups was not very striking. On the whole it seemed that the chromatid breaks were nonrandomly distributed which was also revealed when similar analysis was made for the distribution of chromatid break is five group of chromosomes induced by the treatment of log culture of P. aeruginosa in parental generation (Table 3).

Table 17

Compiled data of frequency distribution of centric dissociation in different particular groups of chromosomes in liver cells of 15 day old F_1 embryos of different mothers mated at different weeks with the P. aeruginosa treated and control male parents (100 cells/ indv. were counted). Control data are in brackets)

Fix.	No.of mother	No.of indiv.	Total No.	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V	Total
1st wk	*	*	*	*	*	*	*	*	*
	(2)	(14)	(1400)	(-)	(-)	(5)	(7)	(17)	(29)
2nd wk	2	10	1000	-	-	7	-	14	21
	(2)	(10)	(1000)	(1)	(-)	(4)	(3)	(11)	(19)
3rd wk	2	13	1300	-	-	1	4	9	14
	(2)	(11)	(1100)	(-)	(-)	(4)	(2)	(18)	(24)
4th wk	2	15	1500	-	2	5	8	57	72
	(2)	(15)	(1500)	(-)	(3)	(2)	(4)	(15)	(24)
5th wk	*	*	*	*	*	*	*	*	*
	(2)	(11)	(1100)	(-)	(1)	(-)	(6)	(15)	(22)
6th wk	*	*	*	*	*	*	*	*	*
	(2)	(13)	(1300)	(-)	(-)	(-)	(7)	(16)	(23)
7th wk	1	8	800	-	-	4	8	12	24
	(2)	(15)	(1500)	(-)	(-)	(-)	(2)	(7)	(9)
Total	7	46	4600	-	2	17	20	92	131
	(14)	(89)	(8900)	(1)	(3)	(15)	(31)	(99)	(150)

* No pregnant female.

Table 18

Group-wise and region-wise distribution of chromatid breaks at 15 day gestation in F_1 progeny of different mothers mated at different weeks to the P. aeruginosa treated male parents

Region	Group					Total	
	I	II	III	IV	V	Obs.	Expect.
Proximal	1	4	12	8	1	26	30.33
Middle	5	6	14	-	-	25	30.33
Distal	13	10	13	4	-	40	30.33
Total Obs.	19	20	39	12	1	91	
Exp./No.	9.1	18.2	45.5	13.65	4.55		91

The region-wise distribution of chromatid breaks in the liver cells of F_1 embryos of the treated series (Table 18) also showed in the combined data that the distal region was more vulnerable than the proximal and middle region when the observed and expected numbers were compared. The same trend was also seen in the bone marrow chromosome aberration in adults treated with log culture of P. aeruginosa (Table 3).

The occurrence of the same trends of nonrandom distribution of breaks in chromatids in different groups and region of bone marrow chromosomes of P. aeruginosa treated specimens and that of liver cells of F_1 embryos at 15 day

gestation of the treated series would suggest that there could be some inherent weaker region in chromosome which would not only break by the presence of inducing agent but also in the next generation which supported the presence of some inherent weaker region in chromosomes advocated by Manna and Mazumdar (1967) by the study of X-ray induced X-chromosome aberrations in grasshopper and karyotype of mice treated with odd mutagens (Manna, 1975). Recently the heritable fragile site in humen cancer chromosomes has been advocated by Michelle and Rowley (1984) which would support the findings of nonrandom distribution of chromatid breaks in F_1 generation.

b) F_1 individuals of 3 month old

The F_1 individuals of different week treated and control lines at an age of 3 months were sacrificed after the mating programme for F_2 generation was over. The bone marrow of both sexes for somatic chromosome aberration study, the testis for the study of meiotic chromosome aberrations and epididymal sperm suspension samples of the male specimens for sperm count and sperm head morphology study were carried out (vide infra).

Chromosome aberrations in bone marrow cells

Control line :

In the first week mating line the bone marrow chromosome preparation made according to method described earlier revealed in five F_1 individuals of a mother the number of chromosome aberrations ranging between 2 and 8 of which the frequency of individual type was very negligible other than few cases of centromeric dissociation. Out of 500 metaphases examined taking 100 plates per specimen, the frequency was 5.6% or 28 of which the individual types were 15 or 3.0% including 13 or 2.60% centromeric dissociation cases (Table 19).

2nd week :

In a 2nd week mating mother, out of 6 F_1 individuals (Table 19) the number of aberrations ranged between 3 and 10. Out of the 600 metaphases of 6 individuals the average aberration frequency was 38 or 6.33% taking both gross and

individual types together of which 22 or 3.60% were individual types including 13 or 2.10% centromeric dissociation cases.

In 7 individuals of another mother of 2nd week control line mating, the bone marrow chromosome aberration number ranged between 2 and 8 (Table 19). In a total of 700 metaphases the average frequency was 48 or 6.80% of which 35 or 5.0% were individual type including 20 or 2.8% centromeric dissociation cases.

3rd week :

In the 5 F_1 individuals of one mother the number of bone marrow chromosome aberrations ranged between 4 and 9 and in a total of 500 metaphases the average frequency was 30 or 6.0% taking both gross and individual types together of which 12 or 2.4% were individual types including 9 or 1.80% centromeric dissociation cases (Table 19).

4th week :

In 6 F_1 individuals of a 4th week mating mother, the number of bone marrow chromosome aberrations ranged between 4 and 9 and out of the total 600 metaphases taking both gross and individual types together, the aberration frequency was 34 or 5.83% of which 23 or 2.83% were individual types including 16 or 2.66% centromeric dissociation cases (Table 19).

In 6 F_1 individuals of a 2nd mother, the number of chromosome aberrations ranged between 3 and 11 in different

individuals (Table 19). In a total of 600 metaphases the average aberration frequency was 33 or 5.50% of which 18 or 3.0% were individual types including 8 or 1.30% centromeric dissociation cases.

Treated line :

1st week :

In 3 F_1 individuals of a 1st week treated line mother, the number of chromosome aberrations was ranging between 31 and 37. In the combined data of 3 individuals out of 300 metaphases the average frequency was 103 or 34.33% of which 69 or 23.0% were individual types including 34 or 11.30% centromeric dissociation cases (Table 19).

2nd week :

In 5 F_1 individuals of a 2nd week treated line mother, the number of chromosome aberrations ranged widely between 8 and 61 and in a total of 500 metaphases, the average frequency was 206 or 41.40% taking both individual and gross type together of which 121 or 24.2% were individual type including 70 or 14.00% centromeric dissociation cases.

In 3 F_1 individuals of another mother of the 2nd week treated line, the number of aberrations ranged between 5 and 12 and in a total of 300 metaphases of the 3 individuals, the average aberration frequency of gross and individual types taken together was 26 or 8.66% of which 16 or 5.1%

were individual types including 15 or 5.0% centromeric dissociation cases (Table 19).

3rd week :

In 3 F_1 individuals of a 3rd week treated line mating mother, the number of bone marrow chromosome aberration ranged widely between 13 and 43. In a total of 300 metaphases, the average frequency was 94 or 31.33% taking both individual and gross types together of which 64 or 21.3% were individual types which included 44 or 14.66% centromeric dissociation cases (Table 19).

4th week :

In 3 F_1 individuals of a 4th week treated line mother, the number of bone marrow chromosome aberration ranged widely between 29 and 70 in different individuals. Out of the total 300 metaphases the average aberration frequency was 135 or 45.0% of which 75 or 25.0% were individual types including 38 or 19.0% centromeric dissociation cases (Table 19).

Centromeric dissociation :

It was mentioned while analysing the aberration frequency data that the frequency of centromeric dissociation was relatively high in the treated series. However, there was differential response in different groups because the frequency was highest in chromosomes belonging to group V and followed by group IV and group III while it was very rare in group I and in group II.

Table 19

Frequency distribution of bone marrow chromosome aberrations assessed in 100 plates per individual after birth in F_1 progeny of different mother mated to the bacterium P. aeruginosa treated and control male parents.

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberration			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps cons.	& Cent. diss.						
							Mother I						
							Control						
	1	M	-	-	-	-	1	1	5	-	5	6	6.0
	2	M	-	-	-	-	5	5	2	1	3	8	8.0
	3	F	1	-	-	-	3	4	2	-	2	6	6.0
	4	F	-	-	-	1	4	5	-	1	1	6	6.0
	5	F	-	-	-	-	-	-	1	1	2	2	2.0
Total			1	-	-	1	13	15	10	3	13	28	5.6
1st wk							Treated						
	1	M	4	-	6	-	18	28	2	7	9	37	37.0
	2	F	1	4	5	-	-	10	6	9	15	35	35.0
	3	F	1	-	14	-	16	31	-	-	-	31	31.0
Total			6	4	25	-	34	69	8	16	24	103	34.33

Table 19 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type Chr.	Frag.	Trans/ Asso.	Phy. type Gaps & const.	Cent. disso.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
						Mother I							
						Control							
	1	M	-	-	-	1	4	5	-	3	3	8	8.0
	2	M	-	-	-	-	-	-	7	-	7	7	7.0
	3	F	1	-	1	1	3	6	4	-	4	10	10.0
	4	F	-	-	1	1	2	4	-	1	1	5	5.0
	5.	F	1	-	-	-	4	5	-	-	-	5	5.0
	6	F	-	-	1	1	-	2	-	1	1	3	3.0
	Total		2	-	3	4	13	22	11	5	16	38	6.33
2nd wk						Treated							
	1	M	4	1	4	-	14	23	11	5	16	39	39.00
	2	M	1	1	2	-	22	26	13	12	25	51	51.00
	3	M	1	1	12	1	16	31	15	15	30	61	61.00
	4	F	3	2	17	-	11	33	15	-	15	48	48.00
	5	F	-	-	1	-	7	8	-	-	-	8	8.00
	Total		9	5	36	1	70	121	54	32	86	207	41.40

Table 19(Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations: Break type: Chr.	Break type: Frag.	Break type: Trans/ Asso.	Phy. type: Gaps & cons.	Phy. type: Cent. disso.	Total	Gross type aberrations: Num. cha.	Gross type aberrations: Others	Gross type aberrations: Total	Grand total	%
							Mother II						
							Control						
	1	M	-	1	1	-	6	8	-	-	-	8	8.0
	2	M	2	1	2	1	-	6	-	-	-	6	6.0
	3	M	-	-	-	-	3	3	-	2	2	5	5.0
	4	F	2	1	-	1	4	8	-	-	-	8	8.0
	5	F	-	-	-	-	-	-	-	2	2	2	2.0
	6	F	-	-	-	2	2	4	1	2	3	7	7.0
	7	F	1	-	-	-	5	6	2	-	2	8	8.0
	Total		5	3	3	4	20	35	3	6	9	44	6.28
2nd wk							Treated						
	1	M	1	-	1	-	6	8	1	3	4	12	12.00
	2	M	-	-	-	-	7	7	-	2	2	9	9.00
	3	F	-	-	-	1	-	1	-	4	4	5	5.00
	Total		1	-	1	1	13	16	1	9	10	26	8.66

Table 19 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.	Cent. disso.						
							Mother I						
							Control						
	1	M	-	-	-	-	2	2	2	4	6	8	8.00
	2	M	-	-	-	-	-	-	-	4	4	4	4.00
	3	F	-	-	-	-	-	-	-	5	5	5	5.00
	4	F	-	-	-	-	4	4	-	-	-	4	4.00
	5	F	2	-	-	1	3	6	1	2	3	9	9.00
Total			2	-	-	1	9	12	3	15	18	30	6.00
3rd wk													
							Treated						
	1	M	2	-	2	1	21	26	6	6	12	38	38.00
	2	F	2	2	8	1	16	29	12	2	14	43	43.00
	3	F	-	-	-	2	7	9	1	3	4	13	13.00
Total			4	2	10	4	44	64	19	11	40	94	31.30

Table 19 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.	Cent. disso.						
							Mother I						
							Control						
	1	M	1	-	-	1	3	5	2	1	3	8	8.00
	2	M	-	-	-	2	6	8	-	1	1	9	9.00
	3	M	-	-	-	-	5	5	-	-	-	5	5.00
	4	F	-	-	-	-	1	1	1	2	3	4	4.00
	5	F	-	-	-	1	1	2	3	-	3	5	5.00
	6	F	1	1	-	-	-	2	-	2	2	4	4.00
	Total		2	1	-	4	16	23	6	6	12	35	5.83
4th wk							Treated						
	1	M	-	-	-	-	24	24	1	4	5	29	29.00
	2	M	-	-	1	-	20	21	5	10	15	36	36.00
	3	F	5	-	2	2	21	30	32	8	40	70	70.00
	Total		5	-	3	2	65	75	38	22	60	135	45.00

Table 19 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. cha.	Other	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps	& Cent. cons. disso.						
							Mother II						
							Control						
	1	M	-	-	-	1	1	2	2	-	2	4	4.00
	2	M	-	1	2	-	1	4	2	-	2	6	6.00
	3	M	-	-	-	-	-	-	3	1	4	4	4.00
	4	F	-	-	-	-	-	-	-	3	3	3	3.00
	5	F	-	1	-	2	6	9	-	2	2	11	11.00
	6	F	-	-	2	1	-	3	1	1	2	5	5.00
	Total		-	2	4	4	8	18	8	7	15	33	5.50
4th wk							Treated						
	1	M	-	-	1	-	16	17	-	4	4	21	21.00
	2	F	-	1	7	1	22	31	13	3	16	47	47.00
	Total		-	1	8	1	38	48	13	7	20	68	34.00

Table 20

Compiled data of frequency distribution of bone marrow chromosome aberrations after birth in F_1 progeny of different mothers mated at different weeks with the bacterium, P. aeruginosa treated and control male parents (Control data are in brackets)

Fix.	No.of mother	No.of F_1 adult	No.of meta.	Individual type aberrations					Gross type aberrations			Grand total	%
				Break type			Phy. type	Total	Num. cha.	Others	Total		
				Chr. Frag.		Trans/ Asso.	Gaps & cont.	Cent. disso.					
1st wk	1	3	300	6	4	25	-	34 69	8	15	24	103	34.33
	(1)	(5)	(500)	(1)	(-)	(-)	(1)	(13) (15)	(10)	(3)	(13)	(28)	(5.60)
2nd wk	2	8	800	10	5	37	2	85 137	55	41	96	233	29.12
	(2)	(13)	(1300)	(7)	(3)	(3)	(8)	(33) (57)	(14)	(11)	(25)	(82)	(6.30)
3rd wk	1	3	300	4	2	10	4	44 64	19	11	30	94	31.33
	(1)	(5)	(500)	(2)	(-)	(-)	(1)	(9) (12)	(3)	(15)	(18)	(30)	(6.00)
4th wk	2	5	500	5	1	11	3	103 123	51	29	80	203	40.66
	(2)	(12)	(1200)	(2)	(3)	(4)	(8)	(41) (41)	(14)	(13)	(27)	(68)	(5.66)
Total	6	19	1900	25	12	83	11	266 393	133	96	240	633	33.21
	(6)	(35)	(3500)	(12)	(6)	(7)	(18)	(79) (125)	(41)	(42)	(83)	(208)	(5.94)

The frequencies in treated and control line specimens were 11.33% against 2.60% in 1st week, 10.37% against 2.53% in 2nd week, 20.33% against 1.8% in 3rd week, 20.60% against 2.0% in 4th week and 13.38% against 2.25% in all weeks taken together. The trend was more or less the same as found in the Pseudomonas treated and nutrient broth injected specimens (Table 21). Anyhow the occurrence of the higher frequency of centromeric dissociation in the bone marrow cells of F_1 individuals of the treated line could be explained as due to some genetic factor but it is yet to be substantiated.

General comment :

The average frequency of chromosome aberrations in different F_1 individuals of the control line varied narrowly between 5.6% and 6.33% while it was ranging widely between 8.66% and 45.0% in F_1 individuals of the treated line (Table 19). In the combined data of individual and gross type aberrations, the average frequency was 34.33% against 5.6% in the first week, 29.12% against 6.3% in the 2nd week, 31.33% against 6.0% in the 3rd week, 40.66% against 5.66% in the 4th week treated and control line respectively. If the aberration data of all weeks were combined, the average frequency was 33.21% in the treated line and 5.94% in the control line indicating very significantly high frequency of chromosome aberrations in the treated line. This could be interpreted as due to the mutagenic factor in F_1 generation which was induced by the treatment of the bacterial culture in the parental generation. Even this was indicated by the chromosome aberration data in liver cells of embryos of 15 day gestation because the frequency in treated line was higher than that of control line (Tables 15 and 16). However,

Table 21

Compiled data of frequency distribution of centric dissociation in different of chromosomes groups in bone marrow of 30 day old F_1 progeny of different mothers mated at different weeks with the bacterium, *P. aeruginosa* treated and control male parent. 100 cells/ind. were counted (Control data are in brackets)

Fix.	No.of mother	No.of ind.	Total No. of met.	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V	Total	%
1st wk	1	3	300	-	-	6	11	17	34	11.33
	(1)	(5)	(500)	(-)	(-)	(-)	(1)	(12)	(13)	(2.60)
2nd wk	2	8	800	-	-	5	27	51	83	10.37
	(2)	(13)	(1300)	(1)	(1)	(7)	(4)	(20)	(33)	(2.52)
3rd wk	1	3	300	2	1	14	14	30	61	20.33
	(1)	(5)	(500)	(-)	(-)	(-)	(-)	(9)	(9)	(1.80)
4th wk)	2	5	500	-	1	21	35	46	103	20.60
	(2)	(12)	(1200)	(-)	(2)	(3)	(4)	(15)	(24)	(2.00)
Total	6	19	2100	2	2	46	87	144	281	13.38
	(6)	(35)	(3500)	(1)	(3)	(10)	(9)	(56)	(79)	(2.25)

the chromosome aberration data of the liver cells of embryos and that of bone marrow cells of 3 month old F_1 individuals of the treated lines showed that the frequency was relatively high in the latter tissues as it was 13.9% against 29.12% in the 2nd week, and 15.07% against 31.33% in the 3rd week and 23.6% against 40.6% in 4th week (Tables 16 and 20). On the otherhand if the average aberration frequency data of the liver in embryos and bone marrow of F_1 adults of the control line compared, it was 6.14% against 5.6% in the 1st week, 7.1% against 6.3% in the 2nd week, 5.1% against 6.0% in the 3rd week, 4.8% against 5.66% in the 4th week respectively (Tables 16 and 20) showing not much difference in the two tissues. Since the aberration frequency in the control line did not differ so widely as that of the treated line referred to before, it could be due to the differential segregation of the mutagenic factor present in the treated line.

Sex and aberration frequency relationship if any :

The chromosome aberration data in liver cells of embryos and bone marrow cells of adult F_1 individuals analysed before revealed that frequency was much higher in F_1 generation of the treated line then that of control. The data have been also analysed here with a view to find out if the sex factor had any differential response (Table 22).

The sex of male and female embryos was determined by the presence of the minute Y in male and its absence in females besides two autosomes of more or less same size in Vth group in the liver cells (Crippa, 1964). The

average frequency of aberration in the combined data of male and female specimens of control line of different weeks did not differ appreciably. The maximum difference of 6.50% found between male and female embryos was in the 1st week. The average aberrations frequency of 42 male and 46 female embryos of seven week control lines was 5.11% in males and 5.81 in females indicating that there was no significant difference in the two sexes and secondly the sex ratio was upto the expectation. The data of male and female embryos in the treated line had some limitation because no implantation was found in 1st, 5th, 6th week and in the remaining four weeks the average frequency of aberration was variable to some extent. The maximum difference found in 4th week treated line was 28.0% in male embryos against 19.75% in female embryos making a difference of 9.25. In other weeks, the difference was not so glaring. In a total of 24 male and 22 female embryos of four weeks, the average aberration frequency was 20.62 in males and 21.37 in females indicating clearly that there was no influence of 'sex factor' and the sex ratio was upto the expectation.

Similar analysis of the chromosome aberration frequency in bone marrow cells of F_1 males and females of control line (Table 22) did not show much difference except in 1st week. In a total of 15 males and 19 females, the average aberration frequency was 4.0 in males and 5.49 in females. Therefore, though the aberration frequency in F_1 female embryos and adults was little higher than that of males but the difference was not significant.

In the 4th week treated line, on the otherhand the difference of 28.34 in the average aberration frequency between F_1 males and female was appreciable but in other weeks, it was not so glaring. However, in a total of 9 males and 13 females of treated lines of four weeks, the average aberration frequency was 34.22 in males and 35.75 in females rendering practically no difference in the aberration frequency between two sexes. Therefore, it could be concluded that the sex factor played no role in influencing the aberration frequency both in control and in treated line embryos and adults. The glaring difference found in some individual week could be due to the limitation of sampling.

Table 22

Sex-wise compiled data of chromosome aberration in liver-cells of 15dday old embryos (Table 15) and bone marrow chromosomes in 3 month old F_1 individuals (Table 19) of control and treated lines of different weeks

In liver of 15 day old embryos					
Control			Treated		
Week	Sex	Average aber.	Week	Sex	Average aber.
1st	6 male	6.50	1st	No	Implantation
	8 female	5.87			
2nd	4 male	6.50	2nd	6 male	13.00
	6 female	7.50		4 female	18.50
3rd	6 male	4.83	3rd	6 male	14.50
	4 female	5.50		9 female	15.57
4th	8 male	4.75	4th	7 male	28.00
	7 female	4.85		8 female	19.75
5th	6 male	4.00	5th &		
	5 female	5.80	6th	No implantation	
6th	6 male	4.66			
	7 female	6.28			
7th	6 male	4.50	7th	5 male	27.00
	9 female	4.88		3 female	31.66
Total	42 male	5.11		24 male	20.62
	46 female	5.81		22 female	21.37

Table 22 (Contd.)

In bone marrow cells					
Control			Treated		
Week	Sex	Average aber.	Week	Sex	Average aber.
1st	2 male	7.00	1st	1 male	37.00
	2 female	4.66		2 female	33.00
2nd	5 male	4.80	2nd	4 male	33.25
	8 fmelae	6.00		4 female	25.00
3rd	2 male	6.50	3rd	1 male	38.00
	3 female	6.00		2 female	28.00
4th	6 male	6.00	4th	3 male	28.66
	6 female	5.23		5 female	57.00
Total	15 male	4.00		9 male	34.22
	19 female	5.49		13 female	35.75

C. Meiotic chromosome aberrations in 3 month old F_1 males :

The F_1 male individuals of 3 month old of both control and treated lines when sacrificed for the study of the bone marrow chromosome aberration, the meiotic chromosome preparations from their testes were also made separately according to the method described before. The frequency of aberration has been assessed in spermatogonial metaphase, diakinesis, metaphase I and metaphase II but the paucity of the dividing cells limited the data.

Spermatogonial metaphase :

The chromosome aberration types in spermatogonial metaphases were of similar nature as described in the Pseudomonas culture treated males described before. The aberrations were individual types consisting of chromatid break, acentric fragment, centric fusion, precocious centromeric separation etc. (PM 86-90; Figs. 76-81) and gross types consisting of numerical changes, stickiness etc. The individual type aberrations were limited in control series in comparison to that of treated series. Anyhow the aberration frequency in males in different weeks for both the control and the treated line was determined to compare the effect.

Control line :

1st week :

Two male individuals of the 1st week control line had no individual type aberration in 150 spermatogonial

Explanation of Photomicrographs

Photomicrographs of some affected spermatogonial metaphase in male germinal cells of F_1 individuals produced after mating of normal females to Pseudomonas culture treated males.

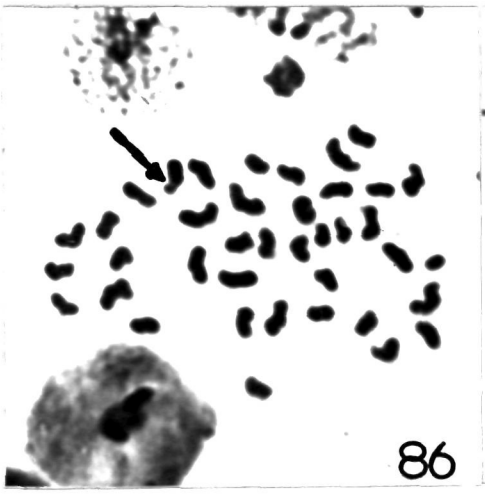
PM 86. Spermatogonial metaphase plate with a constricted chromosome.

PM 87. A fragment of unknown origin.

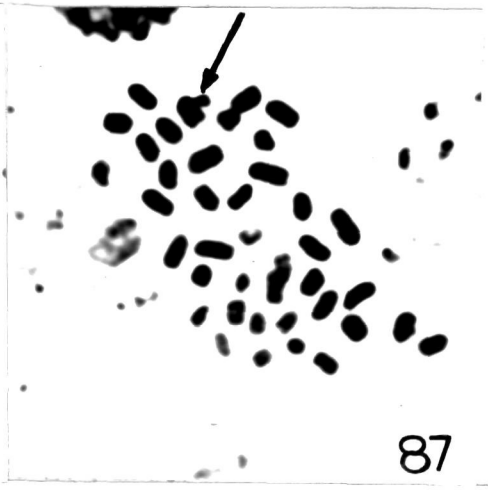
PM 88. Plate showing spiral appearance of chromosome of which are showing a gap and a fragment.

PM 89. A polyploid cells with highly condensed chromosomes.

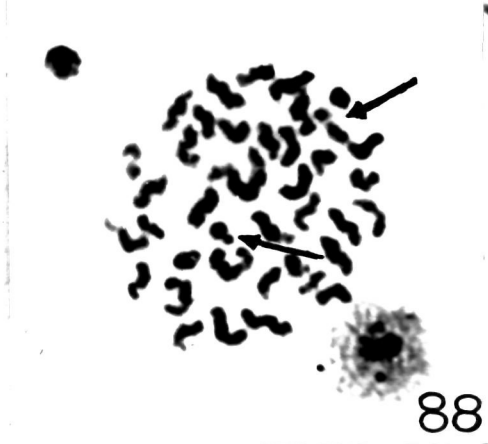
PM 90. Polyploid cell, some showing centric fusion.



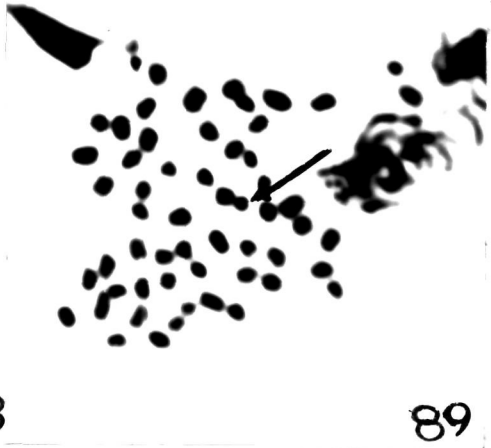
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Explanation of figures

Camera lucida drawing of a part of spermatogonial metaphase in F_1 showing different types of aberrations

Fig. 76. A chromatid break.

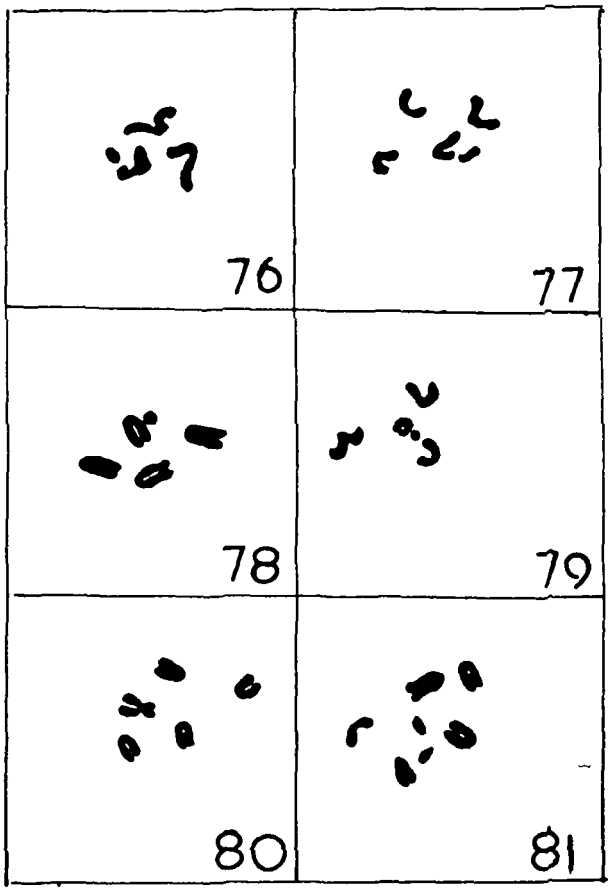
Fig. 77. A chromatid break.

Fig. 78. A fragment.

Fig. 79. A fragment.

Fig. 80. A centric fusion between the chromosomes.

Fig. 81. Precocious centromeric dissoication of a chromosome.


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metaphases. Taking all types together the aberration frequency in two individuals was 9.33% and 6.66% and out of the total 150 plates, the average frequency was 8.0% (Table 23).

2nd week :

In 2 F_1 males of a 2nd week F_1 control line, the total spermatogonial aberration frequency was 7,14% in one individual and 9.41% in another. In the combined data of the two, out of 155 plates the average frequency was 8.38%. In 3 F_1 males of another mother of 2nd week control line, the frequency of aberrations ranged between 2.63% and 23.8%. In one individual it was particularly absent except for a polyploid cell. In a total of 111 spermatogonial metaphases examined in 3 individuals, the average frequency was 10.81% (Table 23).

3rd week :

In $2F_1$ individuals of the 3rd week control line, the aberration frequencies were 16.66% and 9.87%. In the combined data, the average frequency was 12.76% (Table 23).

4th week :

In 3 F_1 individuals of 4th week control line, the frequency of aberrations ranged between 4.0% and 9.0% and out of the total 300 spermatogonial metaphases of three individuals, the average frequency was 6.33%. Three other F_1 individuals of another mother of 4th week control line, the aberration frequencies were 7.27%, 6.89% and 10.6%. In

the combined data, out of 208 spermatogonial metaphases, the average frequency was 8.17%. Therefore, in F_1 individuals of 1st week to 4 week control lines, the average frequency of spermatogonial chromosome aberrations ranged between 6.33% and 12.76% of which the frequency of individual type was conspicuously low. It was absent in F_1 individual of the 1st week control line, 7 in 266 or 2.63% in the 2nd week, 1 in 141 or 0.92% in the 3rd week and 11 in 508 or 2.16% in 4th week control line (Table 23).

Treated line :

As compared to the control line, data of the spermatogonial chromosome aberrations were limited in the treated line but the frequency was significantly high.

1st week :

In one F_1 individual of 1st week, 15 spermatogonial metaphases were observed. The frequency of total aberration was 46.6% of which none was of individual type (Table 23).

2nd week :

In two F_1 individuals out of 57 spermatogonial metaphases examined, the frequency of total aberration was 50.87% of which 5 or 8.77% were individual types (Table 23).

3rd week :

In one F_1 individual of 3rd week treated line, out

of 25 spermatogonial metaphases, the frequency of total aberration was 41.33% of which 8 or 10.6% were individual type (Table 23).

4th week :

In four F_1 individuals of 2 mothers of 4th week, 1 had no aberration out of 24 plates examined while in the remaining 3 individuals, the average frequency was 61.84%. In a total of 135 spermatogonial metaphase plates examined only 3 or 2.2% were individual type (Table 23).

The chromosome aberration data in spermatogonial metaphases of F_1 individuals if combined in control line and compared with that of treated line, the frequency would be found conspicuously high in the treated line. The average frequencies were 46.6% in treated line against 8.0% in the control line of the 1st week, 50.87% against 9.39% in the 2nd week, 41.83% against 12.76% in the 3rd week, 52.2% against 7.08% in the 4th week and 49.01% against 8.54% in the combined data of all weeks (Table 24). Further, the frequency of individual type aberration was relatively low both in control and treated series. It was absent in both cases in 1st week, 5 in 57 or 8.77% in treated against 7. in 266 or 2.63% in control series of 2nd week, 8 in 75 or 10.66% against 1 in 141 or 0.92% in the 3rd week and 3 in 159 or 1.88% against 11 in 508 or 2.16% in the 4th week (Table 24). In the combined data of all weeks, 16 in 306 or 5.22% in the treated series against 19 in 1065 or 1.50% in the control series (Table 24). Therefore, frequency of individual type aberration was higher in the treated line of 2nd and 3rd week mating and also in the average of all 4 weeks while it was little lower in the 4th week.

Table 23

Frequency distribution of chromosome aberrations in Stermatogonial metaphase cells of 30 day old F_1 males of control and bacterium P. aeruginosa. Treated mating male parents of different weeks

Fix.	Ind.	Cells coun.	Individual type aberrations: Break type: Chr.	Frag.	Trans. /Asso.	Phy.type Gaps & Cons.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
							Mother I					
							Control					
	1	75	-	-	-	-	-	7	-	7	7	9.33
	2	75	-	-	-	-	-	4	1	5	5	6.66
	Total	150	-	-	-	-	-	11	1	12	12	8.00
1st wk							Treated					
	1	15	-	-	-	-	-	2	5	7	7	46.60
	Total	15	-	-	-	-	-	2	5	7	7	46.60
2nd wk							Control					
	1	70	1	1	-	-	2	2	1	3	5	7.14
	2	85	-	-	1	2	3	3	2	5	8	9.41
	Total	155	1	1	1	2	5	5	3	8	13	8.38

Table 23 (Contd.)

Fix.	Ind.	Cells coun.	Individual type aberrations: Break type Chr.	Frag.	Trans./ Asso.	Ph.type Gaps & cons.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
						Mother I						
2nd wk						Treated						
	1	22	-	-	-	-	-	2	11	13	13	59.09
	2	35	2	1	2	-	5	6	5	11	16	45.71
Total		57	2	1	2	-	5	8	16	24	29	50.87
						Mother II						
						Control						
	1	21	-	-	-	1	1	2	2	4	5	23.80
	2	38	-	-	-	-	-	1	-	1	1	2.63
	3	52	-	-	-	1	1	5	-	5	6	11.53
Total		111	-	-	-	2	2	8	2	10	12	10.81
						Mother I						
3rd wk						Control						
	1	60	-	-	-	-	-	4	6	10	10	16.66
	2	81	-	-	-	1	1	3	4	7	8	9.87
Total		141	-	-	-	1	1	7	10	17	18	12.76
						Treated						
	1	75	2	1	3	2	8	8	15	23	31	41.33
Total		75	2	1	3	2	8	8	15	23	31	41.33

Table 23 (Contd.)

Fix.	Ind. No.	Cells coun.	Individual type aberrations: Break type Chr.	Frag.	Trans/ Asso.	Phy.type Gaps & Cons.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
4th wk							Mother I					
							Control					
	1	100	-	1	-	-	1	3	2	5	6	6.00
	2	100	1	-	-	-	1	3	-	3	4	4.00
	3	100	1	-	-	1	2	4	3	7	9	9.00
	Total	300	2	1	-	1	4	10	5	15	19	6.33
							Treated					
	1	45	-	-	-	-	-	22	13	35	35	41.33
	2	30	1	-	-	-	1	20	1	21	22	73.33
	3	24	-	-	-	-	-	-	-	-	-	-
	Total	99	1	-	-	-	1	42	14	56	57	57.57
							Mother II					
							Control					
	1	55	-	-	-	2	2	-	2	2	4	7.23
	2	87	-	1	1	-	2	3	1	4	6	6.89
	3	66	2	-	-	1	3	4	-	4	7	10.60
	Total	208	2	1	1	3	7	7	3	10	17	8.17
							Treated					
	1	60	-	-	2	-	2	23	1	24	26	43.33
	Total	60	-	-	2	-	2	23	1	24	26	43.33

The frequency of chromosome aberration in spermatogonial metaphases specially treated line males was high mainly due to the presence of gross types, the genetical significance of which was not very clear. On the otherhand, the frequency of individual type aberration was relatively low but the genetical significance of the break type ones was known. In spite of the low frequency they were relatively more in the treated line. Therefore, the chromosome aberration frequency was found higher in bone marrow cells (Table 21) as well as in spermatogonial metaphases in the treated lines. This could suggest that the mutagenic factor was transmitted for mating of Pseudomonas treated males with virgin females in parental generation for which frequency of aberration both in somatic and germinal chromosome was higher than that of control.

Diakinesis :

Like spermatogonial metaphases, not very many diakinesis cells were encountered per individual of both control and treated F_1 males (Table 25). The aberration were individual types consisting of chromosome and chromatid breaks, fragments, translocation or bivalent association, precocious separation of autosomes and sex chromosome and gross types showing stickiness, polyploidy etc. (PM 91-95; Figs. 82-91).

Control lines :

1st week :

Two F_1 individuals of 1st week control line had the

Explanation of Photomicrographs

Photomicrographs of some affected Diakinesis in male germinal cells of F_1 individuals produced after mating of normal females to *Pseudomonas* culture treated males

PM 91. Two bivalents each with a chromosome break.

PM 92. Two bivalents each with a chromatid break.

PM 93. A translocation or bivalent association.

PM 94. 18 bivalents, one with a chromatid break and a fragment.

PM 95. Sticky bivalents showing interbivalent connection.


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Explanation of figures

Camera lucida drawing of a part of diakinesis nuclei of F_1 males showing different types of aberration.

Fig. 82. A bivalent with a chromosome break.

Fig. 83. A bivalent with a chromosome constriction.

Fig. 84. A bivalent with a chromatid break.

Fig. 85. A bivalent with a chromosome break.

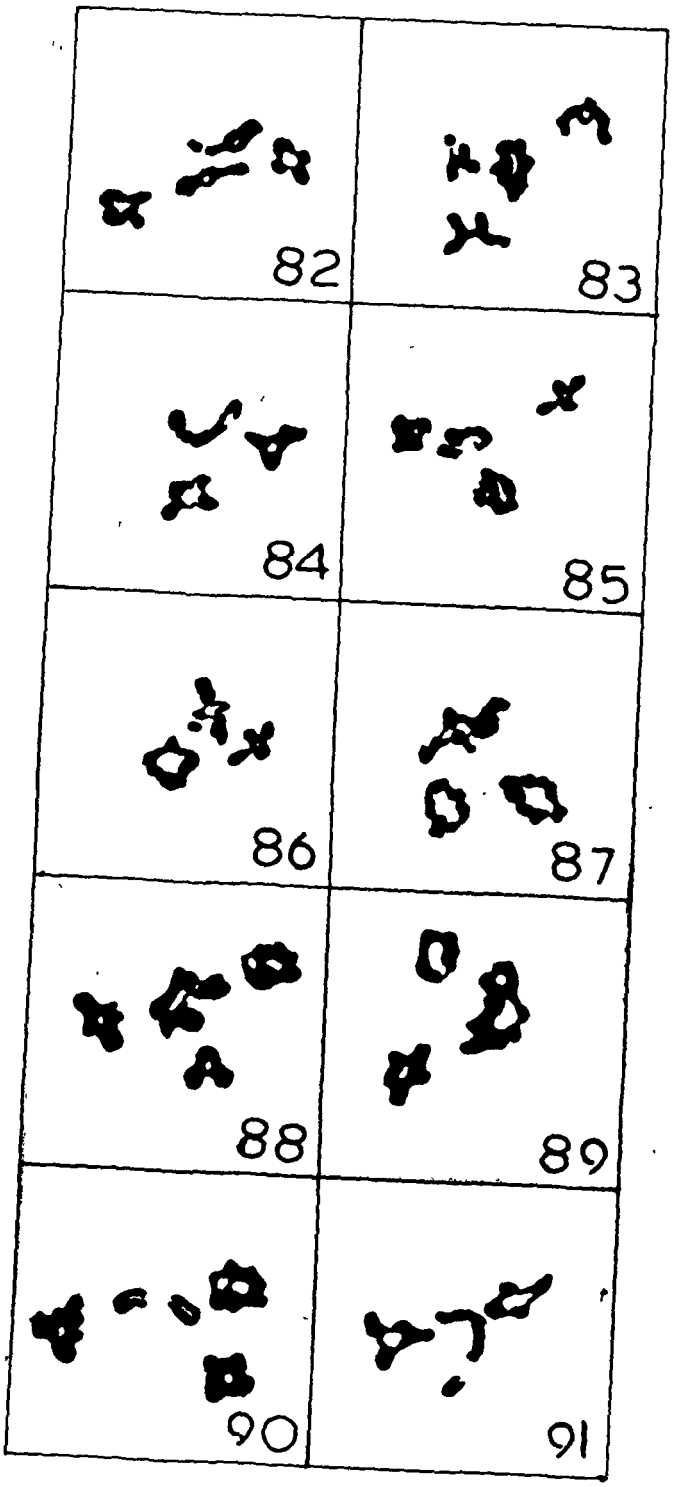
Fig. 86. A fragment of unknown origin.

Figs. 87 and 88. A translocation or bivalent association.

Fig. 89. A multivalent.

Fig. 90. Precocious separation of the autosomes and a multivalent.

Fig. 91. Precocious separation of X and Y.


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frequency of aberration of 10.0% and 5.72% of which individual types other than some instances of precocious separation of autosome and sex chromosome pair were absent. Thus out of total 75 diakinesis nuclei, the total frequency was 8.0% of which 3 or 4% were cases of precocious separation (Table 25).

2nd week :

In the 2nd week control line 2 F_1 individuals of one mother and 3 of another mother had aberration frequency ranging between 5.71% and 14.28% of which the frequency of individual type was relatively low (Table 25).

3rd week :

Two F_1 individuals of a 3rd week mother, the aberration frequencies were 3.33% and 7.27% of which the individual types consisted only some precocious separation cases (Table 25).

4th week :

Six F_1 individuals of 2 mothers in the 4th week control line had the aberration frequency in 3 individuals of one mother ranging between 6.66% and 7.69% with an average of 7.11% determined from 225 cells and in other 3 individuals of the 2nd mother, had the frequency varying between 7.36% and 14.28% with an average of 10.58% determined from 189 diakinesis cells (Table 25).

Table 24

Compiled data of chromosome aberration in spermatogonial metaphase cells of F_1 males of control and bacterium, P. aeruginosa treated mating parents of different weeks. Control data are in brackets.

Fix.	Ind. No.	Cells coun.	Individual type aberrations					Gross type aberrations			Grand total	%
			Break type			Ph.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Ass.	Gaps & cons.						
1st wk	1	15	-	-	-	-	-	2	5	7	7	46.60
	(3)	(150)	(-)	(-)	(-)	(-)	(-)	(11)	(1)	(12)	(12)	(8.00)
2md wk	2	57	2	1	2	-	5	8	16	24	29	50.87
	(5)	(266)	(1)	(1)	(1)	(4)	(7)	(13)	(5)	(18)	(25)	(9.39)
3rd wk	1	75	2	1	3	2	8	8	15	23	31	41.33
	(2)	(141)	(-)	(-)	(-)	(1)	(1)	(7)	(10)	(17)	(18)	(12.76)
4th wk	4	159	1	-	2	-	3	65	15	80	83	52.20
	(6)	(508)	(4)	(2)	(1)	(4)	(11)	(17)	(8)	(25)	(36)	(7.08)
Total	8	306	5	2	7	2	16	83	51	134	150	49.01
	(15)	(1065)	(5)	(3)	(2)	(9)	(19)	(48)	(24)	(72)	(91)	(8.54)

Treated lines :

The number of male individuals available for the study of diakinesis cells was less than that of control.

1st week :

In one individual of the 1st week treated line out of 40 cells encountered, the aberrations frequency was 25.0% of which 5 or 12.5% were individual type. The latter was of mainly multivalent or bivalent association.

2nd week :

In the two F_1 individuals of the 2nd week treated line, the frequency of aberration in diakinesis cells was 64.0% in one and 33.33% in another individual and the average frequency was 44.28% (Table 25).

3rd week :

In one F_1 individual of 3rd week treated line, the frequency of aberration in diakinesis was 34.28%.

4th week :

In the 4th week treated line 3 individuals of one mother had the aberration frequency in 147 diakinesis cells ranging between 27.69% and 40.0% and the average was 32.65%. In 1 F_1 individual of another mother the aberration frequency was 34.28% (Table 25).

As observed in the spermatogonial metaphase, the frequency of chromosomal aberration at diakinesis stage did not vary very widely in the control lines of four weeks mating. The average frequency ranged between 5.88% and 9.05% (Table 26). On the other hand, in the treated line the frequency was relatively high. In the compiled data the frequencies in treated and control lines were 25.0% against 8.0% in the 1st week, 44.28% against 9.05% in the 2nd week, 34.28% against 5.88% in the 3rd week, 32.43% against 8.69% in the 4th week and 34.24% against 8.45% in the average of all weeks taken together (Table 26). However, the higher frequency was partly due to the occurrence more cases of precocious separation of some autosomes and the X and Y of the sex pair. The break type of aberrations like chromosome break, fragment of unknown origin, translocation or association etc. were in low frequency in the treated series while they were very rare or absent in control line (Table 26). Even with the limitation of data, on the whole it was apparent that the aberration frequency in chromosomes of diakinesis cells was higher in treated lines than that of control. In other wards the occurrence of higher frequency of chromosome aberration in F_1 generation of the treated lines would lead us to think that it was due to mutagenic factor originated by the treatment of Pseudomonas culture in male parent was transmitted to the F_1 generation through the chromosome vehicle.

Metaphase I

The types of chromosome aberrations found in metaphase I plates were more or less the same as found in the diakinesis stage. There were both individual type and gross type aberrations (PM 96-100; Figs. 92-97).

Explanation of Photomicrograph

Photomicrographs of some affected metaphase I in male germinal cells of F_1 individuals produced after mating of normal females to *Pseudomonas* culture treated males.

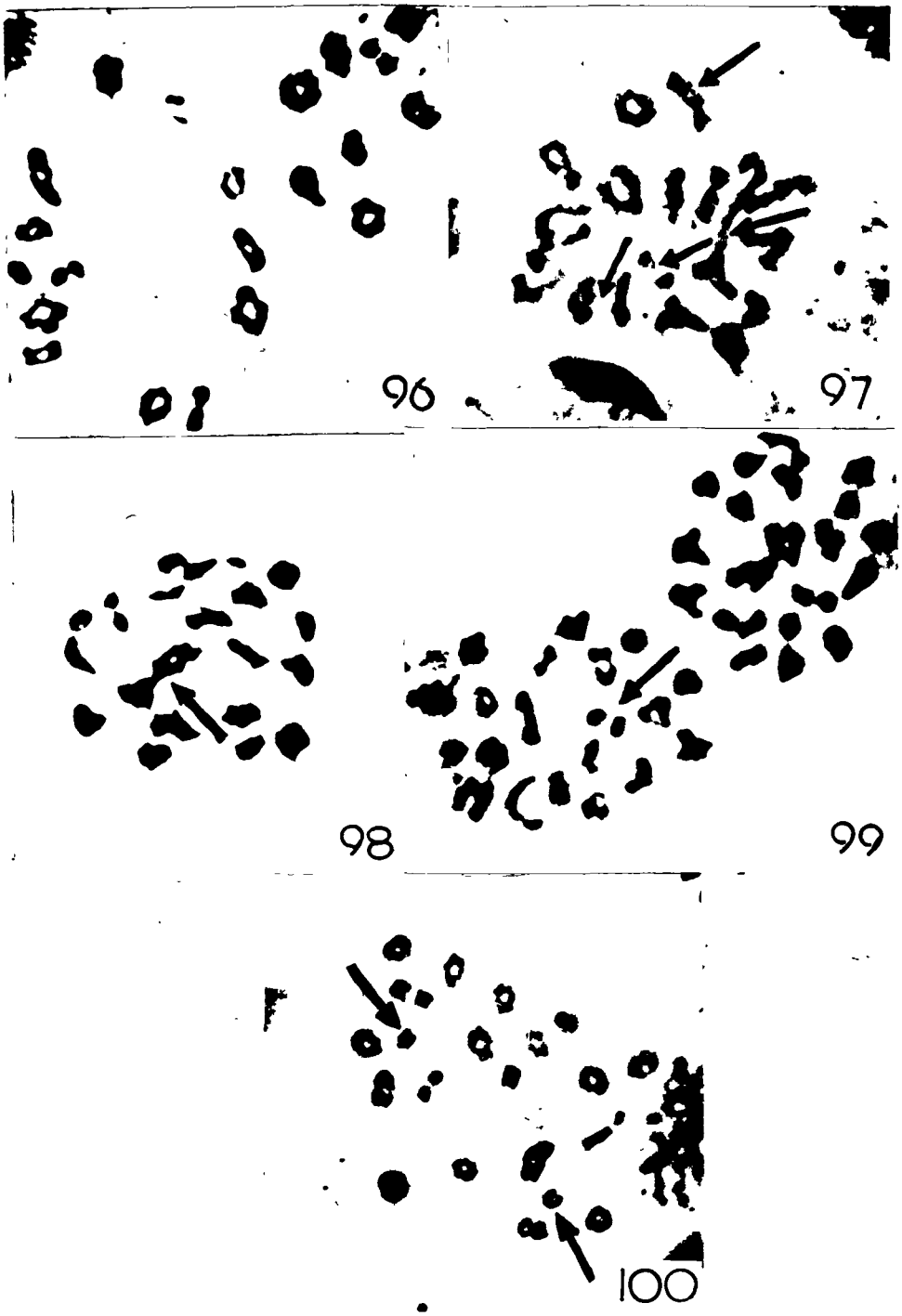
PM 96. Aneuploid metaphase I plates.

PM 97. Two chromatid breaks, one translocation and the precocious separation of an autosome.

PM 98 . A quadrivalent of association.

PM 99. Two plates, one with precocious separation of autosome.

PM 100. 20 bivalents, of which one showing precocious separation.


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Explanation of figures

Camera lucida drawing of a part of metaphase I plates showing different types of aberrations.

Fig. 92. A chromatid break.

Fig. 93. A chromatid break.

Fig. 94. A chromatid break.

Fig. 95. A bivalent association or translocation.

Fig. 96. A bivalent association.

Fig. 97. The precocious separation of autosomes and sex chromosomes.

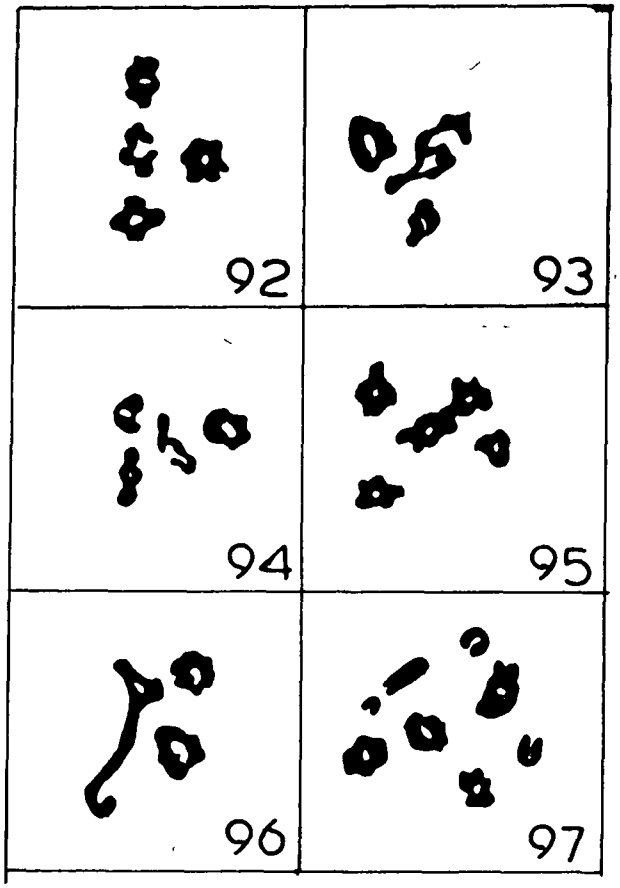

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Table 25

Frequency distribution of chromosome aberration in diakinesis cells of F_1 males of control and the bacterium, P. aeruginosa treated mating parents of different week

Fix.	Ind. No.	Cells coun.	Individual type aberrations: Break type: Chr.	Frag.	Mute/ Asso.	Pre.sep Aut.	Sex	Total	Gross type aberrations: Num. ch.	Others	Total	Grand total	%
1st wk								Mother I					
								Control					
	1	40	-	-	-	1	-	1	-	3	3	4	10.00
	2	35	-	-	-	1	1	2	-	-	-	2	5.72
	Total	75	-	-	-	2	1	2	-	3	3	6	8.00
								Treated					
	1	40	1	-	4	-	-	5	-	5	5	10	25.00
	Total	40	1	-	4	-	-	5	-	5	5	10	25.00
2nd wk								Control					
	1	50	1	-	-	-	-	1	-	2	2	3	6.00
	2	45	-	-	-	1	-	1	-	3	3	4	8.88
	Total	95	1	-	-	1	-	2	-	5	5	7	7.36
								Treated					
	1	25	2	1	1	3	1	8	1	7	8	16	64.00
	2	45	2	-	2	1	3	8	4	3	7	15	33.33
	Total	70	4	1	3	4	4	16	5	10	15	31	44.28

Table 25 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Pre.sep.		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Mute/ Asso.	Aut.	Sex						
						Mother II							
2nd wk						Control							
	1	49	2	-	-	1	-	3	2	2	4	7	14.28
	2	70	1	-	-	1	1	2	-	2	2	4	5.71
	3	40	-	-	-	2	-	2	2	1	3	5	12.50
	Total	159	3	-	-	4	1	7	4	5	9	16	10.06
						Treated							
	1	Reduced testes											
	2	Do											
						Mother I							
3rd wk						Control							
	1.	30	-	-	-	-	1	1	-	-	-	1	3.33
	2	55	-	-	-	1	1	2	-	2	2	4	7.27
	Total	85	-	-	-	1	2	3	-	2	2	5	5.88
						Treated							
	1	35	2	1	1	3	-	7	1	4	5	12	34.28
	Total	35	2	1	1	3	-	7	1	4	5	12	34.28

Table 25 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type Chr.	Frag.	Mute/ Asso.	Pre.sep. Aut.	Sex.	Total	Gross type aberrations: Num. ch.	Others	Total	Grand total	%
4th wk													
								Mother I					
								Control					
	1	65	-	-	-	2	-	2	1	2	3	5	7.69
	2	75	-	-	2	-	-	2	-	3	3	5	6.66
	3	85	2	-	-	-	1	3	2	1	3	6	7.05
Total		225	2	-	2	2	1	7	3	6	9	16	7.11
								Treated					
	1	35	3	1	-	-	-	4	3	7	10	14	40.00
	2	47	3	-	4	-	-	7	4	5	9	16	34.04
	3	65	3	-	3	2	-	8	3	7	10	18	27.69
Total		147	9	1	7	2	-	19	10	19	29	48	32.65
								Mother II					
								Control					
	1	49	-	1	-	-	3	4	3	2	5	7	14.28
	2	45	1	-	1	-	-	2	2	2	4	6	13.33
	3	95	-	-	-	-	-	-	5	2	7	7	7.36
Total		189	1	1	1	-	3	6	8	6	14	20	10.58
								Treated					
	1	35	2	1	1	-	1	5	2	5	7	12	34.28
Total		35	2	1	1	-	1	5	2	5	7	12	34.28

Control line :

1st week :

In the first week control line two F_1 male individuals had out of 100 plates each 8.0% and 7.0% aberrations of which 3.0% and 5.0% were individual types respectively (Table 27).

2nd week :

In the 2nd week control line, 100 plates from each of two F_1 individuals of one mother had 1.0% in one and 8.0% aberration in another individual. On the other hand, three F_1 males of a 2nd mother, the aberration frequency varied between 4.0% and 8.0%. The average frequency was 6.0% (18 in 300 plates) (Table 27).

3rd week :

In two F_1 individuals of the 3rd week control line the aberration frequency determined in 100 plates each was 1.0% and 3.0% and the average frequency was 2.0% (Table 27).

4th week :

In three F_1 male individuals of 4th week control line the frequency of aberration determined from 100 plates each varied between 5.0% and 9.0% and in 3 individuals of another mother the aberration frequency ranged between 5.0% and 8.0% (Table 27).

Treated line :

1st week :

In the 1st week treated line one F_1 male individual had out of 100 plates 30.0% aberrations.

2nd week :

In two F_1 male individuals of 2nd week treated line out of 100 plates each, there were 34.0% and 36.0% aberrations (Table 27).

3rd week :

One F_1 individual of 3rd week treated line had out of 100 metaphase plates 42.0% aberrations.

4th week :

In three F_1 male individuals of 4th week treated line out of 100 metaphase plates, the aberration frequency ranged between 15.0% and 73.0% while 1 individual of another mother had 43.0% aberrations (Table 27). Therefore, at metaphase I the frequency of chromosome aberration was much higher than that of control. The compiled data of chromosome aberration frequency at metaphase I (Table 28) of four week control and treated lines would show that the average chromosome aberration frequencies were 30.0% in the treated line against 7.5% in control line of the 1st week, 35.0% against 5.4% in the 2nd week, 42.0% against 2.0% in the 3rd week, 38.25% against 6.8% in the 4th week and 36.87% against

Table 27

Frequency distribution of chromosome aberration in metaphase I cells of F_1 males of bacterium, P. aeruginosa treated and control males mating parents of different weeks to virgin females

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Pre.sep.		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Mute/ Asso.	Aut.	Sex						
1st wk						Mother I							
						Control							
	1	100	-	-	-	3	-	3	-	5	5	8	8.00
	2	100	-	-	1	2	2	5	1	2	2	7	7.00
	Total	200	-	-	1	5	2	8	1	6	7	15	7.50
						Treated							
	1	100	5	1	2	6	5	19	7	4	11	30	30.00
	Total	100	5	1	2	6	5	19	7	4	11	30	30.00
2nd wk						Control							
	1	100	-	-	-	-	-	-	-	1	1	1	1.00
	2	100	-	-	-	3	3	6	1	1	2	8	8.00
	Total	200	-	-	-	3	3	6	1	2	3	9	4.50
						Treated							
	1	100	2	-	3	9	9	23	6	5	11	34	34.00
	2	100	5	-	4	9	8	26	8	2	10	36	36.00
	Total	200	7	-	7	18	17	49	14	7	21	70	35.00

Table 27 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Mute/ Asso.	Pre.sep.: Aut.	Sex	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
2nd wk													
								Mother II					
								Control					
	1	100	-	-	-	3	2	5	1	2	3	8	8.00
	2	100	-	-	-	2	-	2	1	1	2	4	4.00
	3	100	-	-	1	1	2	4	-	2	2	6	6.00
Total		300	-	-	1	6	4	11	2	5	7	18	6.00
								Treated					
	1	Reduced testis											
	2	Do											
3rd wk													
								Mother I					
								Control					
	1	100	-	-	-	-	1	1	-	-	-	1	1.00
	2	100	-	-	-	1	1	2	-	1	1	3	3.00
Total		200	-	-	-	1	2	3	-	1	1	4	2.00
								Treated					
	1	100	3	1	3	8	10	25	7	10	17	42	42.00
Total		100	3	1	3	8	10	25	7	10	17	42	42.00

Table 27 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Mute/ Asso.	Pre-sep.: Aut.	Sex	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
4th wk									Mother I				
									Control				
	1	100	-	-	2	2	-	4	1	-	1	5	5.00
	2	100	-	-	3	3	-	6	-	3	3	9	9.00
	3	100	-	-	-	2	2	4	2	1	3	7	7.00
	Total	300	-	-	5	7	2	14	3	4	7	21	7.00
									Treated				
	1	100	-	-	18	7	4	29	19	25	44	73	73.00
	2	100	1	-	-	1	1	3	10	2	12	15	15.00
	3	100	3	-	-	4	4	11	3	8	11	22	22.00
	Total	300	4	-	18	12	9	43	82	35	67	110	36.66
									Mother II				
									Control				
	1	100	-	1	-	-	1	2	3	-	3	5	5.00
	2	100	-	-	1	3	-	4	2	1	3	7	7.00
	3	100	-	-	-	-	-	-	5	3	8	8	8.00
	Total	300	-	1	1	3	1	6	10	4	14	20	6.66
									Treated				
	1	100	2	1	3	15	3	24	14	5	19	43	43.00
	Total	100	2	1	3	15	3	24	14	5	19	43	43.00

Table 28

Compiled data of frequency distribution of chromosome aberration in metaphase I cells of F_1 males of control and the bacterium, P. aeruginosa treated males mated with normal females at different weeks (Control data are in brackets)

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Pre.sep.		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Mute/ Asso.	Aut.	Sex						
1st wk	1	100	5	1	2	6	5	19	7	4	11	30	30.00
	(2)	(200)	(-)	(-)	(1)	(5)	(2)	(8)	(1)	(3)	(7)	(15)	(7.50)
2nd wk	2	200	7	-	17	18	17	49	14	7	21	70	35.00
	(5)	(600)	(-)	(-)	(1)	(9)	(7)	(17)	(3)	(6)	(10)	(27)	(5.40)
3rd wk	1	100	3	1	3	8	10	25	7	10	17	42	42.00
	(2)	(200)	(-)	(-)	(-)	(1)	(2)	(3)	(-)	(1)	(1)	(4)	(2.00)
4th wk	4	400	6	1	21	27	12	67	46	40	86	153	38.25
	(6)	(600)	(-)	(1)	(6)	(10)	(3)	(20)	(13)	(8)	(21)	(41)	(6.80)
Total	8	800	21	3	33	59	44	160	74	61	135	295	36.87
	(15)	(1500)	(-)	(1)	(8)	(25)	(14)	(48)	(17)	(18)	(39)	(87)	(5.80)

5.8% in the average of all 4 weeks data taken together. If only the frequencies of individual types were compared, there were 19.0% against 8.0% in 1st week, 24.50% against 8.5% in 2nd week, 25.0% against 1.5% in 3rd week, 16.75% against 3.3% in 4th week and 20.0% against 3.1% in the average of all four weeks in the treated and control line respectively. Therefore, the aberration frequency data in the treated line were significantly high than that of control at metaphase I like that of diakinesis stage. Moreover, the average frequency in different weeks was not very widely different as was expected (Tables 29 and 30). The aberration frequency data at metaphase I also supported the transmission of the mutagenic factor to F_1 generation in the treated line.

Metaphase II :

Unlike spermatogonial metaphase, diakinesis and metaphase I, the chromosomes of metaphase II appeared somewhat despiralized for which many of them showed beaded appearance. There were also some stickiness of the chromosomes. These made some difficulty for an acurate determination of the chromosome aberration though the frequency was higher in the treated line than that of control. The aberration could also be put under two categories as individual and gross types (PM 101-106; Figs. 98-107).

Control line :

1st week :

In the 1st week control line, two F_1 males had the

Explanation of Photomicrograph

Photomicrographs of some affected metaphase II in male germinal cells of F_1 individuals produced after mating of normal females to *Pseudomonas* culture treated males

PM 101. A chromatid break.

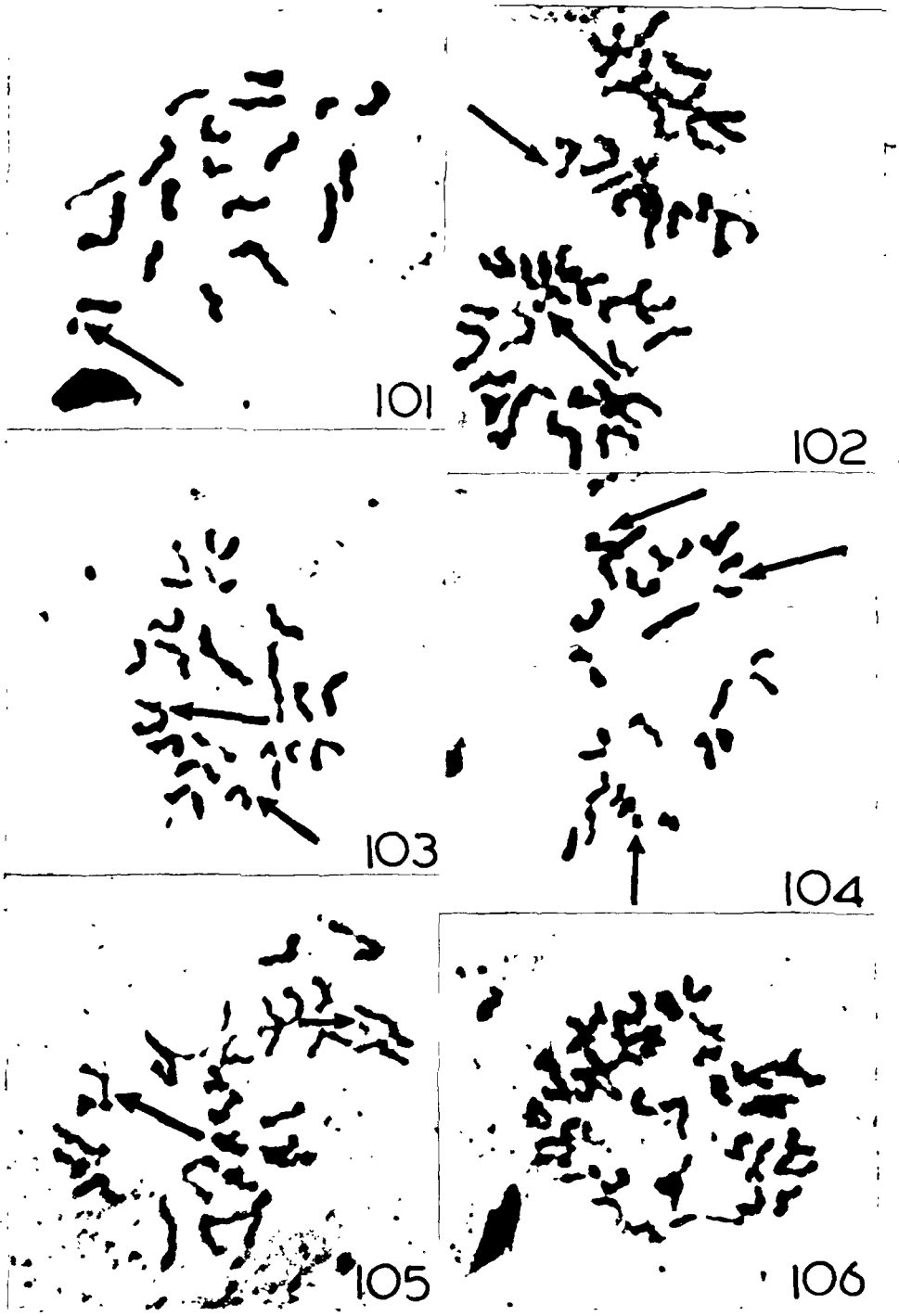
PM 102. A chromatid break and a constriction.

PM 103. A chromatid break and a constrictions.

PM 104. Two fragments and one centromeric dissociation.

PM 105. One break, one fragment and a constriction.

PM 106. A polyploid plate.


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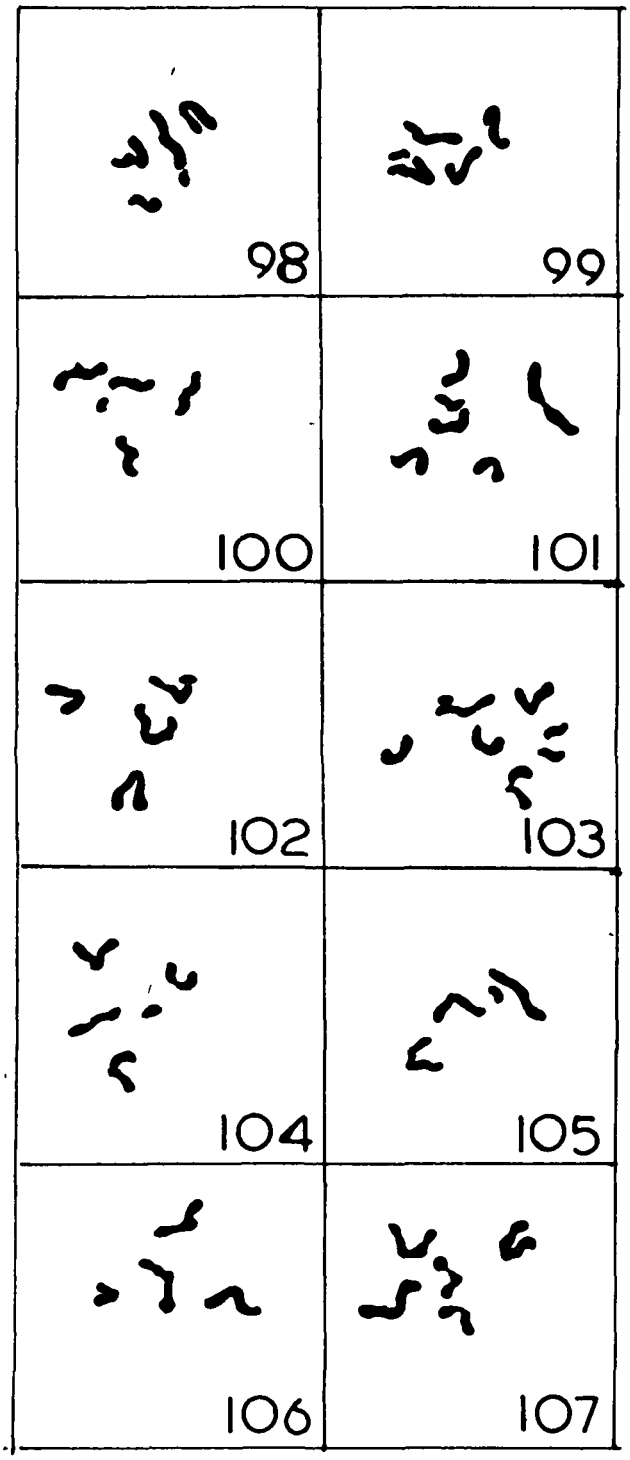
Explanation of figures

Camera lucida drawing of a part of metaphase II plates showing different types of aberrations.

Figs. 98-103. Each with a chromatid break.

Figs. 104-105. Each with a fragment of unknown origin.

Figs. 106-107. A constriction.


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aberration frequencies of 8.33% and 6.15% and all were of gross types. In a total of 125 plates examined the average frequency was 7.20% (Table 29).

2nd week :

In the 2nd week control line two F_1 males had 16.0% and 18.03% aberration frequencies consisting both individual and gross type aberrations. In a total of 136 plates examined the average frequency was 16.91%. In three F_1 male individuals of another mother, the frequency of aberrations ranged between 13.33% and 21.15%. In a total of 135 plates of 3 individuals, the average frequency was 17.77% (Table 29).

3rd week :

In two F_1 individuals of a 3rd week control line mother, the aberration frequencies at metaphase II were 5.26% and 7.27%. In a total of 112 plates the average frequency was 6.25% (Table 29).

4th week :

In three F_1 individuals of a 4th week control line mothers, the aberration frequencies at metaphase II ranged between 10.76% and 13.33%. In a total of 189 plates, the average frequency was 12.16%. In another three F_1 individuals of a different control line mother, the aberration frequencies ranged between 10.25% and 12.72%. In a total of 199 plates of these 3 individuals, the average frequency was 12.06% (Table 29). Thus in the

control line the average aberration frequency appeared to be more than what was found in spermatogonial metaphase, diakinesis and metaphase I. This was possibly due to some difficulty in determining the aberrations as mentioned already.

Treated line :

1st week :

One treated line F_1 male of 1st week had 30 plates showing various types of aberrations and the frequency of all types taking together was 53.33%.

2nd week :

Two treated line F_1 individuals of a 2nd week had the aberration frequencies of 59.52%, 66.0%. The average was 63.04% (Table 29).

3rd week :

In one F_1 individual of 3rd week treated line, the frequency of aberration was found to be 58.82% in 68 plates examined.

4th week :

In 3 F_1 individuals of a 4th week treated line, the aberration frequencies ranged between 26.66% and 68.28%. In a total of 63 metaphase II plates the average frequency

was 49.2%. In another one F_1 individual of a different mother had the aberration frequency of 21.60% (Table 29).

The frequency was relatively higher than that of other stages. In the treated line the frequency ranged widely between 21.60% and 66.00% (Table 29) which was higher than that of control. Thus a comparison of the average data of 4 week control and treated lines would show that in 1st week, the average aberrations frequency was 53.33% in treated line against 7.2% in the control line; 63.04% against 17.34% in the 2nd week, 58.82% against 6.25% in the 3rd week, 39.0% against 12.11% in the 4th week and 52.75% against 12.27% in all weeks taken together (Table 30). Therefore, the frequency of aberration at metaphase II in the treated line was strikingly higher than that of control line.

If the data of net increase in the aberration frequency in 4 week treated lines were compared between spermatogonial metaphase, diakinesis, metaphase I and metaphase II (Tables 23, 25, 27 and 29), they were 36.6%, 17.0%, 22.5% and 46.13% in the 1st week, 41.48%, 35.23%, 29.60% and 45.7% in 2nd week, 28.57%, 28.40%, 40.0% and 52.57% in 3rd week, 45.12%, 23.74%, 31.45% and 26.89% in the 4th week respectively. If the aberration data of all weeks were combined, the net increase in the average frequency was 40.47% in spermatogonial metaphase, 25.79% in diakinesis, 31.07% in metaphase I, and 40.48% in metaphase II (Tables 24, 26, 28, 30). The data clearly showed that the net increase was considerably high and therefore the limitation of the data would not make much difference if only we conclude that the treated line had higher frequency than that of control line. Further, the spermatogonial metaphase appeared to be more susceptible and the next one was metaphase II. In the other words, the

mitotic chromosomes like that of bone marrow cells, spermatogonial metaphase and metaphase II appeared more vulnerable than that of chromosomes of diakinesis and metaphase I.

The result of the lethal test presented before has indicative of the induction of mutation for the treatment of log-culture of Pseudomonas to male parents which has also been supported by the occurrence of the higher frequency of chromosome aberration observed in the living individuals of F_1 generation. Therefore, the log culture of Pseudomonas contained some genotoxic agents which might have induced chromosomal mutation in mice.

Table 29

Frequency distribution of chromosome aberration in metaphase II cells of F_1 males of bacterium, P. aeruginosa treated and control males mating parents of different weeks

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type Chr.	Frag.	Trans. Asso.	Phy.type Gaps & cons.	Total	Gross type aberration: Num. cha.	Others	Total	Grand total	%
1st wk							Mother I					
							Control					
	1	60	-	-	-	-	-	3	2	5	5	8.33
	2	65	-	-	-	-	-	1	3	4	4	6.15
	Total	125	-	-	-	-	-	4	5	9	9	7.20
							Treated					
	1	30	4	2	1	1	8	-	8	8	16	53.33
	Total	30	4	2	1	1	8	-	8	8	16	53.33
2nd wk							Mother I					
							Control					
	1	75	3	1	-	1	5	2	5	7	12	16.00
	2	61	1	2	-	-	3	4	4	8	11	18.03
	Total	136	4	3	-	1	8	7	9	15	23	16.91
							Treated					
	1	42	3	2	2	-	7	7	11	18	25	59.52
	2	50	6	4	3	1	14	4	15	19	33	66.00
	Total	92	9	6	5	1	21	11	26	37	58	63.04

Table 29 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Trans. /Asso.	Phy.type Gaps & cons.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
2nd wk							Mother II					
							Control					
	1	45	1	-	-	1	2	2	2	4	6	13.33
	2	38	2	1	-	-	3	1	3	4	7	18.42
	3	52	-	2	-	2	4	2	5	7	11	21.15
	Total	135	3	3	-	3	9	5	10	15	24	17.77
							Treated					
	1	Reduced testis										
	2	Do										
3rd wk							Mother I					
							Control					
	1	57	-	-	-	1	1	2	-	2	3	5.26
	2	55	-	-	-	-	-	1	3	4	4	7.27
	Total	112	-	-	-	1	1	3	3	6	7	6.25
							Treated					
	1	68	8	2	-	2	12	3	15	18	40	58.82
	Total	68	8	2	-	2	12	3	15	18	40	58.82

Table 29 (Contd.)

Fdx.	Ind. No.	Cells count.	Individual type aberrations					Gross type aberrations			Grand total	%
			Break type			Ph.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans. /Asso.	Gaps & cons.						
4th wk							Mother I					
							Control					
	1	65	2	1	-	-	3	2	2	4	7	10.76
	2	45	1	-	-	1	2	-	4	4	6	13.33
	3	79	-	-	-	2	2	2	6	8	10	12.65
Total		189	3	1	-	3	7	4	12	16	23	12.16
	1	20	-	-	-	-	-	-	9	9	9	45.00
	2	15	-	-	-	1	1	1	2	3	4	26.66
	3	28	1	-	-	-	1	2	15	17	18	64.28
Total		63	1	-	-	1	2	3	26	29	31	49.20
							Mother II					
							Control					
	1	105	3	2	-	2	7	2	4	6	13	12.38
	2	55	-	3	-	-	3	-	4	4	7	12.72
	3	39	1	1	-	-	2	-	2	2	4	10.25
Total		199	4	6	-	2	12	2	10	12	24	12.06
							Treated					
	1	37	-	-	-	-	-	6	2	8	8	21.60
Total		37	-	-	-	-	-	6	2	8	8	21.60

Table 30

Compiled data of frequency distribution of chromosome aberrations in metaphase II cells of F_1 males of control and bacterium, P. aeruginosa treated mating parents of different (Control data are brackets)

Fix.	No.of Ind.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Trans/	Phy.type Gaps &	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
1st wk	1	30	4	2	1	1	8	-	8	8	16	53.33
	(2)	(125)	(-)	(-)	(-)	(-)	(-)	(4)	(5)	(9)	(9)	(7.20)
2nd wk	2	92	9	6	5	1	21	11	26	37	58	63.04
	(5)	(271)	(7)	(6)	(-)	(4)	(17)	(11)	(19)	(30)	(74)	(17.34)
3rd wk	1	68	8	2	-	2	12	3	15	18	40	58.82
	(2)	(112)	(-)	(-)	(-)	(1)	(1)	(3)	(3)	(6)	(7)	(6.25)
4th wk	4	100	1	-	-	1	2	9	28	37	39	39.00
	(6)	(388)	(7)	(7)	(-)	(5)	(19)	(6)	(22)	(28)	(47)	12.11)
Total	8	290	22	10	6	5	43	23	77	100	153	52.75
	(15)	(896)	(14)	(13)	(-)	(10)	(37)	(24)	(49)	(73)	(110)	(12.27)

D. Sperm count in 3 month old F_1 males :

With a view to finding out if there was any effect on sperm number and sperm head morphology in the epididymal sperm samples of F_1 treated line males against that of control line, these tests were carried out in the same 3 month old F_1 male individuals as they were used for the chromosome aberration study in the somatic and germinal cells described before.

Sperm depletion test :

Epididymal sperm suspensions of treated and control lines male individuals were prepared separately according to the method described before and the average count was scored.

Result and comment :

In first week control line, count of sperms in left and right epididymes in two F_1 male individuals did not show an appreciable difference. The average count was 22.90 $\pm$ 0.34 and 16.62 $\pm$ 0.37 and in the combined data of the two sides the mean average was 21.26 (Table 31).

In five F_1 individuals of 2nd week control lines, the sperm count in the left and right epididymes differed in some cases and it was 18.60 $\pm$ 0.25 and 26.32 $\pm$ 0.49 as the maximum in one individual. While in some cases it was very negligible as 17.36 $\pm$ 0.65 in the right and 17.42 $\pm$ 0.23 in left epididymes. Anyhow the average count of sperms

in the left and right epididymis of 5 individuals were combined and the mean values were 19.90 for the right epididymes and 22.26 for the left epididymes (Table 31). The combined average of the two sides was 21.17 which was very close to that of 1st week control line (21.26). In two F_1 individuals of 3rd week control line, the mean number of sperm count did not vary much between the right and left epididymes of each individual and the average was found to be 14.02 ± 0,43 in one individual and 18,44 ± 0.61 in another individual. The mean value of 2 individuals was 16,23 which was little lower than that of 1st and 2nd week F_1 individuals. In the 4th week control line the sperm count of the 6 individuals showed some amount of difference between the right and left epididymes. The maximum difference was 24,04 ± 0.45 in the right epididymes and 17.64 ± 0.65 in the left epididymis in the same individual. In some other individual the difference was very negligible as the values were 25,32 ± 0.35 and 24.88 ± 0.46. In the combined data of all 6 individuals, the average mean frequency was 24.17 in the right epididymes and 21.29 in the left epididymes and 22.78 was the average of the left and right epididymes combined. Therefore, in spite of the differences in the sperm count in different individuals the mean average value did not differ considerably as it was 21.26 in the first week 21.17 in the 2nd week and 22.78 in the 4th week but it was 16.23 in the 3rd week (Table 31).

The data of sperm count in the treated line had certain limitation because very few males were available for the study. In one individual of 1st week treated line, the average sperm count was 8.80 ± 0.66 in the right, 4.52 ± 0,46 in the left and 6.66 ± 0.48 in the combined data of right

and left epididymes which clearly indicated that the treated line male had significant reduction in sperm count than that of control as the mean value was 6.66 (Table 31) against 21.26 in the control line (Table 31). Two F_1 male individuals of 2nd week treated line showed some degree of difference in the sperm count though the difference was not so significant between left and right side. The mean number of sperms of the right and left epididymes taken together was 5.74 ± 0.36 in one individual and 14.84 ± 0.75 in another. The average of the individuals taken together was 10.29 which was significantly lower than the average of 21.17 of 5 individuals of 2nd week control line. In one F_1 male of 3rd week treated line the mean count was 1.66 ± 0.23 in the right epididymes and 12.50 ± 0.23 also in left epididymis making the mean value of 6.68 ± 0.80 of the left and the right sides combined which was very significantly lower than the average of 16.23 obtained in the 3rd week control line. In the 4th week treated line the mean value of sperm count in 5 individuals differed between the right and left epididymes. In one individual the maximum difference was 22.04 ± 0.95 in the right epididymes and of 9.68 ± 0.73 in the left epididymes. In some other, the difference was negligible as it was 22.04 ± 0.70 in the right epididymes and 22.84 ± 0.69 in the left (Table 31). If the mean values of the left and right epididymes of different individuals were combined, it was found to range between 4.04 ± 0.25 and 22.44 ± 0.49. Anyhow, if the mean values of sperm count of 5 individuals were combined, the average mean was 14.85 in the treated line against 22.78 of the control line (Table 31).

The data (Table 31) of sperm count revealed that barring a few, in most cases the difference in the mean value

Table 31

Mean average sperm count in the epididymes of F_1 in different mothers mated to bacterium P. aeruginosa treated and control males parents at different weeks

Fix.	No.of indiv.	Control			No.of Indiv.	Treated		
		Right epidi. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R & L comb. (Mean value ± S.E.)		Right epid. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R&& Le.comb. (Mean value ± S.E.)
				Mother I				
1st wk	1	22.96 ± 0.40	22.84 ± 0.57	22.90 ± 0.34	1	8.80 ± 0.66	4.52 ± 0.46	6.66 ± 0.48
	2	19.32 ± 0.36	19.88 ± 0.66	19.62 ± 0.27				
	Average	21.14	21.36	21.36		8.80	4.52	6.66
2nd wk	1	21.08 ± 0.39	24.16 ± 0.56	22.64 ± 0.38	1	6.68 ± 0.53	4.80 ± 0.43	5.74 ± 0.36
	2	18.60 ± 0.25	26.32 ± 0.49	22.46 ± 0.61	2	10.16 ± 0.62	19.52 ± 0.35	14.84 ± 0.75
					Average	8.42	12.16	10.29

Table 31 (Contd.)

Fix.	No.of Indiv.	Control			No.of Indiv.	Treated		
		Right epidi. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R & L comb. (Mean value ± S.E.)		Right epidi. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R & L comb. (Mean value ± S.E.)
2nd wk					Mother II			
	3	20.32 ± 0.36	24.64 ± 0.44	22.48 ± 0.42	3	Reduced testis	Absent	-
	4	17.36 ± 0.65	17.92 ± 0.23	18.04 ± 0.32	4	"	Reduced testis	-
	5	22.20 ± 0.40	18.28 ± 0.17	20.28 ± 0.35				
	Average	19.90	22.26	21.17				
					Mother I			
3rd wk	1	14.80 ± 0.65	13.60 ± 0.61	14.02 ± 0.43	1	1.66 ± 0.23	12.50 ± 0.23	6.68 ± 0.80
	2	19.44 ± 1.05	17.84 ± 0.61	18.44 ± 0.61				
	Average	17.12	15.72	16.23		1.66	12.50	6.68

Table 31 (Contd.)

Fix.	No.of Ind.	Control			No.of Indiv.	Treated		
		Right epidi. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R & L comb. (Mean value ± S.E.)		Right epidi. (Mean value ± S.E.)	Left epidi. (Mean value ± S.E.)	R & L comb. (Mean value ± S.E.)
				Mother I				
4th wk	1	20.48 ± 0.44	16.24 ± 0.73	18.36 ± 0.54	1	13.36 ± 0.48	20.00 ± 0.77	16.68 ± 0.65
	2	24.04 ± 0.45	17.64 ± 0.65	21.14 ± 0.64	2	22.04 ± 0.95	9.68 ± 0.73	15.86 ± 1.04
	3	25.32 ± 0.35	24.88 ± 0.46	25.10 ± 0.29	3	15.88 ± 0.80	14.64 ± 0.84	15.26 ± 0.58
				Mother II				
	4	22.75 ± 0.84	17.52 ± 0.56	20.14 ± 0.62	4	4.80 ± 0.37	3.24 ± 0.26	4.04 ± 0.25
	5	29.00 ± 0.86	23.16 ± 0.72	26.58 ± 0.65	5	22.04 ± 0.70	22.84 ± 0.69	22.44 ± 0.49
	6	23.44 ± 0.78	27.32 ± 0.57	25.38 ± 0.55				
Average		24.17	21.29	22.78		15.62	14.08	14.85

of the left and the right epididymal samples did not differ to a great extent. Further, in different week control lines, the mean average did not differ appreciably but in the treated line it differed quite a bit. The occurrence of the lower count of sperms in the treated line than that of control would definitely indicate that the effect of the treatment of the log-culture to male parents was transmitted in the F_1 treated line males.

E. Sperm head morphology in 3 month old F_1 males :

The sperm head morphology in the control and treated lines F_1 males of 4 week matings was also conducted taking 600 sperms per individual. The smear preparation of sperms stained with Giemsa was made according to the method described before. In both control and treated males, there were sperms with both normal and abnormal head morphology (PM 107 a-j) but there was difference in frequency. Sperms with abnormal head morphology were very much similar to those of parental generation. In other words, it had indicated that the abnormal sperm head morphology was not directly related to the toxic effect of the bacteria but it might have induced by certain genetic changes for which higher frequency of sperm with abnormal head morphology was obtained in the F_1 treated line males. The quantitative data (Table 32) of the sperms with abnormal head morphology in 4 week control line males would show that the frequency was not very high and the average of 2 individuals taken together was 0.83% in the 1st week, that of 5 individuals was 1.80% in the 2nd week, that of 2 individuals was 0.66% in the 3rd week and that of 6 individuals was 1.08% in the 4th week (Table 32). Further the average frequency of sperms with abnormal head morphology did not vary much in different week control lines.

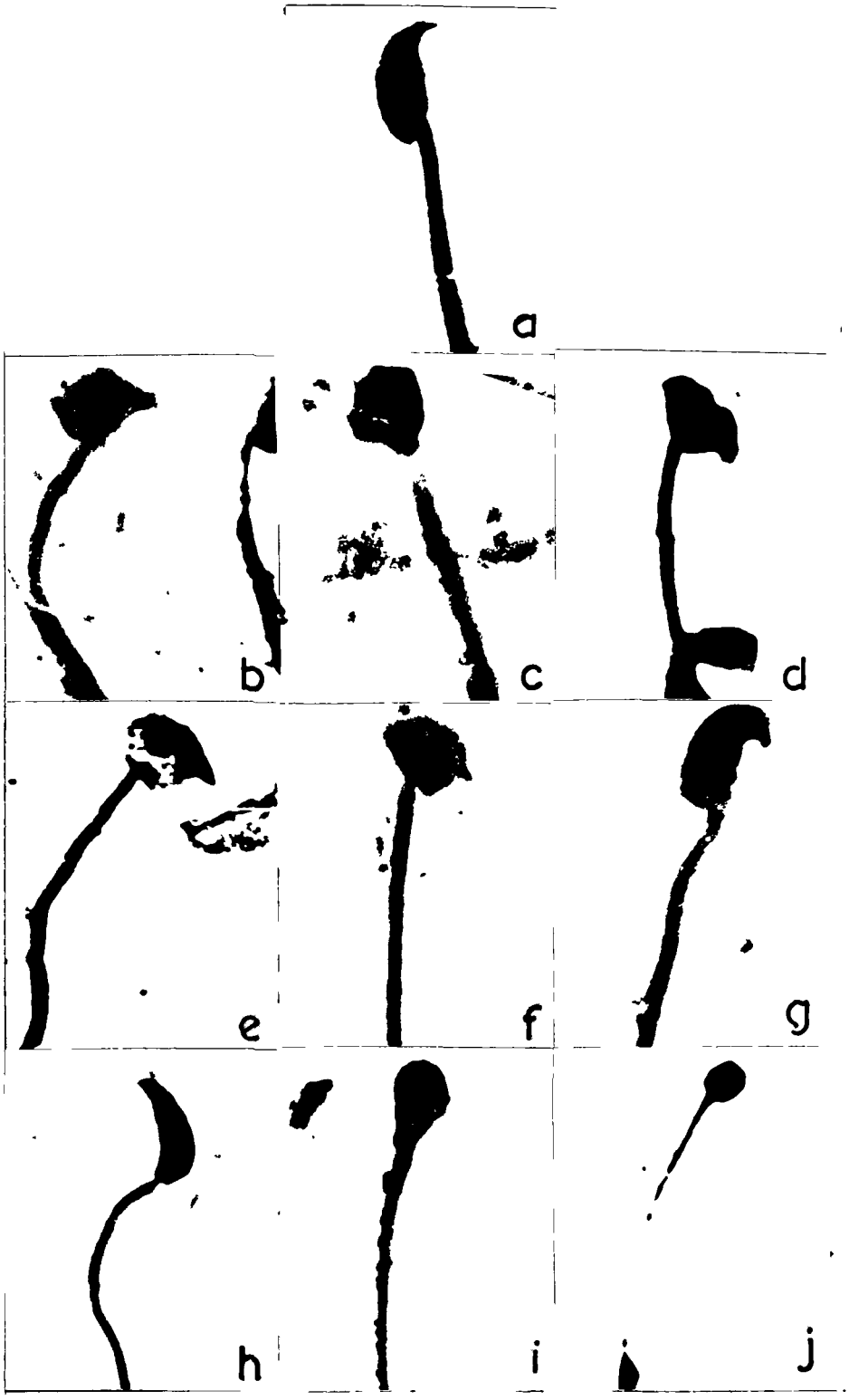
In the treated line, however the average frequency of sperms with abnormal head morphology was quite high though there was some limitation in the data (Table 32). The average frequency of sperms with abnormal head morphology was 11.66% determined from one F_1 individual of the 1st week treated line, 13.58% in two individuals of the 2nd week treated line, 10.33% in one individual of the 3rd week treated

Explanation of Photomicrographs

Photomicrographs of sperm with abnoraml head of F_1 individuals produced after mating of normal females to Pseudomonas culture treated males.

PM 107a. A normal sperm head.

PM 107 b-j. Different types of sperm with abnormal head morphology.



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line and 17.62% in four individuals of the 4th week treated line. Therefore, the frequency of sperms with abnormal head morphology was distinctly higher than that of respective control and the maximum difference was found in 4th week (Table 32). Further as compared to controls the average frequency in 4 week treated lines was quite variable which ranged between 10.33% and 17.62%. This could be due to the random segregation of chromosome containing the mutated loci. That the sperm head morphology could be under genetic control was mentioned before (Wyrobek, 1979). The occurrence of the higher frequency of abnormal sperms obtained in F_1 males in different week treated lines than that of control might be taken as a supportive evidence of the mutagenic potentiality of the bacterium P. aeruginosa in mice systems.

Therefore, the bone marrow chromosome aberrations in F_1 male and female and in in meiotic chromosome aberrations in spermatogonial metaphase diakinesis, metaphase I and metaphase II, sperm count and sperms with abnormal head morphology shown significant difference between treated and control lines on the whole indicated the mutagenic potentiality of log culture of P. aeruginosa which was transmitted to F_1 generation.

Table 32

Frequency distribution of sperm with abnormal head morphology in 3 month old F_1 progeny of different mothers mated at different weeks to the P. aeruginosa treated and control males.

Fix. time	Control				Treated			
	No.of indiv	Total No. sp.head counted	Total No.abn sp.head counted	%	No.of Ind.	Total No. sp.head counted	Total No.of abn.sp. head counted	%
				Mother I				
1st wk	1	600	6	1.00	1	600	70	11.66
	2	600	4	0.66				
Total	2	1200	10	0.83	1	600	70	11.66
2nd wk	1	600	8	1.33	1	600	66	11.00
	2	600	22	3.66	2	600	97	16.16
Total	2	1200	30	2.50		1200	163	13.58
				Mother II				
2nd wk	3	600	9	1.50				
	4	600	11	1.83				
	5	600	4	0.66				
Total		1800	24	1.33				

Table 32 (Contd.)

Fix. time	Control				Treated			
	No.of indi.	Total No. sp.head counted	Total No. abn. sp.head counted	%	No.of Ind.	Total No. sp.head counted	Total No. of abn.sp. head counted	%
				Mother I				
3rd wk	1	600	6	1.00	1	600	62	10.33
	2	600	2	0.33				
Total	2	1200	8	0.66		600	62	10.33
4th wk	1	600	4	0.66	1	600	190	31.66
	2	600	5	0.83	2	600	124	20.66
	3	600	8	1.33	3	600	62	10.33
Total		1800	17	0.94		1800	376	20.88
				Mother II				
4th wk	4	600	7	1.16	4	600	47	7.83
	5	600	5	0.83				
	6	600	10	1.66				
Total		1800	22	1.22		600	47	7.83
Grand total		3600	39	1.08		2400	423	17.62

F. Teratogenic effect :

a) In F_1 festus at 15 day of gestation :

So far as the present author was aware the teratogenic effect for the treatment of log culture P. aeruginosa had not been studied earlier though it was reported that the treatment of P. aeruginosa caused some kinking of the tail and deformities in hind limb etc. in F_1 generation (see Manna, 1980). The present author carried out some studies to detect the teratogenic effect in embryos of 15 day gestation and in F_1 adults of 3 months old while those specimens of the control and treated lines were sacrificed for collecting the tissues for chromosome-aberration study, sperm count and sperm head morphology etc.

Method :

Each of the 15 day old embryos of control and treated lines of different week mating soon after the removal of liver, the entire embryo was fixed separately in absolute alcohol and processed for Alezarine preparation with a view to finding out if there was any skeletal abnormality. The alcohol fixed embryos were kept for 4 to 5 months for proper hardening with changes of absolute alcohol at every month. After 4 to 5 months each embryo was put into 0.1% KOH solution for about 2 hrs, then the embryo was transferred to 0.1% Alezarine solution (0.01 gm Alezarine powder, dissolved in 100 ml of 0.1% KOH solution for staining for 10 to 12 hr). The stained embryos were transferred to dilute glycerine

in distilled water (1:1) and left for overnight. Next day, the embryos were transferred to pure glycerine when the skeletan appeared darkly stained which could be seen through the transparent muscles and the vegetative parts.

Observation and comment :

In a total alezarine preparation of 94 F_1 embryos of 7 week control lines, no skeletal deformity was observed in any one of them. On the otherhand an examination of alezarine transparency of 48 embryos (Table 14) of the treated line resulted for mating of P. aeruginosa treated males in 1st week, 2nd week, 3rd week, 4th week and 7th week to different set of females, only one was found to have the ill-developed last rib (PM 109) which appeared as a reduced bony element in compared to that of normal (PM 108). Therefore, the teratogenic effect was insignificant though it was found only in the treated series. However, for lack of time no critical study of the sections could be made. Therefore, no conclusion was made for the present.

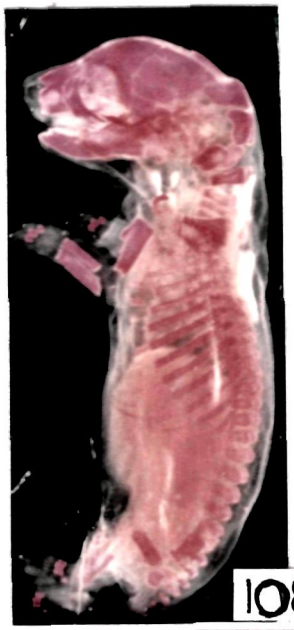
b) In 3 month old F_1 individuals organs :

In a total of 35 control line F_1 individuals sacrificed for bone-marrow chromosome aberration study (Table 20) in 4 week lines, no abnormality was observed in their reproductive organ, and brain. On the otherhand similar study in 19 F_1 adults of 4 week treated lines yielded some abnormalities. In 3 F_1 adults of the first week

Explanation of Photomicrographs

PM 108. Normal structure of the skeletal of mouse.

PM 109. Photomicrograph showing ill develop last rib.



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treated line no abnormality was observed in the two organs mentioned above but on the other hand among 5 F_1 treated line adults of a 2nd week mating mother, 2 F_1 females showed abnormality in the morphological appearance of the brain (PM 111-119). It was round, while the normal shape (PM 110) is oval elongated. Incidentally it might be mentioned that these specimens were found to rest at a corner of the cage and if disturbed they used to make whorl-movement like "Waltz mutation" recorded in mice. However, we could not follow up further and no claim has been made for the specific mutation but just reported the behaviour keeping in view of the future study. In another mother of the 2nd week treated line, three F_1 adults consisting of 1 female and 2 males showed that the female was with one reduced leg (PM 124a), ill-developed uterus and reproductive organ (PM 122 c) and abnormal brain, the two males had ill-developed testes (PM 122 a-b). It was interesting to note that all three individuals obtained from this mother were of dwarf size (PM 123 b, c, d) like dwarf mutation reported in mice. In spite of our best effort no breeding was possible using those 3 dwarf specimens though they lived normally upto 3 months after which they became inactive and showed that the death was nearing by. Therefore, the specimens were sacrificed and some abnormalities were found which have already been mentioned. In the 3rd week treated line, out of 3 F_1 adults obtained, two were females showing abnormal roundish brain morphology and one male had ill developed testes (PM 121). One of the 4 F_1 individuals of 4th week treated line of one mother, 3 showed abnormal brain morphology while 2 F_1 individuals of another mother did not show any form of abnormality. The occurrence of rib

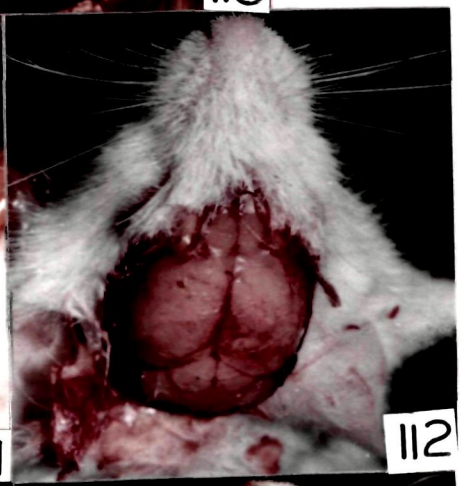
Explanation of Photomicrographs

PM 110. Normal brain.

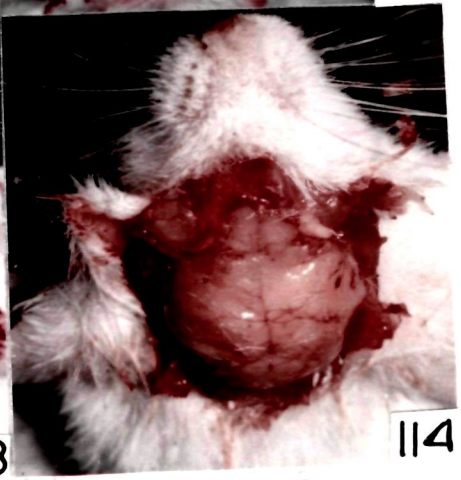
PM 111-114. Different types of abnormal brain.


110


111


112


113


114

Explanation of Photomicrographs

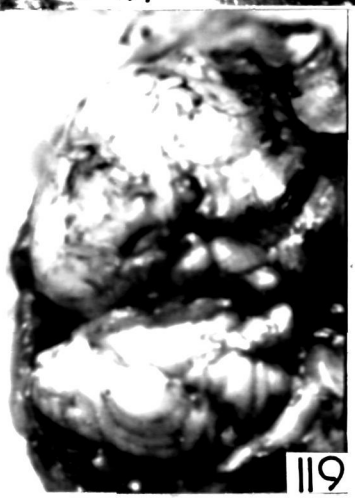
PM 115-119. Different types of abnormal brain.


115


116


117


118


119

Explanation of Photomicrographs

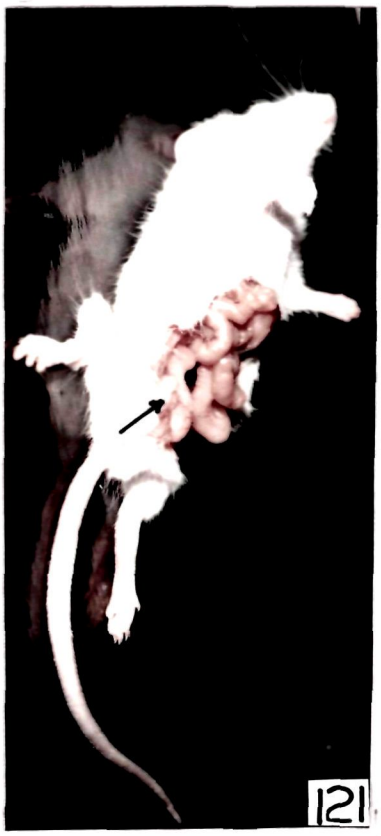
PM 120. Normal structure of the testis.

PM 121-122a,b. Ill develop testis.

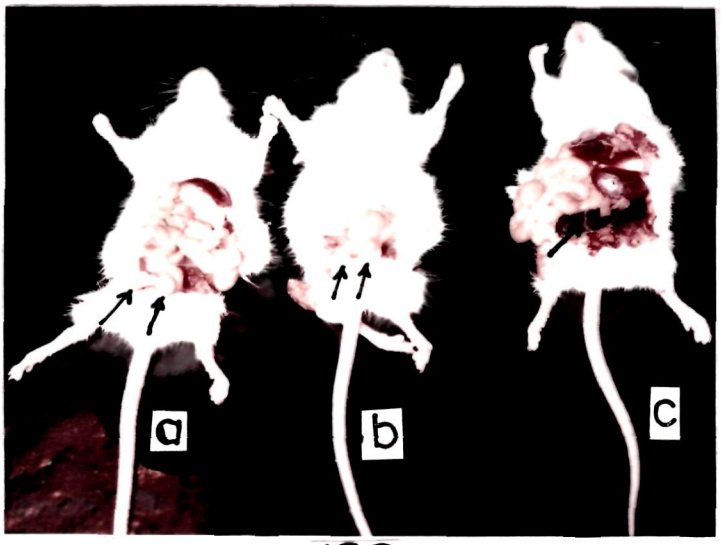
PM 122c. Ill develop uterus.



120



121



122

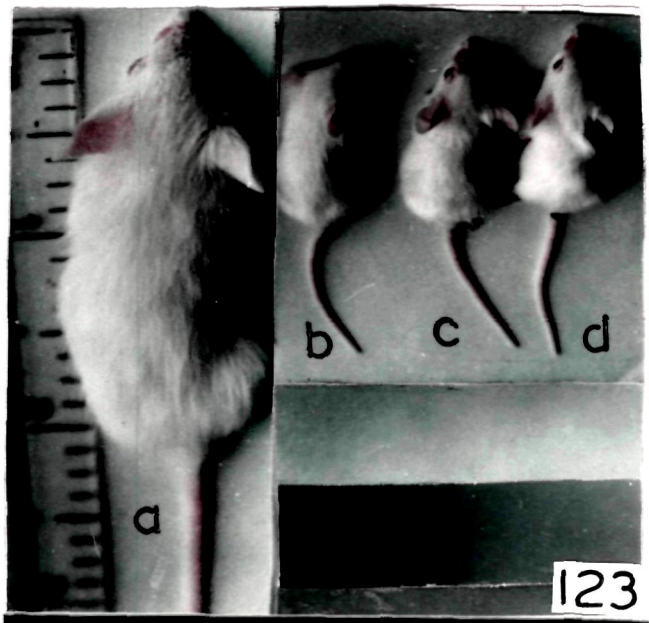
Explanation of Photomicrographs

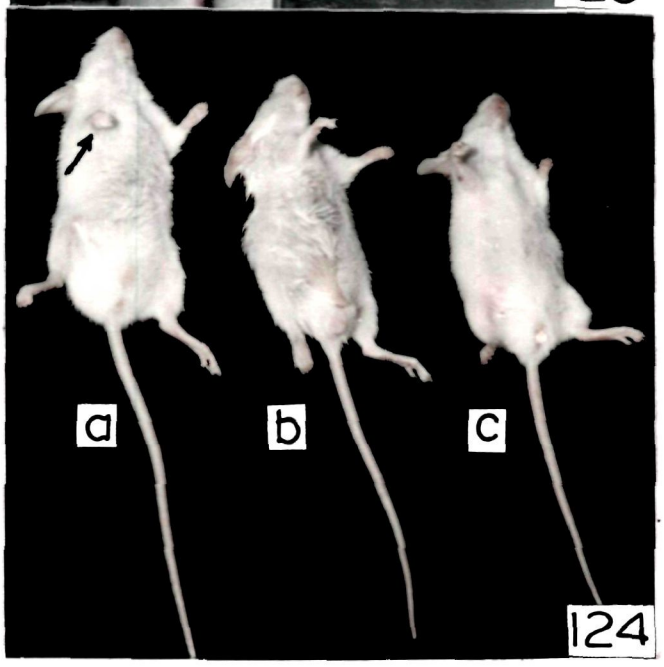
PM 123a. Normal individual.

PM 123b-d Dwarf individuals.

PM 124a. Reduced fore limb.

PM 124b-c. Normal fore limbs.


a
b
c
d
123


a
b
c
124

abnormality in one out of 35 embryos and reduced leg, abnormal brain and reproductive organ found in 8 out of 19 F_1 adults and the complete absence of such abnormalities in the control line would suggest the teratogenic potentiality of the log culture sample of P. aeruginosa experimented on male mice. However, as the sample for the present study of the teratogenic effect was not very large, for which the frequency appeared quite high (8 in 20 or 40%). Anyhow, it was recorded earlier (see Manna, 1980) that the treatment of P. aeruginosa induced some visible changes like kinking of tail, deformities of hind limb in treated mice. All these evidences together with the studies carried out on the progeny after the treatment the log culture of P. aeruginosa to pregnant females presented in last chapter of the thesis would confirm the teratogenic potentiality of the bacterium while its clastogenic and mutagenic potentialities have been shown by various tests.

G. Histological effect :

a) Effect on testis :

The study of the histological section of the testes of F_1 control line individuals of different weeks did not show any difference in the structure as compared to that of normal of control of parental generation. The study of the histological sections of the testes of F_1 individuals of 7 week treated lines showed little difference from that of control. The epithelium of the tubule of the testis was normal (PM 125) like that of control but most of the seminiferous tubules appeared to have lost their compactness (PM 126-129). A number of vacuoles (PM 128) appeared in the tubules and in some cases the lumen was blocked (PM 127). The spermatogonial and spermatocytic cells appeared to be vacuolated than that of control (PM 129).

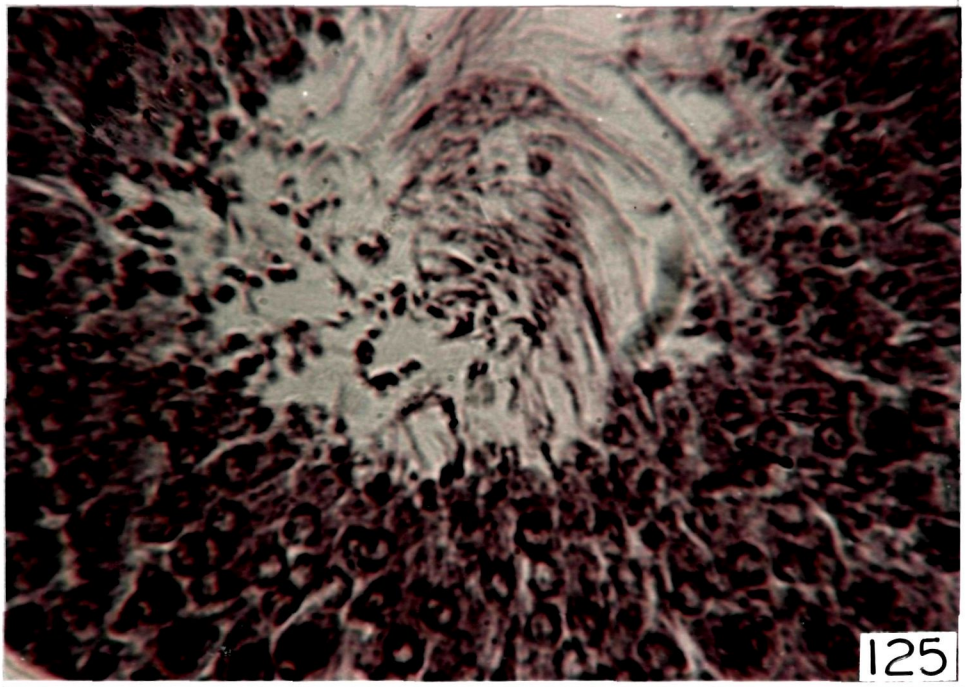
It has been said (Bal, 1984) that the histological changes in the successive generation could be taken as a parameter for the mutagenic potentiality of an agent. In the present study there were some indications in the treated lines while the control line had no effect. Thus when this present finding was added to other evidences, the mutagenic effect induced by injection of the log culture of P. aeruginosa to male parent would further be strengthened. Moreover, the treatment of the log culture reduced considerably in reproductive potentiality which was supported by the effect in the histological structure of the testis.

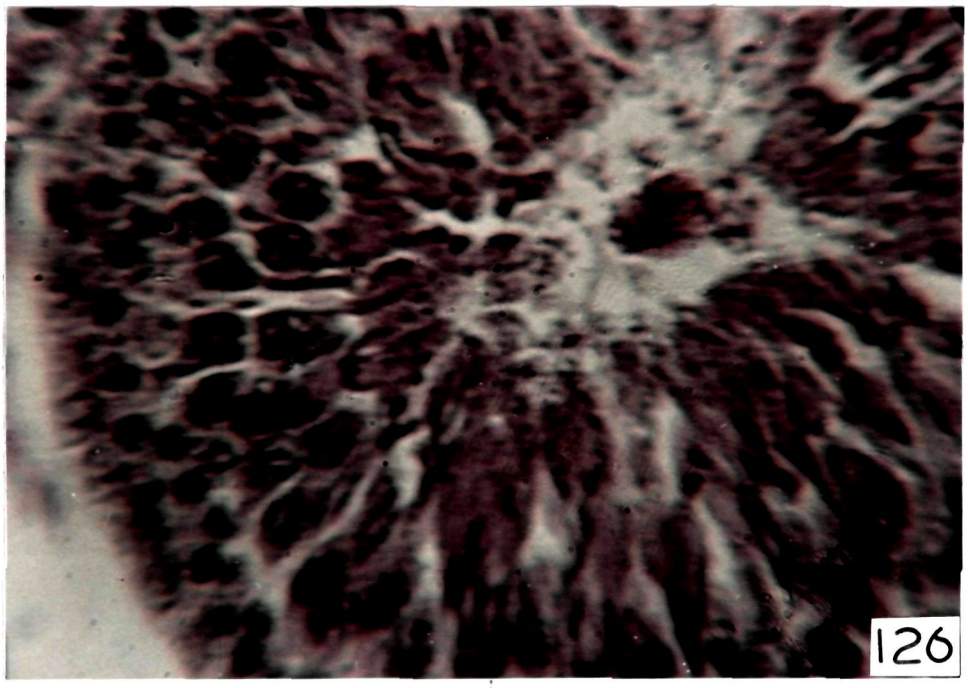
Explanation of Photomicrographs

Photomicrograph of T.S. of Testis in F_1 generation.

PM 125. Normal structure of T.S. of testis.

PM 126. Degeneration of the follicular cells lining.


125


126

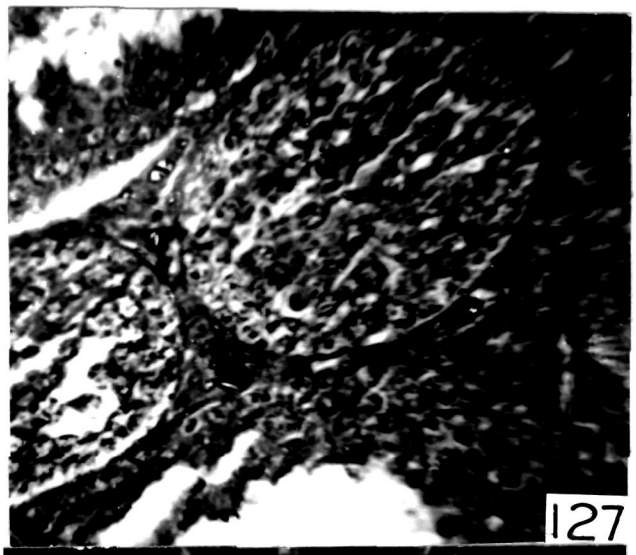
Explanation of Photomicrographs

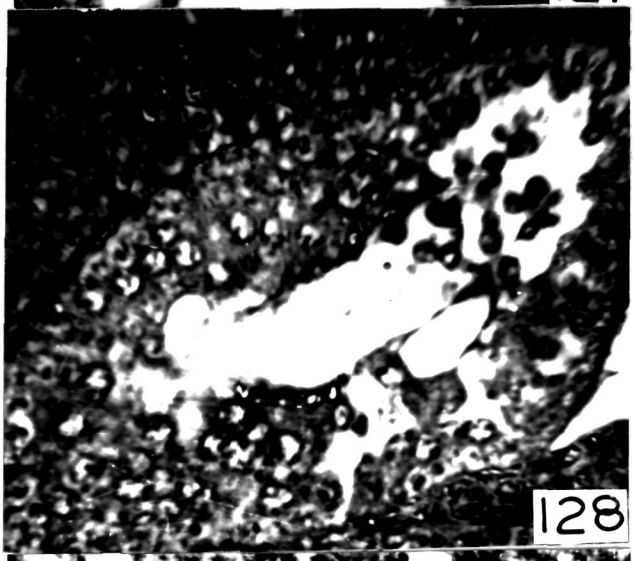
Photomicrographs of T.S. of Testis in F_1 generation.

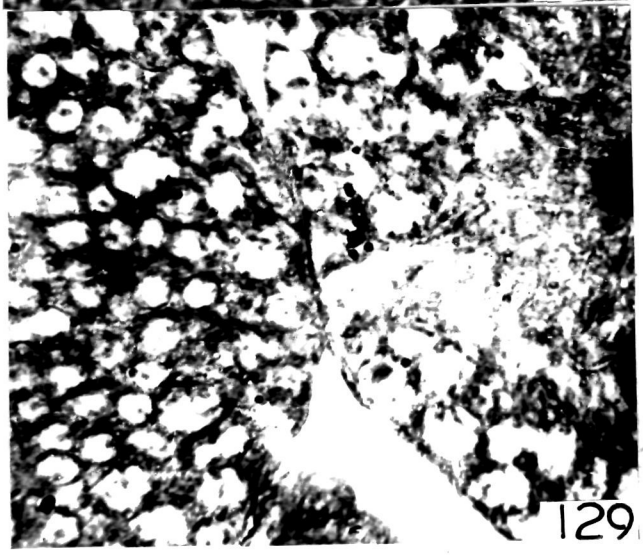
PM 127. Blocked lumen, vacuolation in the tubules and a lumen without spermatozoa.

PM 128. Many vacuolation within the tubules and a lumen without spermatozoa.

PM 129. Vacuolation within the follicular cells.


127


128


129

b) Effect on ovary :

The 2nd week and 4th week treated line F_1 female individuals, which did not have successful mating were used for the present study in order to find out if any histological changes was related to the sterility. The histological sections of the ovaries of the treated lines were compared with that of control line females which did not have successful mating. In the control line the histological sections of the ovary showed some amount of differences from normal structures (PM 130-147). In the control line, the section of the ovaries of 3 females of the 2nd week and 2 females of the 4th week showed variable number of large perivitelline spaces in secondary and Graafian/ maturing follicles. The maximum number of two nucleoli was found in a few cases (Table 33). Further, in some cases a clear perinuclear cavity was seen in the mature follicle (PM 134-136). These changes might not have any genetic significance but they could imply the reason for the failure of regular ovulation. The frequency was found to range between 3.94% and 15.23% in the secondary follicle and between 7.77% and 12.94% in Graafian or maturing follicles (Table 33). In histological sections of the treated line the main differences were attributable to the number of nucleoli and the frequency of abnormalities was remarkably high in most cases that of control. The frequency ranged between 3.89% and 76.47% for secondary follicles and between 8.0% and 96.66% in Graafian follicles or maturing ova, (Table 33). Therefore, though both control and treated line F_1 females did not have offsprings due to some reproductive failure it was obvious in the sections of the ovary of treated line females.

Explanation of Photomicrographs

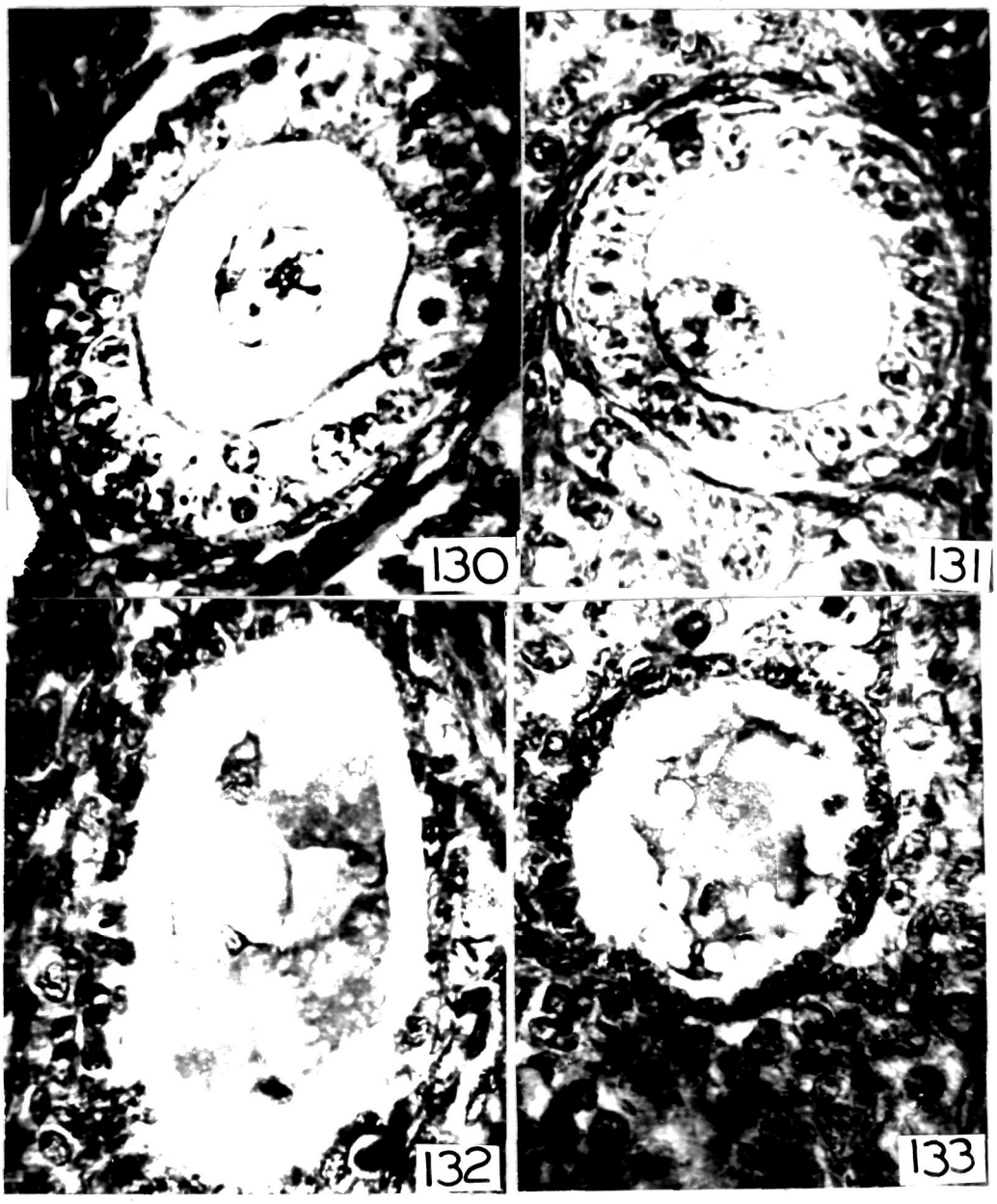
Photomicrographs of the secondary follicles in F_1 females.

PM 130. Secondary follicles with fragmented nucleolus.

PM 131. Secondary follicles with two micronucleoli within a nucleus.

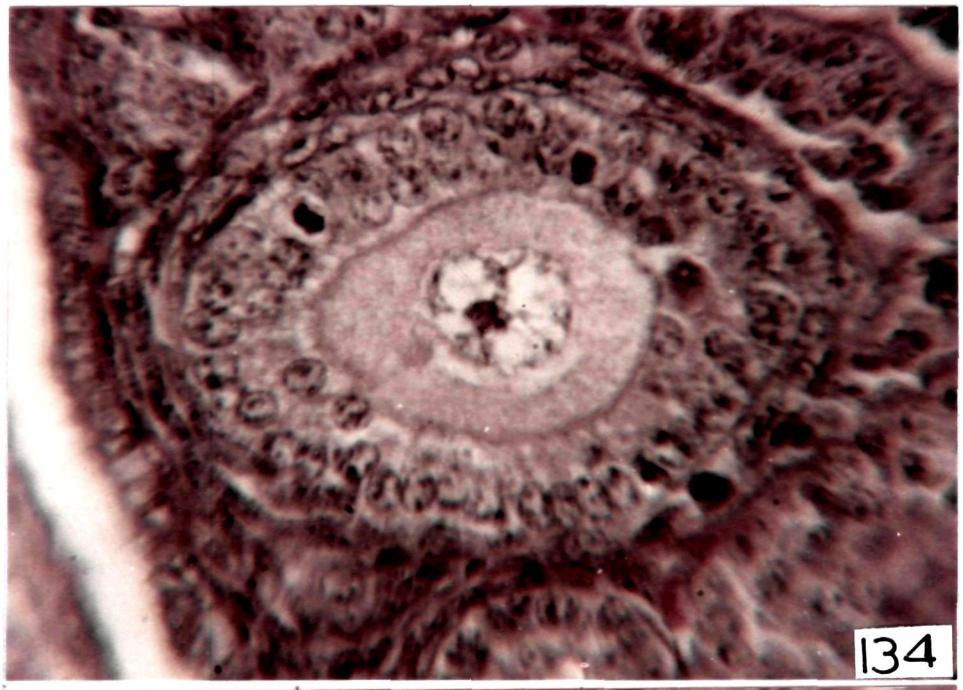
PM 132. Large perivitelline space in secondary follicle with granular cytoplasm.

PM 133. Large perivitelline space with many vacuolation in the cytoplasm of a secondary follicle.

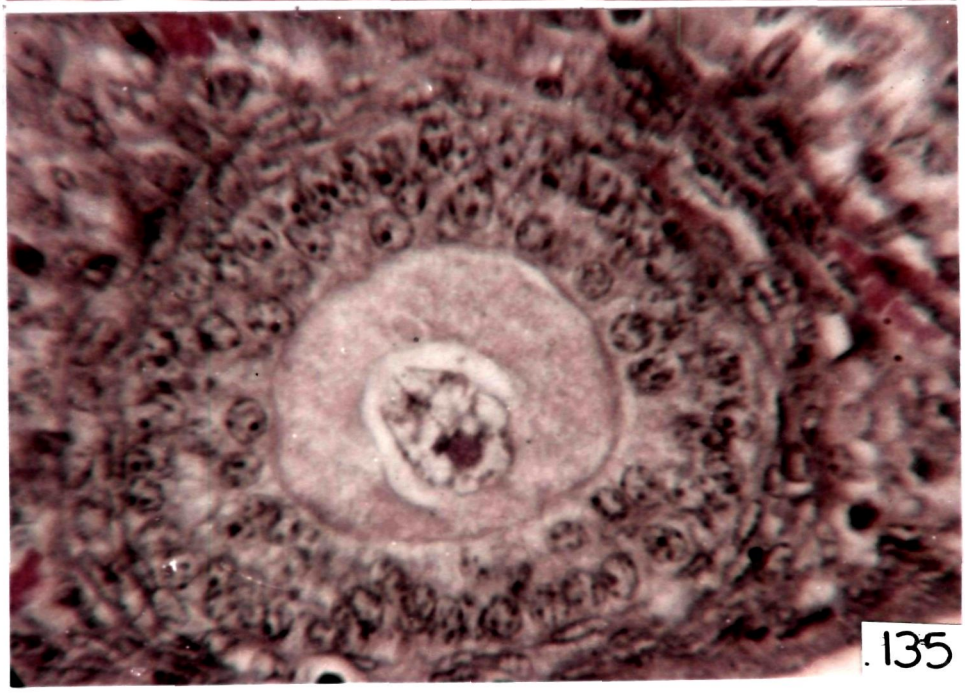

130
131
132
133

Explanation of Photomicrographs

PM 134-135. Perinuclear space within the secondary follicle.



134



135

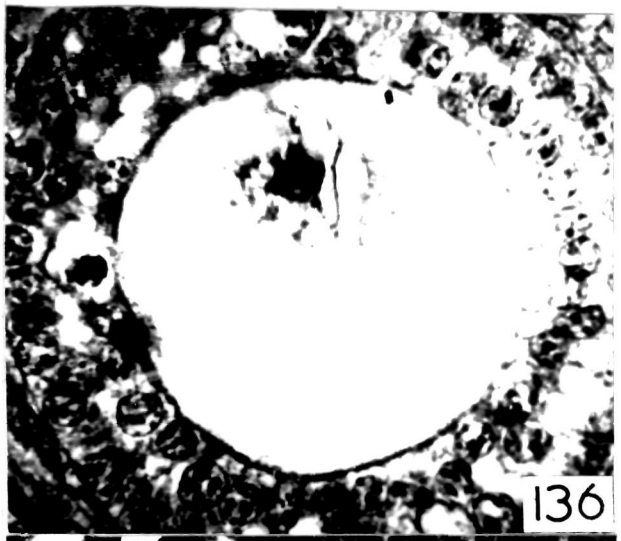
Explanation of Photomicrographs

Photomicrograph of abnormal maturing or/Graafian follicles in F_1 females ovary.

PM 136. Perinuclear space within the follicle.

PM 137. Two micronucleoli within the nucleus.

PM 138. Large perivitelline space and two nucleoli (One mega and one micro) within the nucleus.


136


137


138

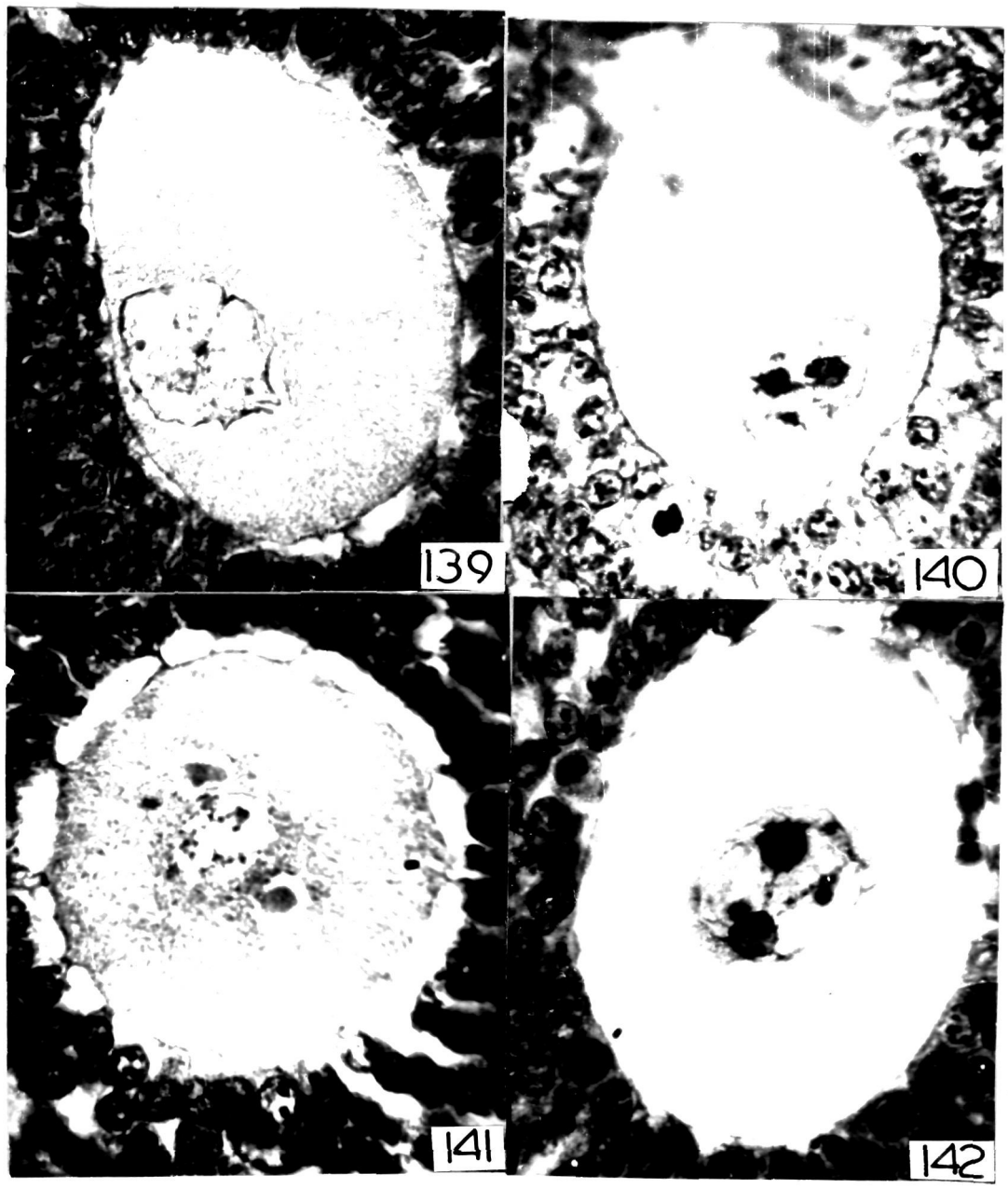
Explanation of Photomicrographs

PM 139. Two micro nucleoli within the nucleus.

PM 140. Two nucleoli within the nucleus with a bridge.

PM 141. Three nuclei within the follicle .

PM 142. Two micro and two macro-nucleoli within the nucelus.


139
140
141
142

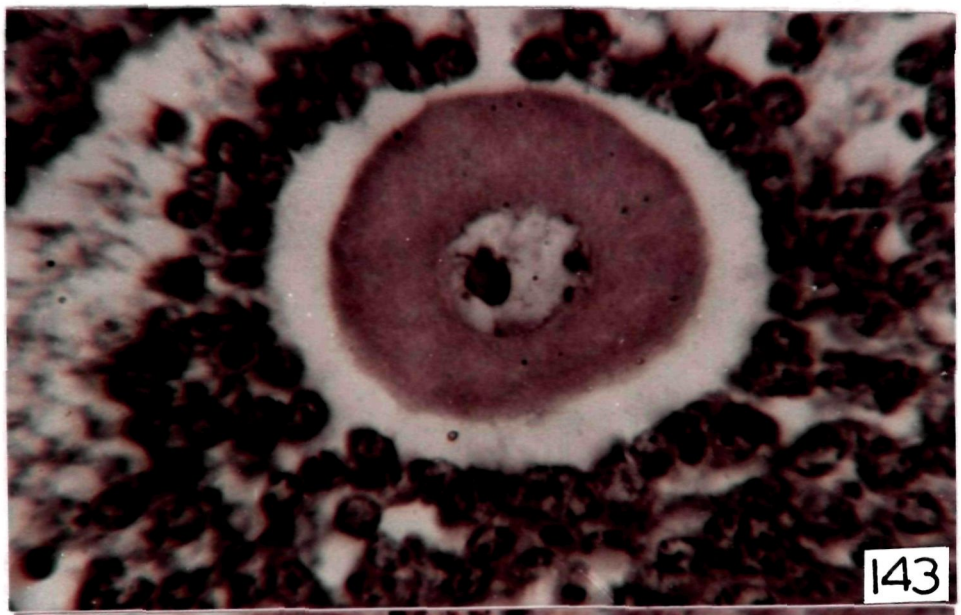
Explanation of Photomicrographs

Photomicrograph of abnormal maturing/Graafian follicles in F_1 females ovary.

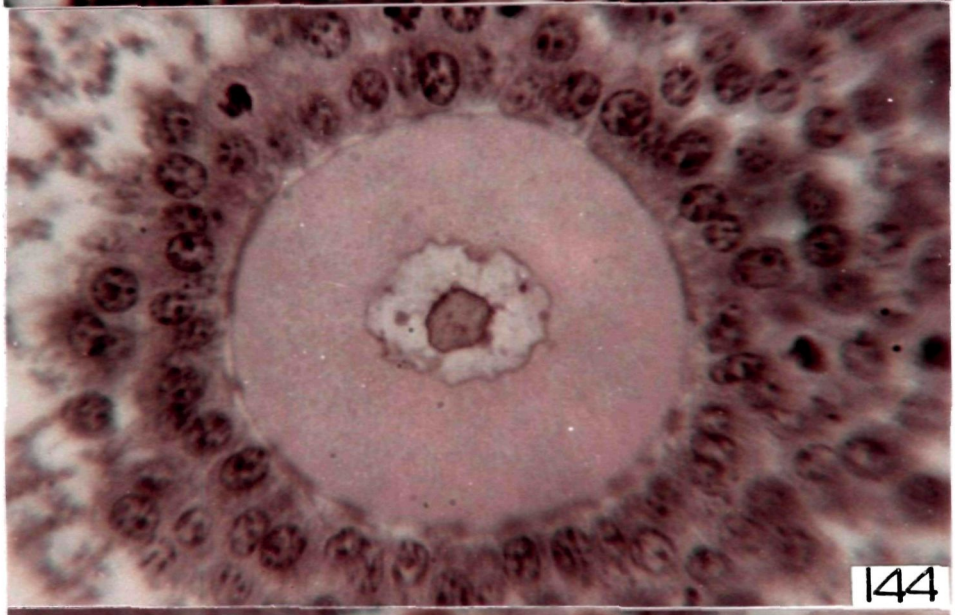
PM 143. Large perivitelline space with two micro and one macro-nucleoli within the nucleus.

PM 144. Two micro- and one macro-nucleoli within the nucleus.

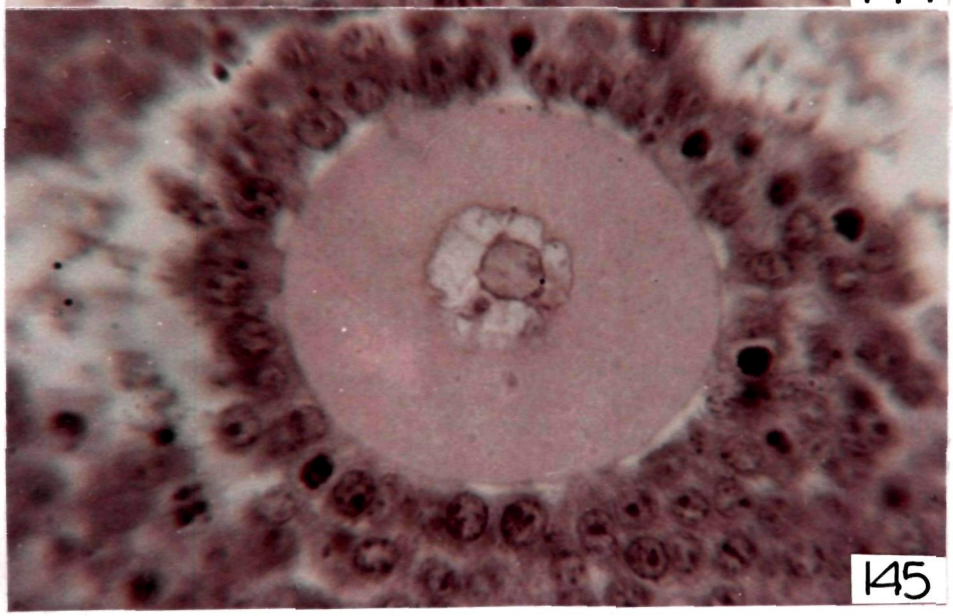
PM 145. One micro- and one macro-nuclei within the nucleus.



143



144



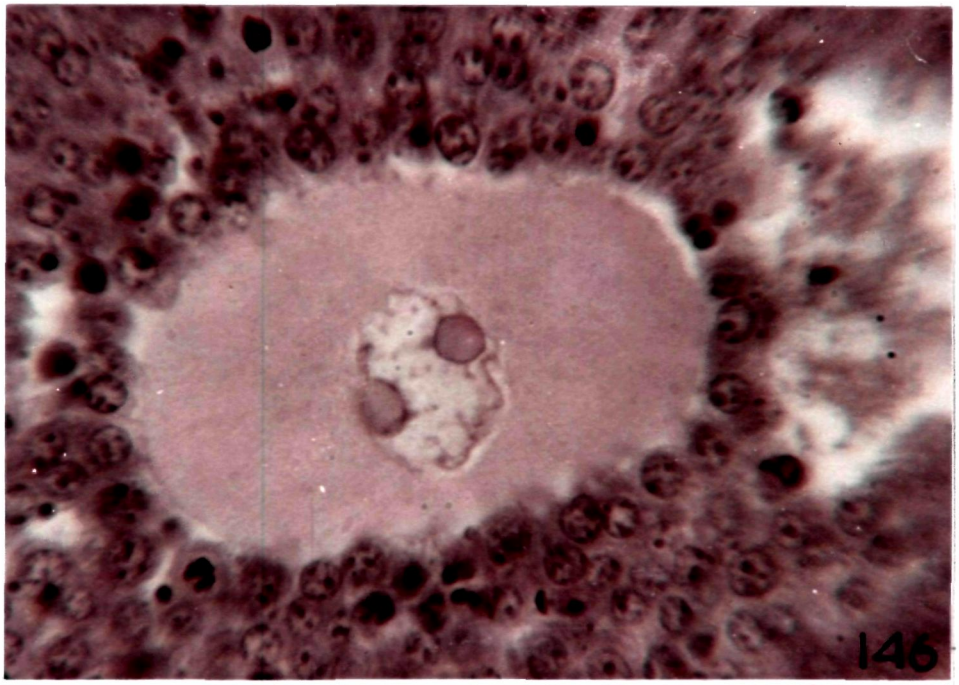
145

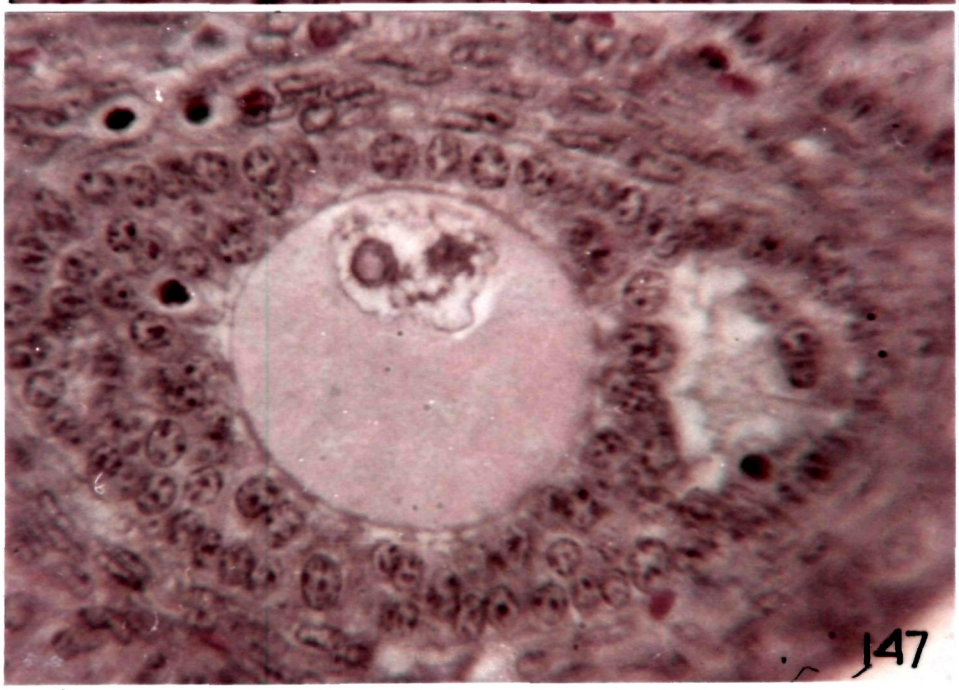
Explanation of Photomicrographs

Photomicrograph of abnormal maturing or/Graafian follicles in F_1 females ovary.

PM 146. Two large nucleoli and chromatin elements within the nucleus.

PM 147. Two large nucleoli near the nuclear membrane.


146


147

c) Effect of uterus :

The study of the histological sections of the uterus of the 2nd and 4th week F_1 control and treated line females mentioned before showed no difference in two lines. This would imply that the sterility could have developed due to the effect in the ovary and/or testes but not due to the accessory part of the reproductive organ.

General comments on Chapter II :

The cytological assays in F_1 generation carried out by various methods mentioned before have shown that the treated line individuals had much higher effect than that of control line.

The lethal test conducted for the post implantation loss at 15 day gestation and at the end of weaning period after parturition suffered from adequate data. In some weeks no pregnancy was found e.g. 5th and 6th week in the treated line while in other week also the data were limited. Thus it seemed that some sterility developed and/or reproductive potentiality was reduced. Though the data of the lethal test suffered limitation because of the sterility infertility problem, even then it showed that the mutagenic index was higher than that of control.

The study of the chromosome aberration in the liver cells of embryos of 15 day gestation and the bone marrow cells of 3 month old F_1 showed significantly high frequency

of aberrations in treated lines than that of the corresponding controls. This would support the mutagenic effect produced for the treatment of log-culture of P. aeruginosa to male parent mice. This was also supported from the result of the meiotic chromosome aberration studied in 3 month old F_1 treated line males against controls and also by the sperm depletion test and the frequency of sperm with abnormal head morphology. Besides the mutagenic effects, the study of teratogenic effects and histological changes in the testes, ovary and uterus also supported genotoxic potentiality of the log culture P. aeruginosa. That the cytological phenomenon is under genetic control was postulated for chromosome pairing Herdeum and Secale (Gupta, 1985a, b) chromosome aberrations by Manna (1984) and so on.

Some lacunae of the present study have been mentioned already which concerned mainly with the limitation of the data in some cases. This was unavoidable because there was a tremendous fall in the reproduction potentiality. Even repeating the experiment using good number of F_1 males and females did not help to improve this draw back. The frequency was found exceedingly high in the treated line which would favour the transmission of mutagenic factor to the F_1 generation.

Table 33

Frequency of ovum abnormality in F_1 female progeny of different mothers mated at different weeks to *P. aeruginosa* treated male parents

Fix.		Stage	Total No.	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Peri-nucleus cavity	Atri. foll.	Total	%
						Control					
2nd wk	1	Gf/M	70	4	1	-	-	-	1	6	8.75
		S.F.	76	3	-	-	-	-	-	3	3.94
		Total	146	7	1	-	-	-	1	9	6.16
						Treated					
	1	Gf/M	75	3	1	-	2	-	-	6	8.00
		S.F.	77	1	1	1	-	-	-	3	3.89
		Total	152	4	2	1	2	-	-	9	5.92
						Control					
	2	Gf/M	90	4	-	-	-	-	3	7	7.77
		S.F.	120	6	-	-	-	4**	-	10	8.33
		Total	210	10	-	-	-	4	3	17	8.09

Table 33 (Contd.)

Fix.		Stage	Total No.	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Peri-nucleus cavity	Atri. foll.	Total	%
						Treated					
2nd wk	2	Gf/M	61	32	20	3	1	-	-	56	83.60
		S.F.	120	50	8	2	-	3*	-	63	52.50
		Total	181	82	28	5	1	3	-	119	65.74
						Control					
	3	Gr/M	85	7	2	-	-	-	2	11	12.94
		S.F.	135	9	-	-	-	3**	-	12	8.88
		Total	220	16	2	-	-	3	2	23	10.45
						Treated					
	3	Gf/M	60	43	5	1	4	5	-	58	96.66
		S.F.	102	41	2	-	-	35**	-	78	76.47
		Total	162	84	7	1	4	40	-	136	83.95

Table 33 (Contd.)

Fix.		Stage	Total No.	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Peri-nucleus cavity	Atri. foll.	Total	%
						Control					
4th wk	1	Gf/M	110	3	1	-	-	2	4	10	9.09
		S.F.	120	7	-	-	-	4**	-	11	9.16
		Total	230	10	1	-	-	6	4	21	9.13
						Treated					
	1	Gf/M	60	36	4	2	2	3	4	51	85.00
		S.F.	122	54	2	-	-	30**	-	86	70.49
		Total	182	90	6	2	2	33	4	137	75.27
						Control					
	2	Gf/M	110	5	2	-	-	2	3	12	10.90
		S.F.	105	9	-	-	-	7**	-	16	15.23
		Total	215	14	2	-	-	9	3	28	13.02

* Tri nucleated

** Cytoplasm abnormal.

CHAPTER III

Cytogenetic Assays in Second Filial (F_2) Generation

A. Lethal test

a) At the end of weaning period of the mother :

It has been mentioned before that the lethal so-called 'dominant test' was conducted in F_1 generation in mice by various worker after mating the treated male parents to virgin females by the assessment of the pre-implantation loss or post-implantation loss. The effect, was presumed to be due to the induction of dominant lethal and, therefore, they did not follow the F_2 generation. On the other hand Manna and his collaborators (Manna, 1980, 1981, 1982; Roy and Manna, 1981; Manna and Patel, 1981, 1985) found that if F_1 individuals of the treated line were selfed, in F_2 generation some lethal effect was indicated.

However, in the present study, the lethal effect was not possible to evaluate when F_1 males and females of the treated line of different weeks were separately mated (Table 34). In the control line the data were extremely limited because the reproductive potentiality of the F_1 individuals was also seriously affected. An analysis of the data of the control line at gestation and after parturition would show (Table 34) that 2 pregnant females in each of 1st, 2nd, 3rd and 4th week yielded all living implants except 1 dead implant in 3rd week. Therefore, the data oviously showed that no lethal factor was operating in the control line. However, out of 13 F_1 females deployed for mating,

8 were pregnant indicating that there was some sterility or else the reproduction was impaired for the effect in the germinal gland and the gametes.

The data of the lethal effect in the 1st to 4th week treated line were seriously handicapped because practically no pregnant female was obtained by selfing F_1 male and females in these weeks. Out of 10 females allowed for mating only two, one of 2nd week and the other of the 4th week were pregnant (Table 34). Both of them had 4 living implants and none was dead. Therefore, nothing could be said about the lethal effect in F_2 generation in the treated line and as such it was absent. A proper judgement was not possible. However, the occurrence of the chromosome aberration, histological changes in the reproductive organs, the sperm head morphology and sperm depletion test might indicate that the effect of bacterium was present in the F_2 generation.

B. Somatic chromosome aberration in F_2 generation

a) In liver of embryos of 15 day gestation :

The liver of living embryos of 15 day gestation obtained from inbreeding of F_1 male and female of 4 week control lines were processed for the study of chromosome aberrations. The preparation of the liver cell of each individual was made according to the procedure described before.

Qualitatively the aberrations were in the form of individual type and gross type with variable frequencies in different individuals. Six F_2 living embryos, 3 males and 3

Table 34

Data of lethal test in F_2 raise from different mothers of treated and control mated with F_1 males

Week	No.of fem. allowed for mat.	No.of preg. fem.	Mother No.	At	Total No.of impl.	No.of live impl.	No.of dead impl.
1st wk			Control line				
	3	2	1	Ges	6	6	-
			2	Partu.	5	5	-
Total	3	2	2		11	11	-
			Treated line				
	2	-	-		-	-	-
			Not impregnated				
Total	2	-	-		-	-	-
2nd wk			Control line				
	4	2	1	Ges	5	5	-
			2	Partu.	6	6	-
Total	4	2	2		11	11	-
			Treated line				
	3	1	1	Partu.	4	4	-
Total	3	1	1		4	4	-
3rd wk			Control line				
	3	2	1	Ges	6	5	1
			2	Ges	7	7	-
Total	3	2	2		13	12	1
			Treated line				
	1	Not impregnated					
	2	Do					
	1	-	-		-	-	-
4th wk			Control line				
	3	2	1	Ges	7	7	-
			2	Partu.	6	6	-
Total	3	2	2		13	13	-
			Treated line				
	1	Not impregnated					
	1	1	1	Partu.	4	4	-

females of a F_1 mother of 1st week control line had very little individual type aberration. If the cases of centromeric dissociation were disregarded, two embryos would have no individual type aberration. The frequency in 6 F_2 embryos ranged between 2.0% and 9.0% and the average was 6.5% (Table 35). In the liver cells of 2nd week control line embryos more or less the same situation was found. Out of 2 males and 3 females of a F_1 mother, the frequency of aberration ranged between 4.0% and 8.0% and the average frequency 6.6%. Similarly in 2 male and 3 female embryos of a 3rd week. Control F_1 mother, the frequency of aberration ranged between 3.0% and 9.0% and the average was 6.4% (Table 35). Further 3 male and 1 female of another F_1 3rd week control line mother had the aberration frequency ranging between 6.0% and 8.0% and the average frequency was 6.75%. In 4 males and 3 females F_2 embryos of a 4th week control line mother, the chromosome aberration frequency ranged between 1.0% and 11.0% and the average was 7.0% (Table 35).

If the aberration data of a total of 27 embryos obtained from 5 mothers of four week control lines were combined out 2700 metaphases examined in liver cells, there were only 10 or 0.3% chromatid breaks, 3 or 0.11% acentric fragments, 3 or 0.11% translocation or associations, 19 or 0.7% gaps and constrictions, 70 or 2.5% centromeric dissociations making a total of 106 or 3.9% individual type aberrations. Further, the cases of centromeric dissociation if disregarded, the average frequency would be reduced to 1.2%. The frequency of gross type was 74 or 2.7%. Therefore, the average frequency of both individual and gross type aberrations taking together was 180 or 6.6%. However, as mentioned before some of the embryos practically had no individual type of

aberration if the cases of centromeric dissociation was disregarded. If the average aberration frequency in different week of control line was compared (Table 35) with that of F_1 generation (Table 16) it would be found 6.5% against 6.14% in the 1st week, 6.6% against 7.1% in 2nd week, 6.5% against 5.1% in 3rd week, and 7.0% against 4.8% in the 4th week respectively. Therefore, the frequency of aberration did not vary appreciably in control line embryos of F_1 and F_2 generation.

It was mentioned before that as the number of pregnant mothers in the treated lines was limited, they were not be sacrificed during gestation. Therefore, in the absence of any data of chromosome aberrations in the treated line F_2 embryos, we could not make any comparison with that of the control data. However, there was another way of comparison because instead of F_2 embryos, some F_2 adult individuals were available. They were sacrificed at the age of 3 months to study the meiotic chromosome aberrations, sperm depletion, sperm head morphology etc. with a view to compare with the corresponding control (vide infra).

b) F_2 individual of 3 month old :

Bone marrow chromosome aberrations :

The bone marrow chromosome aberrations in 3 month old individuals of F_2 had qualitatively the aberrations in the form of individual types and gross types (PM 148-159; Figs. 108-127) in variable frequencies. In three males and two female F_2 individual of a 1st week control line mother, the aberration frequency ranged between 4.0% and 12.0%.

Explanation of Photomicrographs

Photomicrograph of affected metaphases plates of F_2 bone marrow cells of treated line F_2 individuals.

PM 148. Plate showing several individual type aberration as an acentric fragment, a translocation, a constriction, isochromatid gap etc.

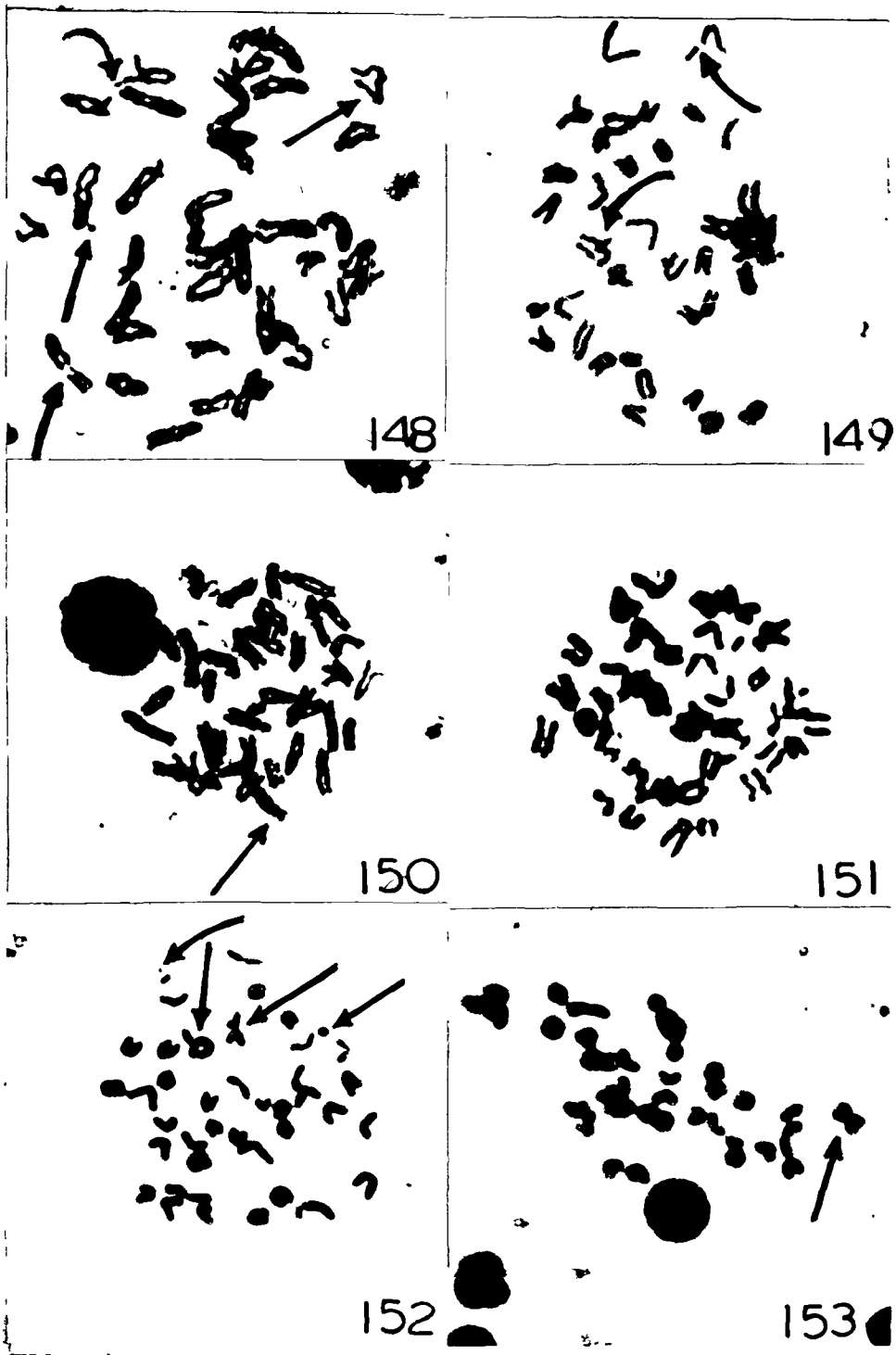
PM 149. A chromatid break in the middle region of a group I chromosome and an acentric fragment.

PM 180. A chromosome with two breaks in a chromatid, a terminal association.

PM 151. A fragment and centromeric dissociation in different groups of chromosomes.

PM 152. Plate showing acentric fusion, a ring chromosome, a fragment and one extra element.

PM 153. A centric fusion.


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Explanation of Photomicrographs

Photomicrographs (PM) of affected metaphases of F_2 bone marrow cells of F_2 treated line individuals.

PM 154. A centric fusion.

PM 155. A ring chromosome.

PM 156. A chromatid break in the distal region Group III chromosome.

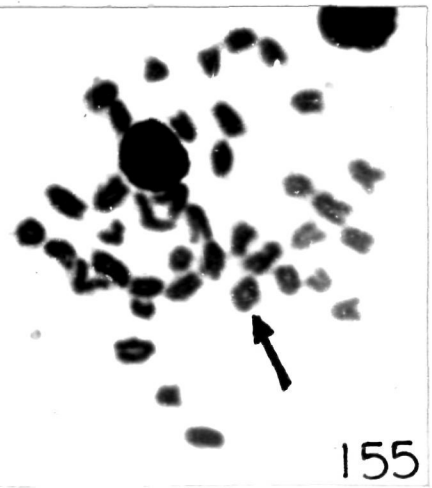
PM 157. Centromeric dissociation in group V chromosome.

PM 158. Sticky metaphase plate.

PM 159. Pulverization.



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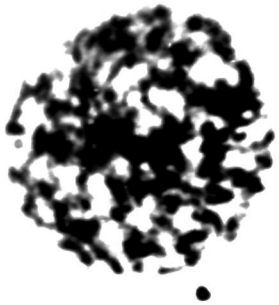
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Explanation of figures

Camera lucida drawing of a part of metaphase plates showing different types of aberrations in F_2 treated line individuals.

Figs. 108. A chromatid break in the middle region of a group I chromosome.

Figs. 109-110. A chromatid break in the distal region of a group I chromosome.

Figs. 111-112. A chromatid break in the middle region of a group II chromosome.

Fig. 113. A chromatid break in the distal region of a group II chromosome.

Figs. 114-115. A chromatid break in the middle region of a group III chromosome.

Fig. 116. A chromatid break in the distal region of a group III chromosome.

Figs. 117-118. A chromatid break in distal region of a group IV chromosome.

Figs.119-120. End to end association between two chromosome.

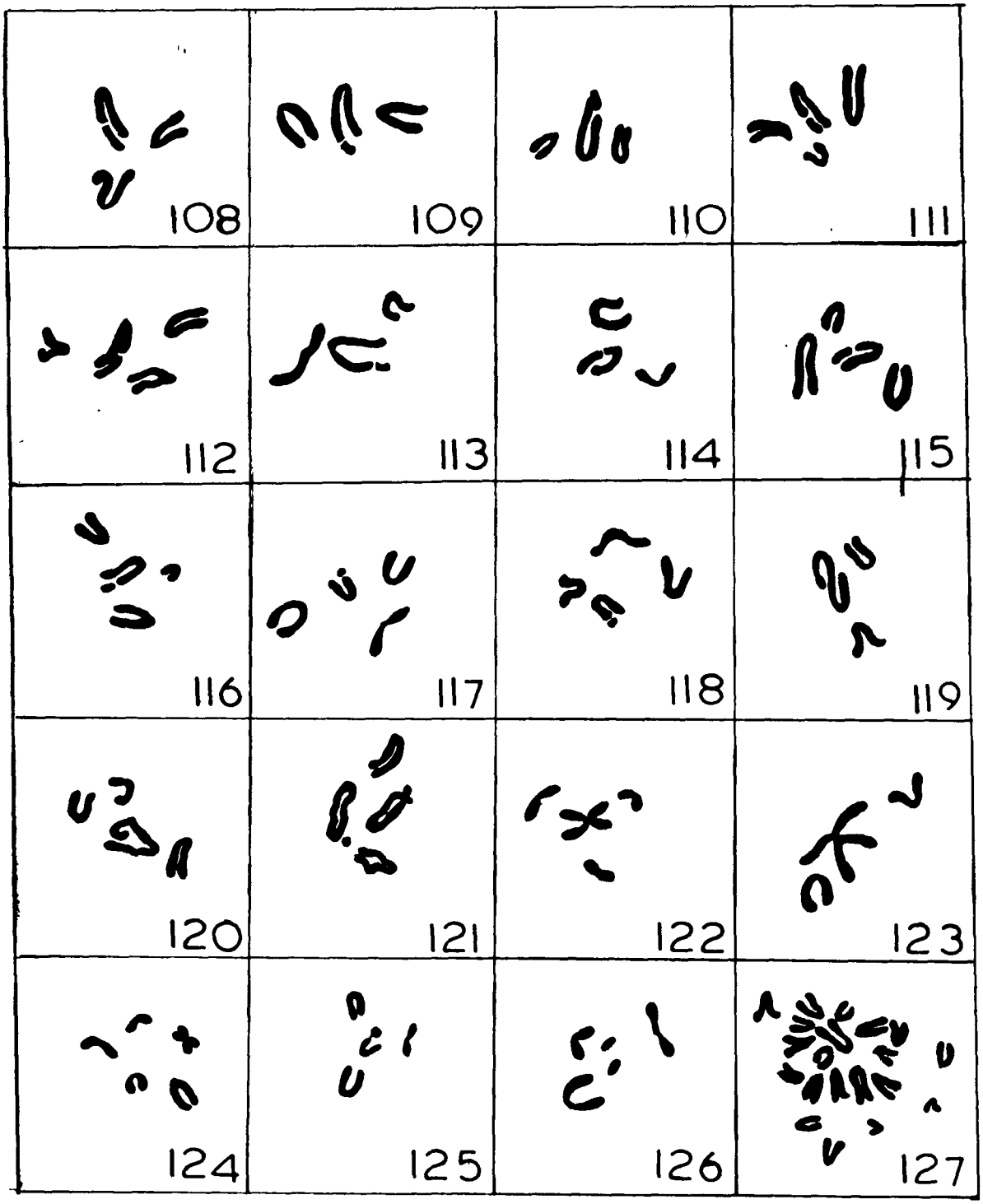
Fig. 121. A ring chromosome and a fragment.

Figs. 122-124. A centric fusion between the chromosomes.

Fig. 125. A constriction.

Fig. 126. The centromeric dissociation of a group V chromosome.

Fig. 127. An aster metaphase.


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The average frequency in these 5 F_2 individuals was 7.6% (38 in 500 metaphases) (Table 36) which was not very different from that of 1st week F_1 embryos of control line (Table 20). Unfortunately no F_2 individual of 1st week treated line was available. Therefore, the data could not be compared.

Two female and 2 male of F_2 individuals of 2nd week control line yielded the bone marrow chromosome aberration frequency in different individuals ranging between 6.0% and 14.0%. In the combined data of 4 individuals, the average frequency was 9.5% (38 in 400 metaphase; Table 36). The frequency appeared to be little higher than that of embryos of 15 day gestation of 2nd week control line. Anyhow, in the 2nd week treated line 2 male and 2 female F_2 adults were available. The chromosome aberration frequency in their bone marrow cells ranged between 15.0% and 56.0% (Table 36). In the combined data of 4 individuals, the average aberration frequency was 37.51% (Table 37) which was much higher than that of control (9.5%) and the net increase in the average frequency of the 2nd week treated line F_2 individuals was 28.0% (Table 37). Qualitatively the aberrations were in the form of chromatid break, centric fusion, ring chromosome, translocation or association, acentric fragment etc. (PM 148-157; Figs. 108-125) and gross type (PM 158, 159) some of which had genetic significance.

Since no treated line F_2 individuals of 3rd week mating line was available, the control line data could not be compared.

Three month old F_2 individuals from both 4th week F_1 control and treated line mating mothers were available

Table 35

Frequency distribution of chromosome aberrations in liver of F_2 embryos at 15 day gestation of F_1 mothers mated to control and treated line F_1 male parents. 100 plates per ind. were assessed.

Fix.	Ind. No.	Sex	Individual type aberrations: Break type: Chr.	Break type: Frag.	Break type: Trans./ Asso.	Phy. type: Gaps & cons.	Phy. type: Cent. disso.	Total	Gross type aberration: Num. cha.	Gross type aberration: Others	Gross type aberration: Total	Grand total	%
						Control							
1st	1	M	-	-	-	-	-	-	-	2	2	2	2.00
wk	2	M	-	-	-	1	3	4	1	-	1	5	5.00
	3	M	-	-	2	1	3	6	2	1	3	9	9.00
	4	F	1	-	-	-	1	2	3	2	5	7	7.00
	5	F	2	-	-	-	4	6	-	2	2	8	8.00
	6	F	-	-	-	-	5	5	3	-	3	8	8.00
Total	6	3M+3F	3	-	2	2	16	23	9	7	16	39	6.50

Not available

*No treated line F_2 embryo was available from 1st, 2nd, 3rd and 4th week F_1 mating parents.

Table 35 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans./ Asso.	Gaps & cons.	Cent. disso.						
2nd wk						Control							
	1	M	-	-	-	-	-	-	-	4	4	4	4.00
	2	M	-	-	-	2	3	5	-	2	2	7	7.00
	3	F	-	-	-	2	5	7	-	1	1	8	8.00
	4	F	-	-	-	-	7	7	-	-	-	7	7.00
	5	F	1	-	-	1	4	6	1	-	1	7	7.00
Total	5	2M+3F	1	-	-	5	19	25	1	7	8	33	6.60
3rd wk						Mother I Control							
	1	M	1	-	-	-	3	4	-	2	2	6	6.00
	2	M	-	2	-	1	-	3	-	2	5	8	8.00
	3	F	-	-	-	1	3	4	-	2	2	6	6.00
	4	F	-	-	-	-	-	-	3	-	3	3	3.00
	5	F	2	-	-	-	4	6	-	3	3	9	9.00
Total	5	2M+3F	3	2	-	2	10	17	3	9	15	32	6.40

Table 35 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans./ Asso.	Gaps & Cons.	Cent. disso.						
3rd wk						Mother II							
						Control							
	1	M	-	-	-	-	3	3	-	3	3	6	6.00
	2	M	-	-	-	2	3	5	1	2	3	8	8.00
	3	M	-	-	-	-	2	2	-	4	4	6	6.00
	4	F	-	-	-	-	-	-	2	5	7	7	7.00
Total	4	3M+1F	-	-	-	2	8	10	3	14	17	27	6.75
4th wk													
						Control							
	1	M	1	-	-	2	3	6	-	1	1	7	7.00
	2	M	-	-	-	-	1	1	-	-	-	1	1.00
	3	M	-	-	-	-	2	2	-	5	5	7	7.00
	4	M	-	-	-	-	5	5	-	2	2	7	7.00
	5	F	-	1	1	2	3	7	-	2	2	9	9.00
	6	F	2	-	1	1	-	4	-	3	3	7	7.00
	7	F	-	-	-	3	3	6	2	3	5	11	11.00
Total	7	4M+3F	3	1	2	8	17	31	2	16	18	49	7.00

(Table 36). In the bone marrow of control line 3 male and 3 female F_2 individuals, the frequency of aberrations in different individuals ranged between 5.0% and 13.0%. In the combined data of 6 individuals the average aberration frequency taking both individual and gross type together was 8.5% (51 out of 600 metaphases). On the other hand an examination, the bone marrow cells of 2 male and 2 female F_2 individuals of 4th week treated line F_1 mother, the frequency of aberration ranged between 16.0% and 58.0% (Table 36). Out of 400 metaphase in 4 individuals the total number of aberration was 143 or 35.75% (Table 37) which was much higher than that of control (8.50%). The net increase was there in the 4th week treated line was 27.25% (Table 37).

General comments :

It has been mentioned before that the chromosome aberration data in F_2 generation could not adequately be obtained for nonviability of pregnant females for which the comparison made here would have some limitation. In the treated line only the data of 2nd week and 4th week were obtained which could be compared with that of the control line. It revealed that the treated line had much higher frequency of aberration than that of control. Therefore, in the present study though the lethal testing in F_2 was possible, the chromosome aberration in bone marrow cells of F_2 adults of the treated line clearly indicated that the mutagenic factor was transmitted to F_2 from F_1 generation.

Table 36

Frequency distribution of chromosome aberration in bone marrow of 3 month old F_2 individuals from mating of F_1 mother of control and treated line F_1 males. 100 plates/ind. were assessed.

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy.type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.	Cent. Disso.						
1st wk													
						Control							
	1	M	-	-	-	-	3	3	2	2	4	7	7.00
	2	M	-	-	-	-	-	-	2	5	7	7	7.00
	3	M	1	1	-	2	3	7	2	3	5	12	12.00
	4	F	-	-	-	2	3	5	-	3	3	8	8.00
	5	F	-	-	-	-	-	-	2	2	4	4	4.00
Total		3M+2F	1	1	-	4	9	15	8	15	23	38	7.60
						Treated							
						Not impregnated							

Table 36 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Phy. type		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & Cons.	Cent. Disso.						
2nd wk							Control						
	1	M	-	-	-	-	5	5	-	4	4	9	9.00
	2	M	-	-	-	3	5	8	2	4	6	14	14.00
	3	F	1	-	1	2	-	4	-	2	2	6	6.00
	4	F	-	-	-	-	4	4	-	5	5	9	9.00
Total		2M+2F	1	-	1	5	14	21	2	15	17	38	9.50
							Treated						
	1	M	4	3	5	3	14	29	8	6	14	43	43.00
	2	M	2	1	2	4	11	20	9	7	16	36	36.00
	3	F	5	1	12	3	17	38	11	7	18	56	56.00
	4	F	-	-	3	3	3	9	1	5	6	15	15.00
Total		2M+2F	11	5	22	13	45	96	29	25	54	150	37.51

Table 36 (Contd.)

Fix.	Ind. No.	Sex	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type				Phy.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans. Asso.	Gaps cons.	& Cent. disso.						
4th wk							Control						
	1	M	-	-	-	-	5	5	-	3	3	8	8.00
	2	M	-	2	-	2	6	10	-	3	3	13	13.00
	3	M	-	-	-	1	3	4	-	3	3	7	7.00
	4	F	-	-	-	1	5	6	-	4	4	10	10.00
	5	F	-	-	-	-	-	-	-	5	5	5	5.00
	6	F	1	-	-	-	7	8	-	-	-	8	8.00
	Total	3M+3F	1	2	-	4	26	33	-	18	18	51	8.50
							Treated						
	1	M	-	-	-	-	7	7	5	4	9	16	16.00
	2	M	5	-	7	2	21	35	5	11	16	51	51.00
	3	F	4	2	3	1	23	33	13	12	25	58	58.00
	4	F	-	-	-	-	6	6	5	7	12	18	18.00
	Total	2M+2F	9	2	10	3	57	81	28	34	62	143	35.70

Table 37

Compiled data of chromosome aberration in bone marrow of 3 month old F_2 individual after birth from mating of F_1 mothers from 1st week to 4th week with control and the bacterium *P. aeruginosa* treated line F_1 males. Control data in brackets.

Fix.	No.of mother	No.of emb-ryos	No.of meta-phase	Individual type aberrations					Total	Gross type aberration		Grand total	%	Net incr. %
				Break type			Phy.type			Num. chan.	Others	Total		
				Ch.	Frag.	Trans/ Ass.	Gaps & const.	Cent. diss.						
1st wk	- (1)	- (6)	- (600)	- (1)	- (1)	- (-)	- (4)	- (9)	- (15)	- (8)	- (15)	- (23)	- (38)	- (6.33)	-
2nd wk	1 (1)	4 (5)	400 (500)	11 (1)	5 (-)	22 (1)	13 (5)	45 (14)	96 (21)	29 (2)	25 (15)	54 (17)	150 (38)	37.50 (9.50)	28.00
4th wk	1 (1)	4 (6)	400 (600)	9 (1)	2 (2)	10 (-)	3 (4)	57 (26)	81 (33)	28 (-)	34 (18)	62 (18)	143 (51)	35.75 (8.50)	27.25
Total	2 (3)	8 (17)	800 (1700)	20 (3)	7 (3)	32 (1)	16 (13)	102 (49)	177 (79)	57 (10)	59 (48)	116 (58)	293 (127)	36.62 (7.40)	29.22

C. Meiotic chromosome aberration in 3 month old F_2 males

Since two F_2 adult males could only be obtained from 2nd week and 4th week treated line from mating of F_1 parents, the present study suffered some limitation as compared to that of F_1 adult males (Table 22). Further in the present study relatively less number of cells in different stages of meiosis was available for the study of chromosome aberration as compared to the corresponding control males.

The preparations of the meiotic chromosomes of testis of 3 month old F_2 3 males each of 2nd and 4th week control line and 2 F_2 males each of the 2nd and 4th week treated line were made according to the method described earlier.

Further as followed before while assessing chromosome aberrations in 100 metaphase I plates per individual of control and treated line males, all the spermatogonial metaphase, diakinesis and metaphase II were also assessed.

Spermatogonial metaphase :

Normal spermatogonial metaphase complement was relatively rare because in most cases the chromosomes had some physiological effect like despiralization, stickiness etc. In spite of such general effects on chromosomes, some individual and gross type aberrations could be scored in the form of chromatid breaks, acentric fragment, centric fusion etc. as individual types and stickiness, numerical change, pulverization etc. as gross type (PM 161-164; Figs. 128-133).

Explanation of Photomicrographs

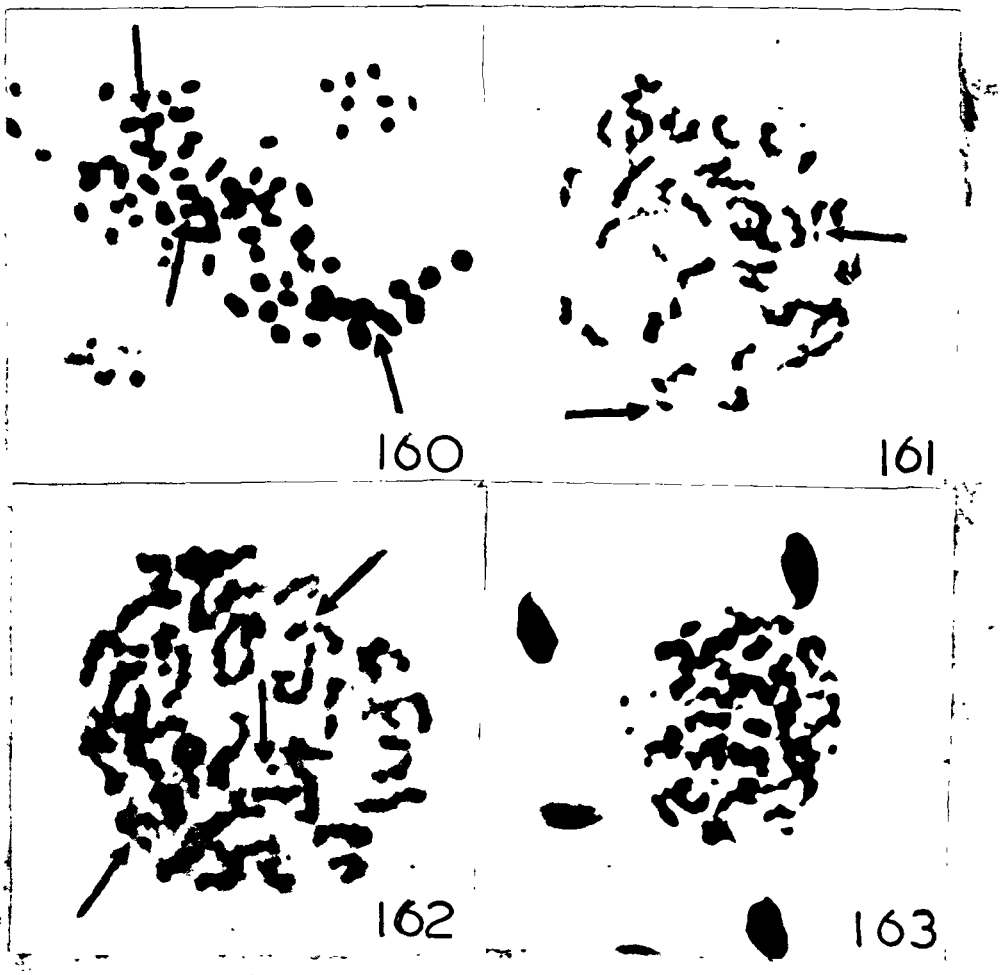
Photomicrographs of affected spermatogonial metaphases in F_2 treated line male mice.

PM 160. Plate with aneuploid number containing.

PM 161. Plate sticky effect with some fragments breaks etc.

PM 162. Plate despiralization effect with some acentric fragment, break etc.

PM 163. Pulverization effect.


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Explanation of figures

Camera lucida drawing of a part of spermatogonial metaphase in F_2 generation showing different types of aberrations.

Fig. 128. A chromatid break.

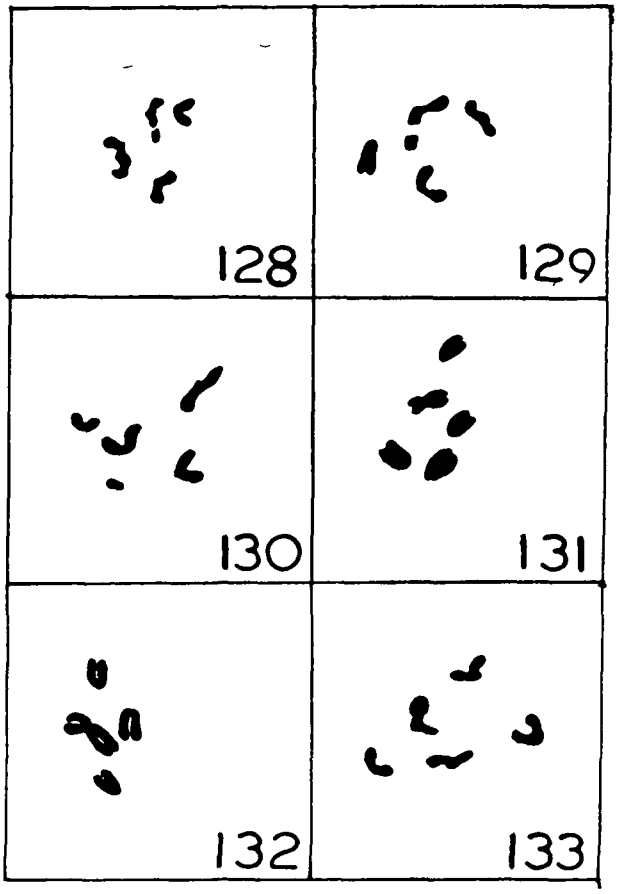
Fig. 129. A chromatid break.

Fig. 130. A fragment of unknown origin.

Fig. 131. Centric fusion between two chromosomes.

Fig. 132. Centric fusion between two chromosomes.

Fig. 133. A constriction.


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The total frequencies of spermatogonial metaphase chromosome aberrations in 2 F_2 males of 2nd week treated line were 29.72% and 60.0% (Table 38). In the combined data of two individuals out of 82 plates there were 4 chromatid breaks, 1 fragment, 1 translocation or association and 3 gaps and constriction making a total of 9 or 10.9% individual type aberration while the frequency of gross type was 29 or 35.3%. The average frequency was 46.34% (Table 39). It was noted that in one out of two testes of one male, there was no individual type aberration. On the other hand in the 2nd week control line 3 F_2 males, the aberration frequency ranged between 9.33% and 13.58% (Table 38). In a total of 271 plates there were 4 chromatid breaks and 4 gaps or constriction making a total of 8 or 3.37% individual type while the number of gross types was 24 or 10.12%. The average frequency was 11.8% which was much lower than that of treated line (46.34%). The net increase was 34.54% (Table 39).

In the 4th week treated line the spermatogonial metaphase chromosome aberration frequencies in two males were 54.54% and 62.85%(Table 38). The frequency of individual type aberration was very low in comparison to that of gross type. In the combined data out of 90 spermatogonial metaphases there were only 4 or 4.4% individual type and 48 or 53.5% gross type aberrations making a total of 57.77% taking both types together (Tables 38-39). On the other hand in the 3 males of the control line, the spermatogonial metaphase aberration frequency ranged between 10.52% and 20.0% (Table 38). In a total of 247 spermatogonial metaphases of 3 individuals, there were only 5 or 2.2% individual type and 31 or 12.5% gross type aberrations. The combined frequency was 14.57%

taking both individual and gross types together which was much lower than that of treated line (57.77%). Thus the net increase was about 43.20 in the treated line (Table 39).

The data of 2nd week and 4th week were combined (Table 39) to have the larger sample. It showed that out of the total 172 spermatogonial metaphases there were only 13 or 7.55% individual type and 77 or 44.76% gross type aberrations. The average frequency was 52.32% (Table 39). On the other hand in the control line out of 518 spermatogonial metaphases, there were 14 or 2.50% individual type and 55 or 10.6% gross type making a total of 68 or 13.12%. In the combined data of both types, the net increase of the aberration frequency in the spermatogonial metaphase of the treated line was 39.20% which was significantly high.

Comments :

The analysis of aberration data in the spermatogonial metaphase of control and treated line showed significant increase in both type of aberrations in the treated line over control. This would definitely indicate that the mutagenic effect was continued in F_2 generation in spite of the fact that there was considerable reproductive failure in the treated line. Thus the aberration data which could be studied only in two weeks supported the view that the mutagenic factor induced by the treatment of log culture of *P. aeruginosa* to male grand parent was transmitted to F_2 generation which yielded higher frequency of chromosome aberrations.

If the present data of spermatogonial metaphase chromosome aberration were compared with that of F_1 generation (Table 23) it would be found that the frequencies between treated and control lines were 50.87% against 9.39% in the 2nd week, 52.2% against 8.08% in 4th week and 51.53% against 8.23 in the average respectively. While in F_2 generation they were 46.34% against 11.80% in 2nd week; 57.27% against 14.57% in 4th week and in the average of two weeks 52.32% against 13.12% (Table 39). Therefore, the data of chromosome aberration in spermatogonial metaphase in F_1 and F_2 generation did not vary appreciably. This could be explained if it was assumed that the higher frequency of aberration in F_1 and F_2 generation was due to the transmission of the mutagenic factor induced by the treatment of P. aeruginosa culture to male parents.

Diakinesis :

The study of chromosome aberrations in the diakinesis nuclei of the same treated and control line males showed both individual and gross type aberrations though the number of individual type aberrations appeared relatively more. The individual type aberration consisted of chromatid breaks, fragment of unknown origin, bivalent association or translocation etc. (PM 164-166; Figs. 134-139).

In the two male individuals of 2nd week treated line the frequencies of total aberration were 14.92% and 48.0% (Table 40). If the data were combined, out of 92 diakinesis nuclei there were 14 or 15.21% individual type and 8 or 8.66% gross type making a total of 22 or 23.87%. On the other hand

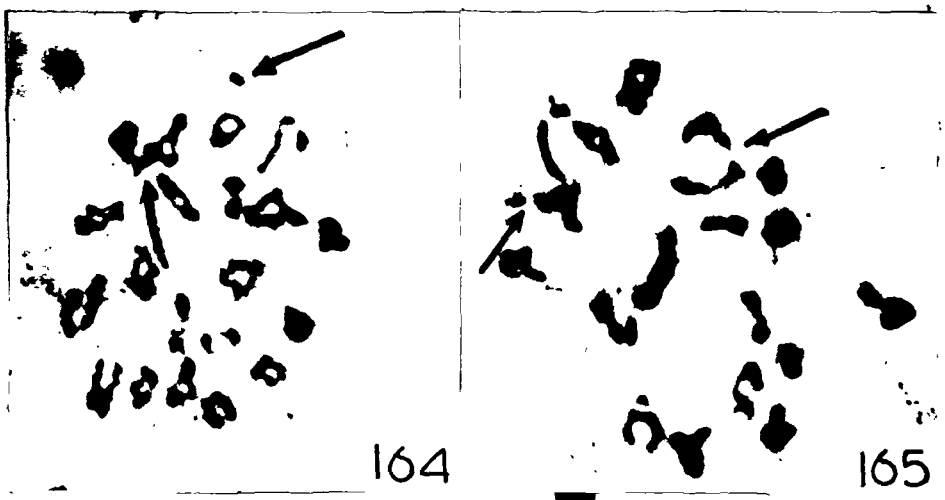
Explanation of Photomicrographs

Photomicrographs of affected diakinesis in F_2 treated line male mice.

PM 164. Translocation or terminal association between two bivalents and a fragment.

PM 165. A chromosome break and a interbivalent connection.

PM 166. Precocious separation of an autosomal bivalent.



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Explanation of figures

Camera lucida drawing of a part of diakinesis plate showing different types of aberrations.

Fig. 134. A chromatid break.

Fig. 135. A chromosome break.

Fig. 136. A chromatid break.

Fig. 137. A fragment of unknown origin.

Fig. 138. A fragment of unknown origin.

Fig. 139. A precocious separation of autosomes and sex chromosomes.

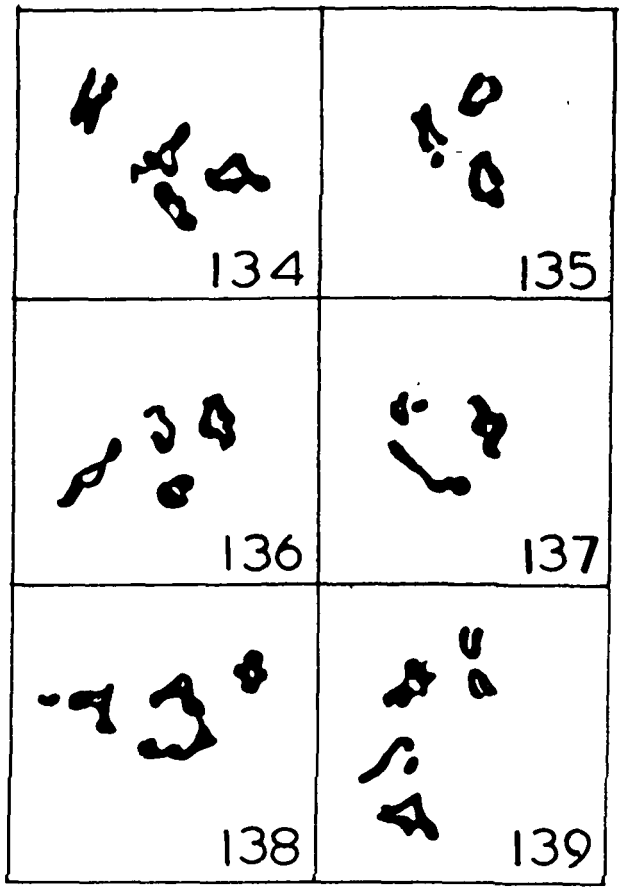

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Table 38

Frequency distribution of chromosome aberration in spermatogonial metaphase cells of F_2 male of control and the bacterium P. aeruginosa treated line mice of 2nd and 4th week

Fix.	Ind. No.	No.of cells	Individual type aberrations					Gross type aberrations			Grand total	%
			Break type			Phy.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.						
2nd wk						Control						
	1	75	2	-	-	-	2	3	2	5	7	9.33
	2	115	2	-	-	3	5	5	4	9	14	12.17
	3	81	-	-	-	1	1	5	5	10	11	13.58
Total	3	271	4	-	-	4	8	13	11	24	32	11.80
						Treated						
	1	37	-	-	-	-	-	4	7	11	11	29.72
	2	45	4	1	1	3	9	11	7	18	27	60.00
Total	2	82	4	1	1	3	9	15	14	29	38	46.34

Table 38 (Contd.)

Fix.	Ind. No.	No.of cells	Individual type aberrations					Gross type aberrations			Grand total	%
			Break type			Phy.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.						
4th wk						Control						
	1	95	-	-	-	3	3	7	-	7	10	10.52
	2	65	-	-	-	-	-	-	13	13	13	20.00
	3	87	-	2	-	-	2	4	7	11	13	14.94
Total	3	247	-	2	-	3	5	11	20	31	36	14.57
						Treated						
	1	55	-	-	-	-	-	13	17	30	30	54.55
	2	35	1	1	-	2	4	10	8	18	22	62.85
Total	2	90	1	1	-	2	4	23	25	48	52	57.77

Table 39

Compiled data of chromosome aberration in spermatogonial metaphase cells of F_2 male of control and bacterium, P. aeruginosa treated line mice of 2nd and 4th week (Control data in brackets)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Trans./ Asso.	Phy.type Gaps.& cons.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%	Nett incr. %
2nd wk	2	82	4	1	1	3	9	15	14	29	38	46.34	34.54
	(3)	(271)	(4)	(-)	(-)	(4)	(9)	(13)	(11)	(24)	(32)	(11.80)	
4th wk	2	90	1	1	-	2	4	23	25	48	52	57.77	43.20
	(3)	(247)	(-)	(2)	(-)	(3)	(5)	(11)	(20)	(31)	(36)	(14.57)	
Total	4	172	5	2	1	5	13	38	38	77	90	52.32	39.20
	(6)	(518)	(4)	(2)	(-)	(7)	(14)	(24)	(31)	(55)	(68)	(13.12)	

in control line the frequency of aberration in diakinesis nuclei of 3 F_2 males ranged between 6.5% and 15.55%. In a total of 197 diakinesis nuclei there were 5 or 2.53% individual type and 9 or 4.56% gross type aberrations (Table 40) making a total of 14 or 7.10% in the average of two types taken together (Table 41). Thus the frequency of aberration in treated line had a net increase of 16.8% over the control (Table 41).

In two F_2 males of 4th week treated line the total aberration frequency at diakinesis stage was 19.67% and 29.72%. When the data of the 2 individuals were combined, out of 98 diakinesis nuclei there were 12 or 12.24% individual type and 11 or 11.22% gross type making a total of 23 or 23.46% aberrations (Table 40). On the otherhand in 3 F_2 males of the control line, the aberration frequency in the diakinesis nuclei ranged between 7.89% and 14.28%. In the combined data out of 164 diakinesis nuclei, there were 6 or 3.65% individual type and 11 or 6.70% gross type (Table 40) making a total of 17 or 10.36% aberrations. Therefore, the net increase in the aberration frequency in the treated line over control was 13.10% (Table 41). If the aberration data in two weeks were combined to have a larger sample, the average frequency would be 23.68% in the treated line against 8.35% in the control line indicating the net increase of 15.33% (Table 41). If the aberration frequency in diakinesis nuclei of F_1 individuals were considered, it was 44.28% in the treated line against 9.05% in the control line in the 2nd week and 32.43% against 8.62% in the 4th week control line (Table 26). In the combined data of 2nd and 4th week it was 38.35% in the treated line against 8.87% in the control line indicating the net increase of 29.48% while in F_2 generation it was 25.33%.

Therefore, the study of the aberration frequency in diakinesis nuclei showed also higher effect in the treated line than that of control when the data were compared. This was expected since the spermatogonial metaphase plates the frequency of aberration was higher in the treated line. The frequency of aberration in diakinesis nuclei in F_2 generation was little lower than that of F_1 generation which could be due to the non-random distribution of the mutagenic factor carried on chromosomes in successive generation.

Metaphase I

The chromosome aberration study at metaphase I in the same 2 males each of 2nd and 4th week F_2 treated line and 3 each of 2nd 4th week control line F_2 males was carried out from the same prepared slides.

The metaphase I plates also contained both individual and gross type chromosome aberrations (PM 167-169; Figs. 140-145). The individual types were in the form of chromatid break, acentric fragment, translocation, precocious separation of autosomal bivalent or sex chromosome etc. while the gross type included stickiness and aneuploidy etc. The frequency of chromosome aberrations in 100 metaphase I per individual revealed in 2 F_2 males of 2nd week treated line 9.0% and 27.0% and in the combined data of 2 males, out of 200 metaphases, there were 26 individual type and 10 gross type making a total of 36 or 18.0% aberrations. Correspondingly in 2nd week control line 3 F_2 males, the frequency of aberration ranged between 5.0% and 11.0% (Table 42). In a total of 300 metaphase I of 3 males, the individual type aberrations were

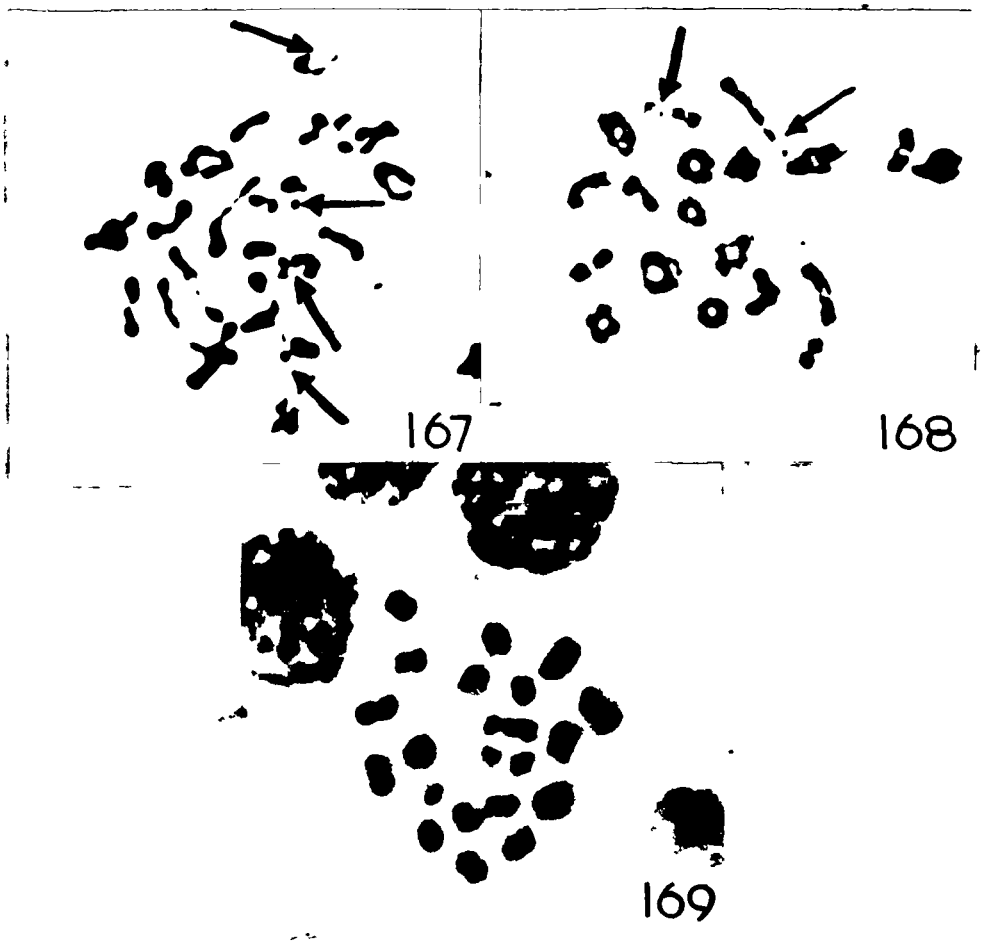
Explanation of Photomicrographs

Photomicrographs of affected metaphase I of F_2 male mice, Mus musculus of the culture of bacterium Psecudomonas treated line.

PM 167. Some chromatid breaks and fragments of unknwon origin and some bivalents stretched out.

PM 168. Some chromosome break and gap.

PM 169. Precocious separation of an bivalent.


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Explanation of figures

Camera lucida drawing of a part in metaphase I plate of F_2 males showing different types of aberrations.

Fig. 140. A chromosome break.

Fig. 141. A chromatid break and a bivalent association.

Fig. 142. A fragment of unknown origin.

Fig. 143. A bivalent with the borken chiamsa.

Figs. 144-145. Precocious separation of autosomal bivalent and sex chromosomes.

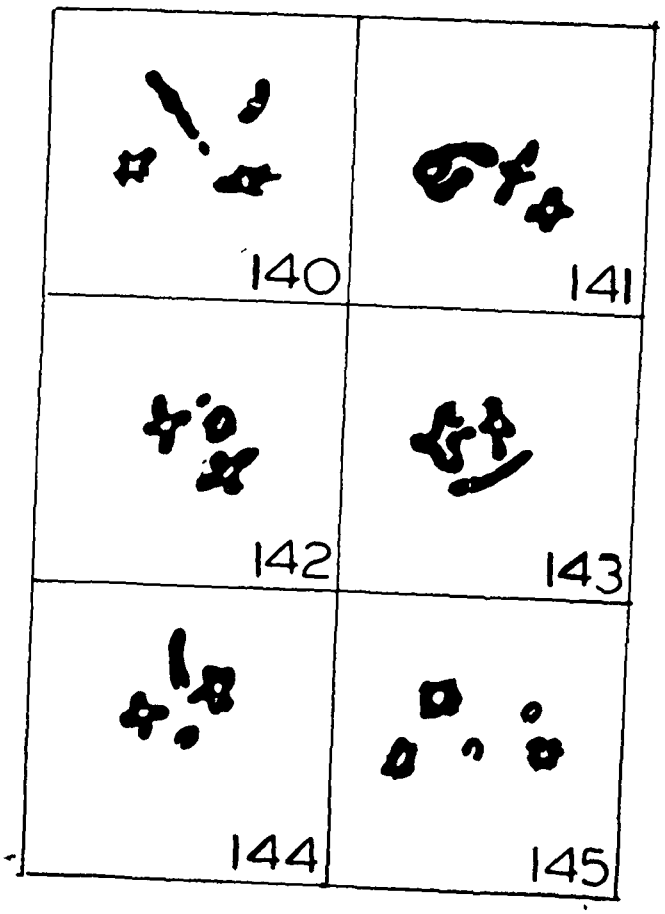

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Table 40

Frequency distribution of chromosome aberration in diakinesis cells of 3 month old F_2 male of control and the bacterium P. aeruginosa treated line mice of 2nd and 4th week

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Mut./ Asso.	Pre-sep.: Aut.	Sex	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
2nd wk						Control							
	1	65	1	-	-	-	-	1	1	1	2	3	4.61
	2	87	2	-	-	-	-	2	-	2	2	4	4.59
	3	45	-	-	1	1	-	2	2	3	5	7	15.55
Total		197	3	-	1	1	-	5	3	6	9	14	7.10
						Treated							
	1	67	-	-	3	2	1	7	-	3	3	10	14.92
	2	25	2	-	2	2	1	7	-	5	5	12	48.00
Total		92	2	-	5	4	2	14	-	8	8	22	23.91

Table 40 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Pre-sep.		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Mut./ Asso.	Aut.	Sex						
4th wk						Control							
	1	39	-	-	-	1	-	1	3	-	3	4	10.25
	2	76	-	-	-	2	-	2	-	4	4	6	7.89
	3	49	-	1	-	1	1	3	2	2	4	7	14.28
Total		164	-	1	-	4	1	6	5	6	11	17	10.36
						Treated							
	1	37	2	1	2	2	-	7	-	4	4	11	29.72
	2	61	-	-	5	-	-	5	-	7	7	12	19.67
Total		98	2	1	7	2	-	12	-	11	11	23	23.46

Table 41

Compiled data of chromosome aberration in diakinesis cells of 3 month old F_2 male of control and bacterium P. aeruginosa treated line mice of 2nd and 4th week (Control data are in brackets)

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%	Net incr %
			Break type			Phy.type		Total	Num. cha.	Others	Total			
			Chr.	Frag.	Mut/ Asso.	Aut.	Sex							
2nd wk	2	92	2	-	5	4	2	14	-	8	8	22	23.91	16.81
	(3)	(197)	(3)	(-)	(1)	(1)	(-)	(5)	(3)	(6)	(9)	(14)	(7.1)	
4th wk	2	98	3	-	7	2	-	12	-	11	11	23	23.46	13.10
	(3)	(164)	(-)	(1)	(-)	(4)	(1)	(6)	(5)	(6)	(11)	(17)	(10.36)	
Total	4	190	5	-	12	6	2	26	-	19	19	45	23.68	13.33
	(6)	(371)	(3)	(1)	(1)	(5)	(1)	(11)	(8)	(12)	(20)	(31)	(8.35)	

14 or 4.6% and that of gross types 9 or 3.0%. The frequncy in the both types of aberration taken together was 7.66%. Therefore, the frequency was much lower than that of treated line and the net increase in the treated line was 9.20% (Table 43).

In the 4th week mating line, 2 F_2 males of treated series had aberration frequency of 8.0% and 26.0%. In the combined data of 2 individuals out of 200 metaphase I, there were 11 or 5.5% individual type and 23 or 11.5% gross type and the two types taken together the frequency was 34 or 17.0%. Correspondingly the aberration frequency in 3 F_2 control line males ranged between 4.0% and 12.0% (Table 42). In a total of 300 metaphase I, therefwere 16 or 5.3% individual types and 11 or 3.5% gross types and the two types taken together were 27 or 9.0% aberrations. Therefore, the frequency was much lower in the control line and the net increase in the treated line was 8.0% (Table 43). If the data (Table 42) of 2nd week and 4th week treated and control line were combined separately to have larger sample (Table 43), it would be found that in treated line out of 400 metaphase I of 4 individuals, there were 37 or 9.25% individual type and 33 or 8.25% gross types and the two types taken together were 70 or 17.5% aberrations. In the combined data of 6 individuals of control line F_2 males out of 600 plates there were 30 or 5.0% individual types and 20 or 3.33% gross types and the two types taking together were 50 or 8.30%. Therefore, the net increase of the aberration frequency in the treated line was 9.2% (Table 43).

The analysis of the chromosome aberration data of metaphase I of 4 treated line males showed much higher frequency than that of control line indicating that the

mutagenic factor also present in F_2 generation. If the aberration data of diakinesis and metaphase I stages were compared it would be found that the frequency was little higher in diakinesis than that of metaphase I. This was perhaps due to sampling error. Leaving aside the quantitative difference in strict sense, on the whole it could be concluded that both in diakinesis and at metaphase I of the treated line the frequency of aberration was significantly higher than that of control line.

Metaphase II

A number of second division metaphase plates encountered while assaying chromosome aberrations in 100 metaphase I. plates per individual in 2 F_2 males each in 2nd and 4th week treated line and 3 each of 2nd and 4th week control line. The chromosomes of metaphase II in general appeared little despiralized with uneven surface and variable degree of stickiness. This factor sometime stood in the way of acurate determination of the aberration frequency.

The aberrations in chromosome of second division metaphase plates of control and treated line F_2 males could also be put under two main categories as individual and gross types (PM 170-175; Figs. 146-155). The individual type aberrations consisted of chromatid break, fragment of unknown origin, centric fusion, terminal association or translocation, gaps, constriction etc. while the gross types were in the form of stickiness, polyploidy etc.

Explanation of Photomicrographs

Photomicrographs of some representative chromosome aberrations in metaphase II of F_2 male individuals from upting of treated line F_1 male and females.

PM 170. Chromatid break and centric fusion.

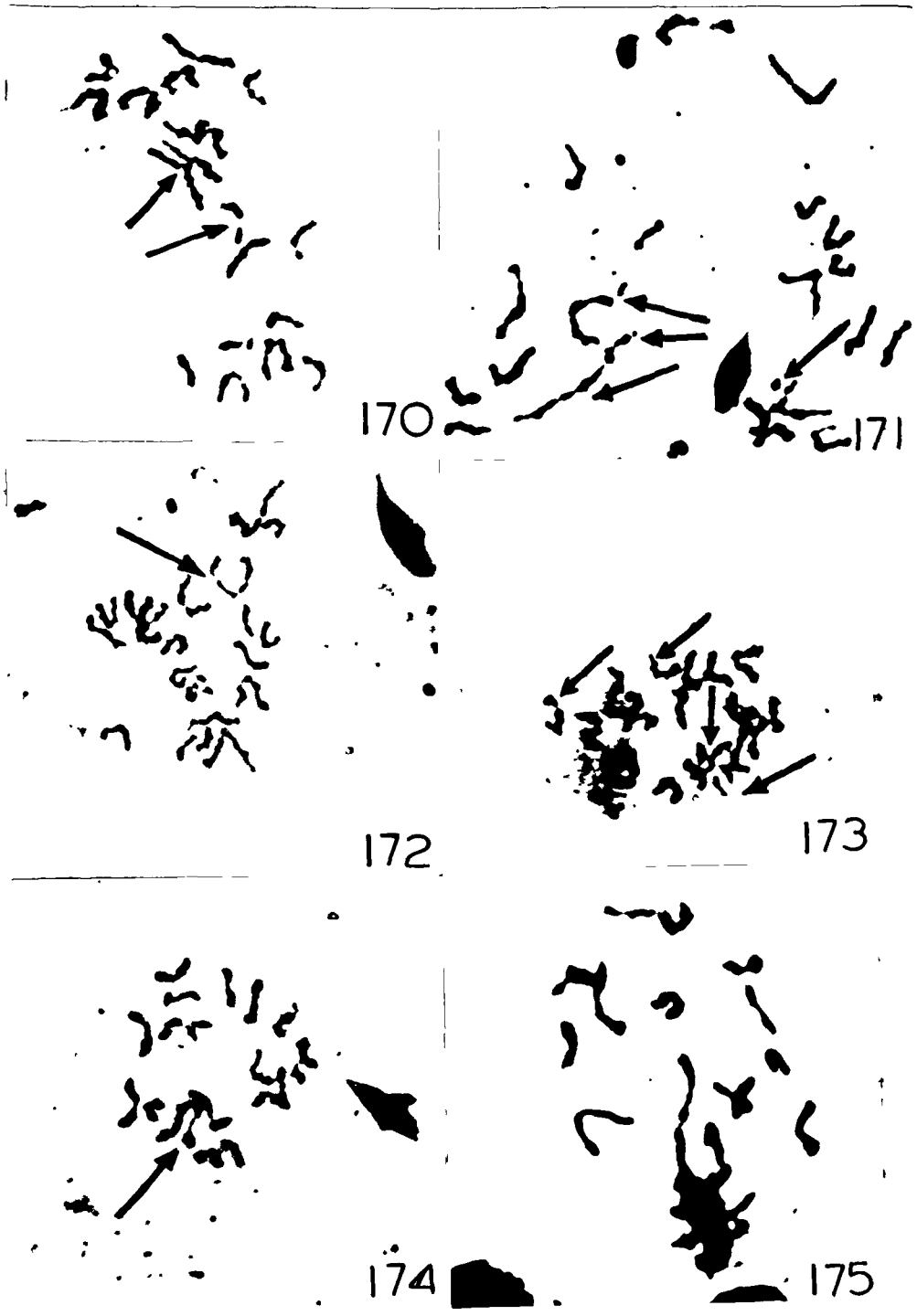
PM 171. Some chromatid break acentric fragment, constrictions and terminal fusion.

PM 172. Sub-chromatid break and terminal association with uneven outlines of chromosomes.

PM 173. Chromatid break with the displaced fragments and constriction.

PM 174. An ellastic constriction.

PM 175. Partial stickiness.


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Explanation of figures

Camera lucida drawing of a part of metaphase II plate showing different types of aberrations in treated line F_2 male individuals

Fig. 146. A chromatid break and a centric fusion.

Figs. 147-149. Each with a chromatid break.

Figs. 150-152. Each with the fragment of unknown origin.

Fig. 153. Beaded appearance in chromosome.

Fig. 154. A chromatid constriction.

Fig. 155. The centromeric dissociation in a chromosome.

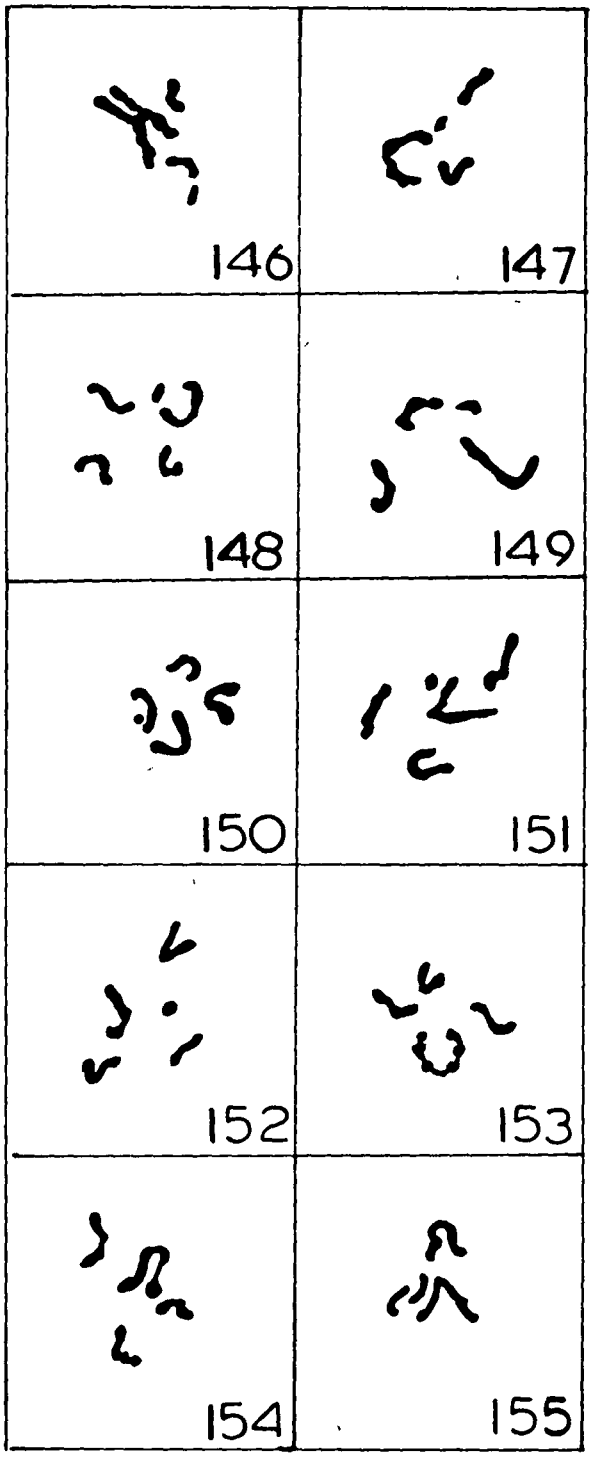

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Table 42

Frequency distribution of chromosome aberration in metaphase I cells of 3 month old F_2 male of control and bacterium, P. aeruginosa treated line mice of 2nd and 4th week.

Fix.	Ind. No.	Cells count.	Individual type aberrations						Gross type aberrations			Grand total	%
			Break type			Pre-sep.		Total	Num. cha.	Others	Total		
			Chr.	Frag.	Mut./ Asso.	Aut.	Sex						
2nd wk						Control							
	1	100	-	-	1	2	2	5	-	-	-	5	5.00
	2	100	-	-	-	2	1	3	3	1	4	7	7.00
	3	100	-	-	2	2	2	6	-	5	5	11	11.00
Total		300	-	-	3	6	5	14	3	6	9	23	7.66
						Treated							
	1	100	1	-	-	2	5	7	-	2	2	9	9.00
	2	100	5	1	3	7	3	19	-	8	8	27	27.00
Total		200	6	1	3	9	8	26	-	10	10	36	18.00

Table 42 (Contd.)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Mut./ Asso.	Pre-sep.: Aut.	Sex	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%
4th wk								Control					
	1	100	-	-	2	1	-	3	1	-	1	4	4.00
	2	100	-	-	4	-	3	7	2	3	5	12	12.00
	3	100	-	-	-	2	4	6	3	2	5	11	11.00
Total		300	-	-	6	3	7	16	6	5	11	27	9.00
								Treated					
	1	100	1	-	-	1	1	3	3	2	5	8	8.00
	2	100	2	-	4	-	2	8	7	11	18	26	26.00
Total		200	3	-	4	1	3	11	10	13	23	34	17.00

Table 43

Compiled data of chromosome aberration in metaphase I cells of 3 month old F_2 male of control and bacterium P. aeruginosa treated line mice of 2nd and 4th week (Control data are in bracket)

Fix.	Ind. No.	Cells count.	Individual type aberrations: Break type: Chr.	Frag.	Mut/ Asso.	Pre-sep.: Aut.	Sex.	Total	Gross type aberrations: Num. cha.	Others	Total	Grand total	%	Net inc%
2nd wk	2	200	6	1	3	9	8	26	-	10	10	36	18.00	10.34
	(3)	(300)	(-)	(-)	(3)	(6)	(5)	(14)	(3)	(6)	(9)	(23)	(7.66)	
4th wk	2	200	3	-	4	1	3	11	10	13	23	34	17.00	8.00
	(3)	(300)	(-)	(-)	(6)	(3)	(7)	(16)	(6)	(6)	(11)	(27)	(9.00)	
Total	4	400	9	1	7	10	11	37	10	23	33	70	17.50	9.20
	(6)	(600)	(-)	(-)	(9)	(9)	(12)	(30)	(9)	(11)	(20)	(50)	(8.30)	

In the 2nd week treated line out of 88 plates in one male and 50 plates in another male, the total frequency of aberration was 30.68% and 46.0% (Table 44) respectively. In a total of 138 metaphase II plates of two individuals there were 21 or 15.21% individual type, 29 or 20.86% gross type aberrations and the two types taken together, it was 50 or 36.23%. Correspondingly in 3 F_2 in 2nd week control line males, the chromosome aberration frequency in the second division metaphase plates ranged between 12.65% and 20.93% (Table 44). Out of the total 227 metaphase II plates in 3 males, there were 16 or 7.04% individual type and 22 or 9.6% gross type and the two types combined it was 38 or 16.70%. Therefore, the frequency of aberration in the treated line was higher than that of control and the net increase was 19.53% (Table 45).

In 2 F_2 males of 4th week treated line, the frequency of chromosome aberration in metaphase II was out of 37 plates, 24.32% in one individual and out of 32 plates 59.37% in another individual. In the combined data of 2 males out of the total 69 plates there were 5 or 7.24% individual type, 23 or 33.33% gross type and the two types taken together it was 28 or 40.57% (Table 44). Correspondingly in 3 males of 4th week F_2 control line the aberration frequency determined from 59, 57 and 49 metaphase II plates were 18.64%, 17.54% and 24.48% respectively (Table 44). In the combined data out of the total 165 plates of metaphase II there were only 7 or 4.3% individual type, 26 or 15.75% gross type and the two types together, it was 33 or 20.0%. Therefore, the frequency of aberration in the control series was much lower and the net increase in the treated line over control was 20.57% (Table 45).

If the aberration data of the 2nd and 4th week were combined to have larger sample, in 207 metaphase II of 4 treated line males there were 26 or 12.56% individual type, 52 or 25.12% gross type and both types taken togher it was 78 or 37.86% aberrations (Table 45). Corresponding in a total of 392 metaphase II of 6 control line males, there were 23 or 5.86% individual types, 53 or 13.52% gross type and 76 or 19.38% taking both types together. The net increase in the aberration frequency was 18.48% (Table 45) which was significantly high.

Comments :

The chromosome aberration data in spermatogonial metaphase, diakinesis, metaphase I and metaphase II of the control and treated line F_2 males revealed in all cases that the frequency was much higher than that of control. It was true that there were some observational limitation as well as the data were limited from which the frequency was determined. The inclusion of some aberration like centromeric dissociation, precocious separation of bivalents; gaps and constriction under individual types or stickiness under gross types having not much genetic significance had no doubt inflated the frequency but they were considered both in control and treated lines. Even if these types were eleminated, the total frequency would be reduced but the frequency of other types would be found higher in the treated lines than that of controls. Thus disregarding the exact frequency, it could reasonably be concluded that the frequency of aberration was higher in treated line than that of control lines assessed at different stages of meiosis. If this very point was to be explained we

Table 44

Frequency distribution of chromosome aberration in metaphase II cells of F_2 male of control and bacterium P. aeruginosa treated line mice of 2nd and 4th week.

Fix.	Ind. No.	No.of Cells count.	Individual type aberrations					Gross type aberrations			Grand Total	%
			Break type			Phy.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.						
2nd wk						Control						
	1	43	2	2	-	2	6	1	2	3	9	20.93
	2	79	-	-	-	-	-	-	10	10	10	12.65
	3	105	3	5	-	2	10	2	7	9	19	18.09
Total	3	227	5	7	-	4	16	3	19	22	38	16.70
						Treated						
	1	88	1	2	3	5	13	7	7	14	27	30.68
	2	50	5	2	-	1	8	2	13	15	23	46.00
Total	2	138	8	4	3	6	21	9	20	29	50	36.23

Table 44 (Contd.)

Fix.	Ind. No.	No.of cells count.	Individual type aberrations					Gross type aberrations			Grand total	%
			Break type			Phy.type	Total	Num. cha.	Others	Total		
			Chr.	Frag.	Trans/ Asso.	Gaps & cons.						
4th wk						Control						
	1	59	1	1	-	3	5	-	6	6	11	18.64
	2	57	-	-	-	-	-	-	10	10	10	17.54
	3	49	-	-	-	2	2	7	3	10	12	24.48
Total	3	165	1	1	-	5	7	7	19	26	33	20.00
						Treated						
	1	37	1	-	-	1	1	3	5	8	9	24.32
	2	32	3	-	-	1	4	3	12	15	19	59.37
Total	2	69	3	-	-	2	5	6	17	23	28	40.57

Table 45

Compiled data of chromosome aberration in metaphase II cells of F_2 male of control and the bacterium P. aeruginosa treated line mice of 2nd and 4th week (Control data are in brackets)

Fix.	Indi. No.	Cells count	Individual type aberrations					Gross type aberrations			Grand total	%	Net incr %
			Break type			Ph.type	Total	Number Cha.	others	Total			
			Chr.	Frag.	Trans/ Asso.	Gaps & Cons.							
2nd wk	2	138	8	4	3	6	21	9	20	29	50	36.23	19.53
	(3)	(227)	(5)	(7)	(-)	(4)	(16)	(5)	(22)	(27)	(43)	(16.70)	
4th wk	2	69	3	-	-	2	5	6	17	23	28	40.57	20.57
	(3)	(165)	(1)	(1)	(-)	(5)	(7)	(7)	(19)	(26)	(33)	(20.00)	
Total	4	207	11	4	3	8	26	15	37	52	78	37.86	18.48
	(6)	(392)	(6)	(8)	(-)	(9)	(23)	(12)	(41)	(53)	(76)	(19.38)	

would have no other alternative but to think of some mutagenic factor induced by the treatment of log culture of P. aeruginosa in males of parental generation was handed over to the F_2 generation through F_1 generation. In other word the mutagenic factor present in F_1 generation was transmitted in the F_2 generation possibly through chromosomes. The frequency was variable as it was expected for random segregation.

An extensive data could not be scored in F_2 generation because of the higher incidence of reproductive failure occurred in F_1 generation. Anyhow, in spite of various limitation the present study showed more or less a clear indication of the mutagenic potentiality of the log culture of P. aeruginosa treated mice and the inheritance of the factor in F_1 and F_2 generations.

D. Sperm count in 3 month old F_2 males :

The epididymes of 2 each of the 2nd and 4th weeks treated line males and 3 each of 2nd and 4th week control line F_2 males kept separately in 1% sodium citrate solution, while removing the testes of the specimens, were processed for preparing sperm suspension sample following the method described earlier. The mean number of sperm was determined from 25 counts made in the Neubauer haemocytometer chamber.

Observation :

In the 2nd week control line (Table 46) there was not much difference in the mean value between the right

and left epididymes of each individual. In the combined data of the left and right epididymes, the mean values were 20.62 ± 0.34 in 1st individual, 23.08 ± 0.33 in 2nd individual, 21.04 ± 0.46 in the 3rd individual showing not very much difference among different individuals. The average of all was 21.58. In 2 males of the 2nd week treated line the mean value of sperm count in the right and left epididymes also did not differ appreciably in each individual. The mean value of the combined data of sperm count of two sides was 19.72 ± 0.42 in one individual and 21.06 ± 0.37 in the 2nd individual showing no appreciable difference. The average of the two was 20.39 (Table 46). A comparison of the sperm count data of the control versus treated line of the 2nd week did not differ appreciably (21.58 against 20.39) indicating thereby that there was practically no difference between control and treated lines. On the other hand similar study in F_1 generation showed considerable difference as the mean value was 21.17 in the control line against 10.29 in the treated line (Table 31).

The sperm count carried out in epididymal samples of 3 males of 4th week F_2 control line revealed also not much difference between the right and left epididymes in each of the 3 individuals except the individual No. 2 (Table 46). Anyhow the average sperm count in 3 individuals was 24.10 ± 0.36, 22.62 ± 0.56, 22.48 ± 0.39 respectively. In the combined data the average of the three was 23.06. On the other hand in the F_2 treated line the sperm count between the right and left epididymis varied widely. In one, of 4th week treated line male it was 6.52 ± 0.37 and 20.04 ± 0.52 respectively (Table 46). The average of two sides

Table 46

Mean average sperm count in the epididymes of F_2 male of control and bacterium P. aeruginosa treated line mice of 2nd and 4th week

Fix.	No.of indiv.	Right epidi. (Mean value ±S.E)	Left epidi. (Mean value ± S.E)	Right & left comb. (Mean value ± S.E.)
		Control		
2nd wk	1	20.20 ± 0.36	21.0 ± 0.56	20.62 ± 0.34
	2	22.52 ± 0.41	23.65 ± 0.48	23.08 ± 0.33
	3	19.40 ± 0.48	22.68 ± 0.64	21.04 ± 0.46
	Average	20.70	22.42	21.58
		Treated		
	1	19.48 ± 0.64	19.96 ± 0.75	19.72 ± 0.42
	2	21.48 ± 0.60	20.32 ± 0.51	21.06 ± 0.37
	Average	20.48	20.14	20.39
		Control		
4th wk	1	24.52 ± 0.46	23.68 ± 0.60	24.10 ± 0.38
	2	24.88 ± 0.39	20.28 ± 0.82	22.62 ± 0.56
	3	23.44 ± 0.45	21.52 ± 0.60	22.48 ± 0.39
	Average	24.28	21.82	23.06
		Treated		
	1	6.52 ± 0.37	20.04 ± 0.52	13.38 ± 1.03
	2	22.25 ± 0.37	20.12 ± 0.62	21.18 ± 0.39
	Average	14.38	20.08	17.28

taken together was 13.38 ± 1.03. On the other hand the sperm count in the right and left epididymes of 2nd individual did not vary appreciably. It was 22.24 ± 0.37 in the right, 20.12 ± 0.62 in the left and 21.18 ± 0.39 in the average of two slides combined. If the difference in the mean value in two sides of the epididymis of 1st individual was ignored and the data of two individuals were combined, the average would be 17.28 against 23.06 of the control line. In other words the average sperm count in the 4th week treated line was somewhat lower than that of control indicating perhaps some effect was present in the treated line which caused the higher frequency of sperm depletion. Anyhow, the difference was not so glaring as found in F_1 generation (Table 31). In F_1 the average sperm count was 22.78 in the control line against 14.85 in the treated line indicating a difference of 7.93 while it was 5.78 in F_2 generation.

General comments

The sperm count data analysed above showed that there was not much difference in the average of 2nd week control and treated line males while the difference was significant in 4th week having higher average in the control line then that of treated line. A similar study carried out in F_1 male individuals from 1st week to 4th week showed that the control line males had always much higher sperm count than that of treated line and the difference was much more glaring in 1st week, 2nd week and 3rd week data than that of 4th week (Table 31). It was generalized before that the sperm depletion effect was being continued in F_1 generation while in F_2 generation it was not so obvious. This would possibly indicate that the effect was reduced in successive generation possibly due to random segregation of the mutagenic factor.

E. Sperm head morphology in 3 month old F_2 males :

Some part of the sperm suspension sample of the epididymes of both 2nd and 4th week control and treated line F_2 individuals was used for the study of the sperm head morphology. The giemsa stained preparation of the sperm smear was made following the procedure described before.

Observation :

The study of the sperm head morphology of both control and treated line specimens showed sperms with both normal and abnormal head morphology (PM 176a-b). The types of sperm with abnormal head morphology were more or less the same as found in F_1 generation with regard to size, garniture, shape etc. Though there was not much difference in the morphology of sperms between control and treated lines, but the frequency differed.

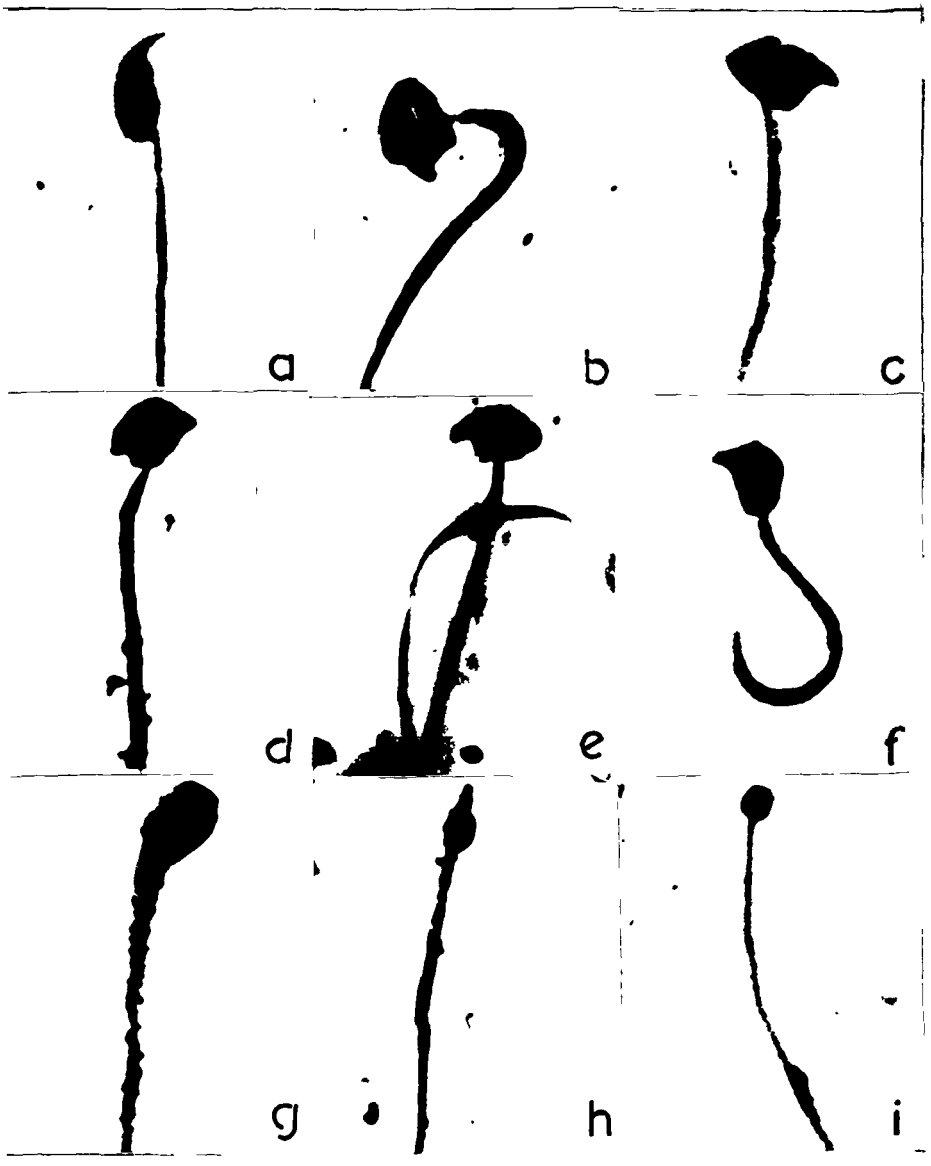
In the 2nd week control line sperm smear of 3 males showed the frequencies of 0.83%, 1.33% and 1.16% (Table 47) indicating not much difference from individual to individual. Out of 1800 sperms, the total number of sperms with abnormal head morphology was 20 or 1.11%. On the other hand, in 2 males of the 2nd week treated line , the frequency of sperms with abnormal head morphology was 5.83% and 13.83% indicating 8.1% difference between the two. In a total of 1200 sperms, the number sperms with abnormal head morphology was 118 and the average frequency was 9.83%. Unlike the result of the sperm count there was significant difference

Explanation of Photomicrographs

Photomicrograph of sperm with abnormal head morphology in F_2 male offspring in the treated line.

PM 176a. Normal sperm head.

PM 176 b-i. Different types of sperms with abnormal morphology.


a
b
c
d
e
f
g
h
i

in the frequency of sperm with abnormal head morphology between 2nd week control and treated line males and the net increase in the treated line over control was 8.72%. Further the frequency did not differ much between individual to individual in the control line while it was very glaring between individual to individual of 2nd week treated line (Table 47).

In the 4th week control line 3 F_2 males the frequency of sperms with abnormal head morphology also did not vary appreciably from individual to individual and the frequencies were 1.83%, 1.50% and 1.16% (Table 47). Out of 1800 sperms, 27 were with abnormal head morphology and the average frequency was 1.50% which was also not so striking different from 2nd week data (1.11%). However, in 2 males of the 4th week F_2 treated line, the frequency of sperms with abnormal head morphology differed to some extent as it was 7.5% in 1 individual and 3.5% in another individual. Out of 1200 sperms of two individuals, 66 were with abnormal head morphology and the average frequency was 5.50% which was higher by 4.0% than that of control (Table 47).

Comment :

If the sperm head abnormality data of the corresponding week in F_1 generation were compared with the present data, it would be found that in F_1 generation the average frequency in the 5 males of two mothers was 1.80% (Table 31) with not much variation among different individuals and in the F_2 generation it was 1.11% (Table 47). Therefore, there was not much difference in frequency of the control line of F_1 and F_2 generation. On the other hand the average frequency of

sperms with abnormal head morphology in 2 males of 2nd week treated line was 13.58% in F_1 (Table 32) while it was 9.83% (Table 47) in the present study of F_2 generation. The average frequency of sperm with abnormal head morphology differed by about 4.0% between F_1 and F_2 generation of treated line. However, in comparison to the respective control, the frequency was significantly high indicating that the mutagenic factor for the treatment of male parent was continued in F_1 and F_2 generation in variable frequency. In other words the mutagenic factor was present in both the generations. A similar comparison of the 4th week data would also reveal that the average frequency of sperms with abnormal head morphology of 6 males was 1.08% between 6 individuals in F_1 and in F_2 generation. The control data of sperm head abnormalities in both F_1 and F_2 generations of 2nd and 4th week F_2 line showed more or less the same trend having in common low frequency and not much difference between individual to individual. On the other hand in the 4th week F_1 treated line males, the average frequency of sperm with abnormal head morphology was 17.62% (Table 32) and in F_2 generation the average frequency was 5.50% in 2 males (Table 47). However, when compared to the respective control, it was found to significantly high in treated line both in F_1 and F_2 generation. Therefore, on the whole the data indicated that the mutagenic factor was transmitted from one generation to the other.

Table 47

Frequency of sperm with abnormal head morphology in F_2 male of control and bacterium, P. aeruginosa treated line mice of 2nd and 4th week

Fix.	No.of Indi.	Total No. of sp.head counted	Total No. of abn. sp. head	%
2nd wk		Control Line		
	1	600	5	0.83
	2	600	8	1.33
	3	600	7	1.16
	Total	1800	20	1.11
		Treated Line		
	1	600	35	5.83
	2	600	83	13.83
	Total	1200	118	9.83
4th wk		Control Line		
	1	600	11	1.83
	2	600	9	1.50
	3	600	7	1.16
	Total	1800	27	1.50
		Treated Line		
	1	600	45	7.50
	2	600	21	3.50
	Total	1200	66	5.50

F. Histological studies :

i) Effect on testis :

Since the reproductive potentiality in F_1 and F_2 generation was greatly affected specially in the treated line, only few testes could histologically be studied. The microtome sections of the testis of F_2 males of both treated and control line were stained with haematoxylin and eosine stain.

Observation :

The histology of testis of the control line male did not show any difference with that of normal individual. On the other hand the histological sections of the testis of treated line male though appeared more or less normal but there was some amount of difference. It was found that the epithelium of seminiferous tubules was normal like that of control but most of them have lost their compactness and having a number of vacuoles (PM 178). Vacuolation was also observed in spermatogonial to spermatocyte cells lining (PM 178). In some cases the lumen of the tubules was large containing no spermatozoa. In some cases the lumen was blocked (PM 177). Further, the appearance of the histological structure of the testis of F_1 and F_2 treated line males did differ appreciably.

ii) Effect on ovary :

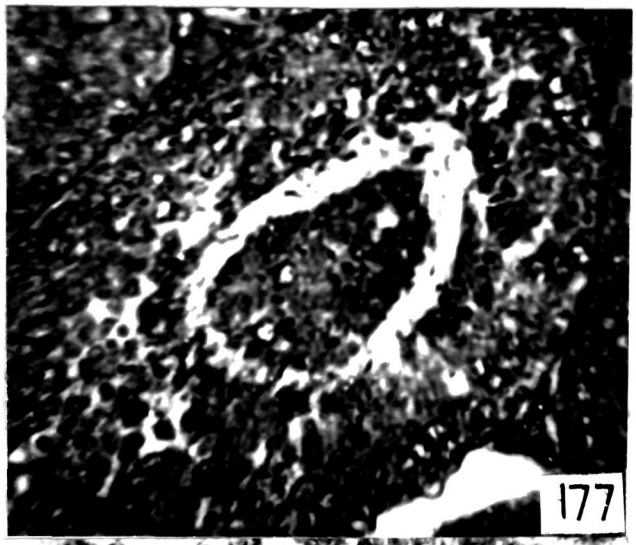
The histological changes in the section of the ovary of 2nd and 4th week control and treated line F_2 females

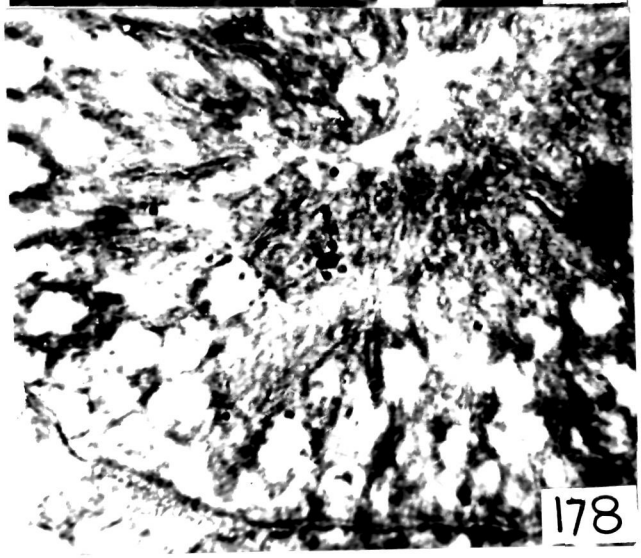
Explanation of Photomicrographs

Photomicrograph of the section of testis of treated line F_2 individuals.

PM 177. Lumen of the follicle is blocked and without spermatozoa.

PM 178. No dividing cells and many vacuoles within the follicular cell lining.


177


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with regard to the structure like perivitelline space, number of nucleoli, abnormal nucleus etc., if any were studied (Table 48). In the control line the sections showed that there was some cases of large perivitelline space and abnormality in the nucleus. On the other hand the sections of the ovary of treated line of 2nd 4th week F_2 females showed variable abnormality (PM 179-184). In some cases the frequency was strikingly different while in others it was not distinguishable from that of control. Thus in 2 females of 2nd week control line the average abnormalities was 6.28% and 8.51% (Table 48) while in the treated line females of 2nd week it was 12.72% and 54.85% (Table 48) indicating some amount of difference in the ovary of the treated females.

Similarly in the 4th week, the average frequency of abnormalities in the section of the ovary of 3 females in F_2 control line was 8.09%, 10.12% and 9.71% (Table 48) while in the treated line it was 11.0% and 14.78%. Therefore, the difference in the treated line was higher than that of control line.

Comments :

The study of the histological section of the ovary did not show any glaring difference though the treated line had some amount of effect.

Effect on uterus :

Histological section of uterus of the F_2 females of control and treated line did not show any glaring difference.

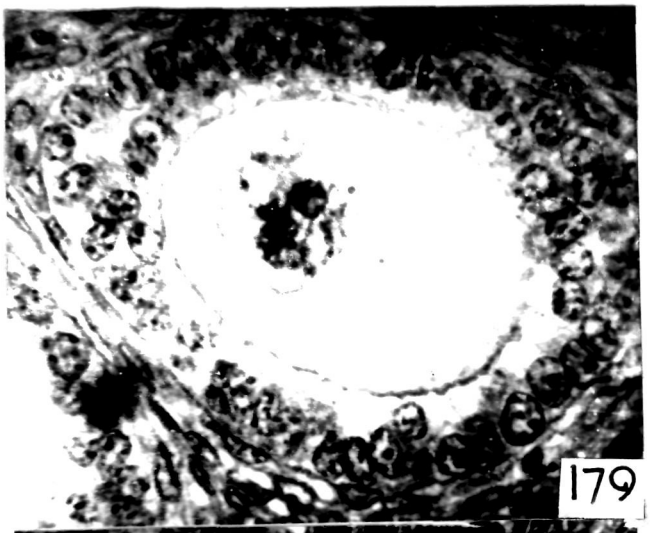
Explanation of Photomicrographs

Photomicrograph of T.S. of ovary of F_2 individuals.

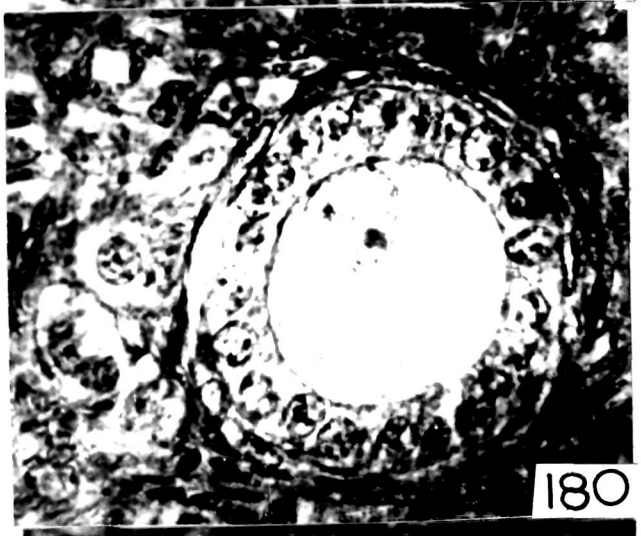
PM 179. Two macro-nucleoli within the nucleus of a secondary follicles of which one in degenerative condition.

PM 180. Two micro-nucleoli within the nucleus of a secondary follicle.

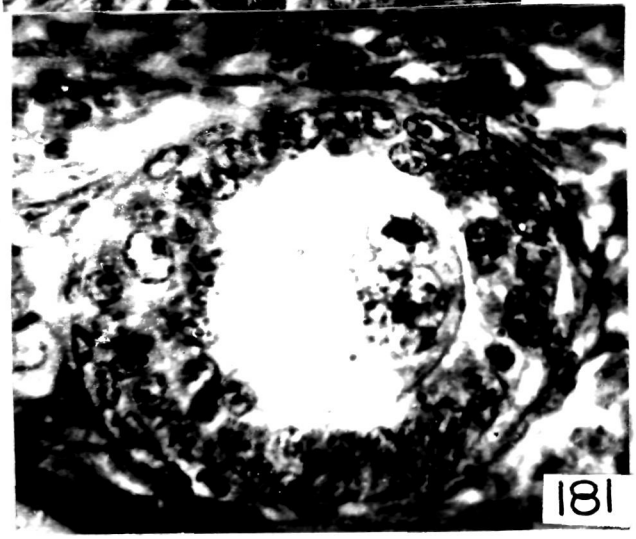
PM 181. Two nuclei with one nucleolus each within a secondary follicles in which one nucleolus is highly fragmented.



179



180



181

Explanation of Photomicrographs

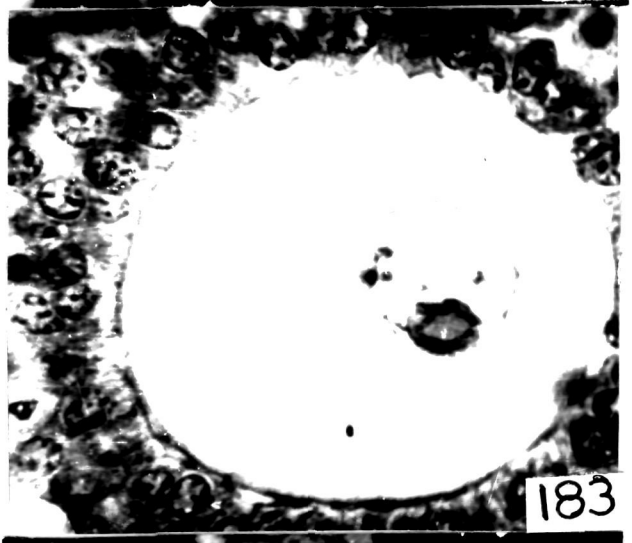
Photomicrograph of the T.S. ovary of F_2 individuals.

PM 182. Three macro-nucleoli with nucleus of a Graafian follicle.

PM 183. One macro- and some micro-nucleoli with a nucleus of a maturing/Graafian follicle.

PM 184. Two prominent macro nucleoli within a nucleus of a Graafian follicle.


182


183


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Table 48

Frequency of abnormal secondary, maturing/grafian follicles in F_2 female offspring for mating of F_1 male and females of control and Pseudomans treated line

Fix.	Ind. No.	Stage	Total	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Abn. nucle.	Atri. falli.	Total	%
					Control						
2nd wk	2	G.T./M	80	3	1	-	-	-	2	6	7.50
		S.F.	95	2	-	-	-	3**	-	5	5.26
	Total		175	5	1	-	-	3	2	11	6.28
					Treated						
	1	G.F./M	98	4	3	-	-	-	1	8	8.16
		S.F.	110	9	1	-	-	4**	-	14	12.72
	Total		208	13	4	-	-	4	1	22	10.57

Table 48 (Contd.)

Fix.	Ind. No.	Stage	Total	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Abn. nucle.	Atri. falli.	Total	%
						Control					
	2	G.f./M	100	4	1	-	-	-	4	9	8.18
		S.F.	125	6	-	-	-	5**	-	11	8.80
		Total	235	10	1	-	-	5	4	20	8.51
2nd wk						Treated					
	2	G.f./M	70	30	11	5	3	2	-	51	72.85
		S.F.	105	18	15	1	-	11**	-	45	42.85
		Total	175	48	26	6	3	13	-	96	54.85
4th wk						Control					
	1	G.f./M	110	6	1	-	-	-	-	7	6.30
		S.F.	100	10	-	-	-	-	-	10	10.00
		Total	210	16	1	-	-	-	-	17	8.09

Table 48 (Contd.)

Fix.	Ind.No.	Stage	Total	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Abn. nucle.	Atri. falli.	Total	%
4th wk						Treated					
	1	G.f./M	115	7	2	-	-	2	3	14	12.17
		S.F.	85	5	-	-	-	3**	-	8	9.41
		Total	200	12	2	-	-	5	3	22	11.00
						Control					
	2	G.f./M	112	8	-	-	-	-	5	13	11.60
		S.F.	125	6	1	-	-	5**	-	11	8.80
		Total	237	14	1	-	-	5	5	24	10.12
						Treated					
	2	G.f./M	105	8	2	1	-	-	2	13	12.38
		S.F.	125	10	1	2	-	8**	-	21	16.80
		Total	230	18	3	3	-	8	2	24	14.78

Table 48 (Contd.)

Fix.	Ind. No.	Stage	Total	Large P.V. space	Nucleus 2	Nucleus 3	Nucleus 4	Abn. nucle.	Atri. falli.	Total	%
4th wk					Control						
	3	G.f./M	117	4	2	-	-	2	2	10	8.54
		S.F.	130	11	-	-	-	3**	-	14	10.76
		Total	247	15	2	-	-	5	2	24	9.71

** Cytoplasm vacuolated

CHAPTER IV

A. Teratogenic effect in P. aeruginosa log culture injected females after showing vaginal plug against nutrient broth injected controls

The teratogenic effect caused by the rubella and cytomegalo viruses have been mentioned before. Brown (1966) made a review of the viral actiology of congenital anomalies associated with rubella, influenza, cytomegalo infections hepatitis, coxsackie, poliomyelitis, ECHO, mumps, measles, small pox and encephalities viruses. Among them the teratogenic effect of rubella, cytomegalo virus, coxsackie, poliomyelitis etc. was claimed while it was not so certain about influenza, infections hepatitis, mumps etc. Desmond et al. (1970) reviewed the work of early growth and development of infants with rubella infected mother. They showed various types of effect. Similarly Hanshaw (1970) reviewed the developmental abnormalities associated with cytomegalo virus infection. Beside viruses, the teratogenic effect of azodyes (Beck and Lloyd, 1966), ionizing radiations (Jacobson, 1970) etc. have also been studied. So far as the present author was aware the teratogenic effect in bacteria infected patients have not been studied though some cases of females infected with bacteria of gonorrhoea, syphilis gave birth blind child

Anyhow the infection of Pseudomonas might have teratogenic effect was indicated by the abnormalities in the progeny of treated mice (Manna and Dey, 1979). In the present study, an attempt has been made to see

the preliminary effect of Pseudomonas culture injected to pregnant mice of different days after the appearance of vaginal plug.

Procedure and observation :

Sexually mature normal males and females were kept together in the breeding cage with food and water. The females were examined every day to see if the vaginal plug had appeared. Such 10 females showing sign of pregnancy in each batch were taken and the log culture of P. aeruginosa was injected intraperitoneally at the rate of 1 ml per 100 gm body weight. Another set of 10 such females after the appearance of vaginal plug were injected with freshly prepared nutrient broth. These two sets of females were sacrificed on 15th day after observing the vaginal plug. It was found that out of 10 control females, 6 were really pregnant which had 35 embryos, all the living showing no apparent embryonic abnormalities. On the other hand all 10 females treated with Pseudomonas culture, was found to have no embryonic implants within their uterus (Table 49) indicating thereby that the fertilized ovum died very likely before they reached to the uterus for implantation. Usually it has been estimated that about 4-5 days are required to reach the fertilized ovum to the uterus for implantation (Rugh, 1962; Bateman and Epstein, 1971). Sometimes it is possible that a normal female might show the vaginal plug as a false pregnancy as found in the control line. But it could be 100% cases as found in the treated line. Therefore, the absence of any female of the treated line having no implanted embryo should indicate that they might have died after or

before implantation. The absence of the scar mark in the uterus of these females ruled out the possibility of post-implantation loss.

Similar study was carried out with different sets of 10 females showing vaginal plug. In the second experiment, on the 2nd day after the appearance of vaginal plug, each of the female of the control series was injected with the nutrient broth. In the treated series each female of another set was intraperitoneally injected with the log culture of P. aeruginosa at the rate of 1 ml per 100 gm body weight. All these females were sacrificed on 15 day after observing the vaginal plug to expose the uterus with a view to counting the number of living and dead embryos as well as any apparent morphological changes in them. In the control series out of 10 females, 7 were really pregnant which had 60 implants of which 3 were dead and 57 were alive (Table 49). After removing the fetal covering the embryos were critically examined under a dissecting binocular. None of the embryo showed any abnormal morphological feature. On the other hand the 10 females of the treated series showed that none of them was having any implant in their uterus (Table 49). Just as the same experienced in one day post-treatment females stated before. Therefore, the same conclusion holds true for the treatment of the 2nd day as well. The fertilization might have taken place at least in some of them but the fertilized eggs could not implant in the uterus indicating the pre-implantation loss.

A set of 10 control females showing vaginal plug was injected on 3rd day with the nutrient broth served as control while another set of 10 females showing vaginal plug were injected on 3rd day with log culture of Pseudomonas served as treated series. On 15 day after the appearance of the vaginal plug they were sacrificed and the uteri were exposed. In the control series, out of 10 females, 6 were really pregnant which had 36 living implants and 1 dead implant. After clearing the fetal covering of the living embryos, none of the 36 showed any conspicuous morphological change. On the other hand in the treated series, out of 10 females sacrificed on 15th day, 2 showed sign of pregnancy which contained only 2 living implants and 2 dead implants (Table 49) and in the other individuals some unknown fluid was found to accumulate in the swelled uterus (PM 185). These two living embryos when examined under dissecting binocular, one appeared to have disproportionately large brain (PM 188) while both the embryos had one fore limb without the formation of digit (PM 187-188). Therefore, the treatment of the log-culture on 3rd day after the formation of vaginal plug showed very interesting result. Though most of the females had fertilized egg destroyed by the effect of the Pseudomonas culture but very few of them escaped and implanted in the uterus. The present study showed that out of 4 embryos which could reach the uterus, 2 died leaving the scar mark and 2 showed the teratogenic abnormality like abnormal brain size (PM 188) and fore limbs without digits (PM 187-188).

On 4th day after showing the vaginal plug, 6

Explanation of Photomicrographs

Photomicrographs showed different types of affect after treatment of *Pseudomonas* culture on different days of pregnancy.

PM 185. Swelled uterus.

PM 186. Uterine horn showed all dead embryos.



185



186

Explanation of Photomicrographs

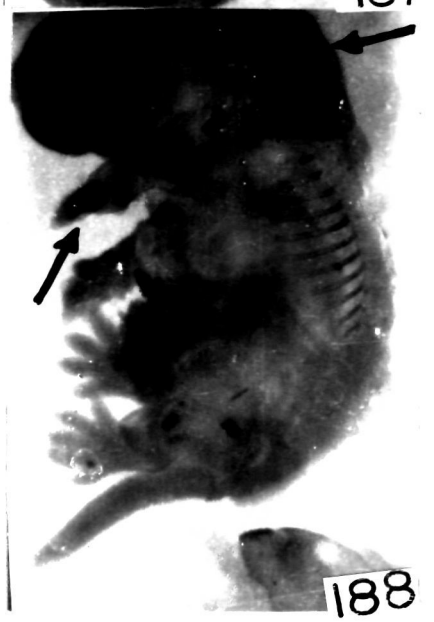
Photomicrographs of affected embryos after treatment of *Pseudomonas* culture on different day of pregnancy i.e. after appearance of vaginal plug.

PM 187. One deformed fore limb.

PM 188. One deformed fore limb and a abnormal brain size.



187



188

females were injected with freshly prepared broth and 8 females were injected with log culture of Pseudomonas.

Table 49

Teratogenic effect in P. aeruginosa log culture injected females showing vaginal plug against nutrient broth injected controls. Control data in bracket.

Days of treat. after plug	No.of plug fem.	No.of preg. fem.	No.of impl.	No.of living impl.	No.of embr. showing teratogenic effect	No.of dead embryos	Remark
1st day	10(10)	*(6)	*(35)	*(35)	*(-)	*(-)	
2nd "	10(10)	*(7)	*(60)	*(57)	*(-)	*(3)	
3rd "	10(10)	2(6)	4(37)	2(36)	2(-)	2(1)	Growth retarded
4th "	8(6)	3(5)	11(35)	1(35)	1(-)	10(-)	Growth
5th "	9(5)	7(4)	45(29)	-(29)	-(-)	45(-)	retarded

*Complete absence of implantation in Pseudomonas culture treated females after mating and appearance of vaginal plug.

Each female was sacrificed on 15 day after observing the vaginal plug. The uteri of 6 control females exposure revealed 5 of them were pregnant and there were 35 implants but none was dead. After clearing the fetal membrane all the 35 embryos did not show any apparent morphological changes. In the treated series, 8 females were sacrificed on 15 day. 3 of them were pregnant which had 11 implants but only one was alive and the remaining 10 were dead (Table 49). The solitary living embryos after clearing its fetal membrane was seen to have retarded growth in comparison to that of the control series.

In another set of experiment 5 females were injected with nutrient broth control series and 9 females within log culture of Pseudomonas in the treated series after 5 days from the date of the appearance of vaginal plug. On exposing the uteri on 15 day of gestation, 4 of 10 control females had 29 implants and none of them was dead. The examination of 29 embryos showed no abnormality in their morphological feature. On the other hand, an examination of the 9 treated females revealed that 7 of them had 45 implants but all were dead (PM 186) having no living implants (Table 49). The data, therefore, showed that most of the treated females had no pre-implantation fetality and it was all due to post-implantation effects.

Comments :

The injection of the log culture of Pseudomonas to mating females after the appearance of vaginal plug on

the 1st to 5th day caused tremendous fetal effect in the form of pre-implantation and post-implantation loss. Only 3 living embryos in the present study were obtained and all of them showed some abnormality. Further the treatment of Pseudomonas culture was totally fatal to the fertilized egg when the bacterial sample was injected on 1st and 2nd day after the appearance of vaginal plut. It was largely fatal from 3rd to 5th day indicated by both pre- and post-implantation loss. Thus with the lapse of time after the appearance of vaginal plug, the treatment (as on 5th day) caused largely the post-implantation loss because by the time the fertilized eggs were implanted into the uteri. If the data of the nutrient broth injected control series was considered, there was perhaps no pre-implantation loss. This was surmised because females which were really pregnant did have many living implants barring one or two dead. Thus out of 196 implants for the injection of the nutrient broth in 1-day to 5th day to different females, only 4 were dead. The remaining 192 living implants had no teratogenic effect. On the other hand the teratogenic effect was very high before and after implantation for the treatment of Pseudomonas culture to pregnant females 1 to 5 days after the appearance of the vaginal plug. It was much higher than that of Pseudomonas treated males mated to different sets of females for 7 week described before.

The mechanism of teratogenic effect has not been investigated at present as the present study was an explorative keeping in view to extend the study in future.

B, Histological studies of uteri of control and treated series pregnant mothers.

It was mentioned before that though the mechanism of action for the production high percentage of mortality and embryonic deformity in Pseudomonas culture treated females has not been critically undertaken but it was looked for if there was any mechanical reason apart from the genotoxic effect. With this in view transverse sections of the uterus of control and treated females were stained in Haemalonglin-Eosin and were studied in some of the pregnant females both control and treated lines of 3rd and 4th day administration.

Observation :

As examination of the histological stained preparation of the uterus of control females showed that the structures were just like normal (PM 189) the detail of which has already been presented in Chapter I. On the other hand the section of the uterus of pregnant females treated on 3rd day with Pseudomonas culture showed on 15th day after that the lumen was very reduced, the epithelial layer was undulated, large number of villi projected into the lumen (PM 192), blood vessels having very narrow cavity, RBC number was limited and so on. The muscular layer was thin and very compact and the cells of the connective tissue were disorganised with many nectrotic cells (PM 190). The structural changes found in the T.S. of uterus of the present study if compared with that of control, the changes were found to be more drastic. The

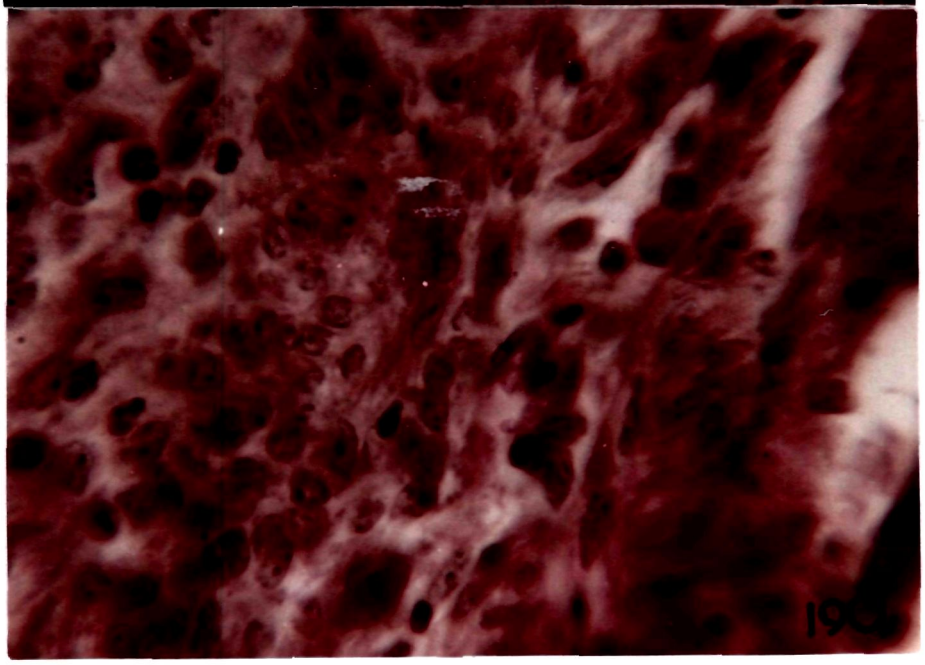
Explanation of Photomicrographs

Photomicrographs of T.S. of uterus after treatment of Pseudomonas culture on different day of pregnancy i.e. after appearance of vaginal plug.

PM 189. Normal structure of uterus.

PM 190. Disorganised connective tissue with many necrotic cells.

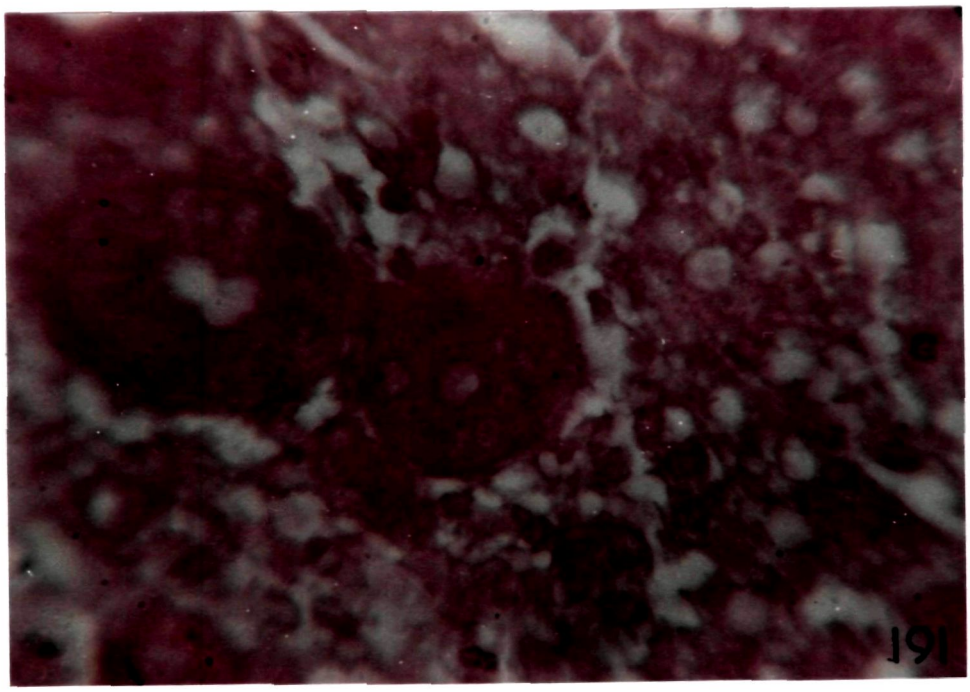




Explanation of Photomicrographs

Photomicrographs of T.S. of uterus after treatment of *Pseudomonas* culture on different day of pregnancy i.e. after appearance of vaginal plug.

PM 191. Disorganised connective tissue layer and many vacuoles in the uterus.


191

Explanation of Photomicrographs

Photomicrographs of T.S. of uterus after treatment of *Pseudomonas* culture on different day of pregnancy i.e. after appearance of vaginal plug.

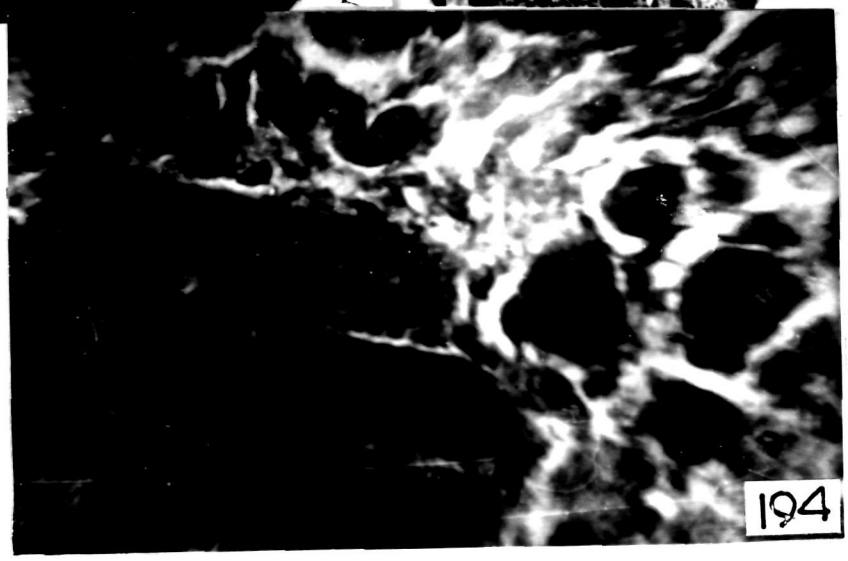
PM 192. Large number of villi projected into the lumen of uterus.

PM 193. Loose villi within the lumen of the uterus.

PM 194. Disorganised permimetrium layer in the uterus.


192


193


194

cells in the uterine epithelium were disorganized, some were loose and found in the lumen, some were cytolized (PM 192). The cells in the perimetrium layer were also disorganised and no definite demarcation of the membrane could be distinghished, etc. They would render some mechanical hindrance in the treated mother beside the genotoxic effect produced by the bacterium.

The histological sections of the pregnant mother at 15 day to whom the log culture was injected on 4 day after observing vaginal plug also showed the changes in the form of very narrow lumen, some detached villi and dead cells within the lumen (PM 192) disorganised connective tissue layer, many vacuoles (PM 190), small number of fibroblasts and blood vessels, muscular layer showing sign of separation of muscular fiber etc. Therefore, the structure of uterus showed greater abnormalities specially by the presence of fragmented villi, vacuolation and pycnotic cells in the myometrium and endometrium layer and which would suggest some mechanical disturbance was also present beside the genotoxic effect.

The histological study made above indicated that there was some abnormalities which might have inflicted as a result of the toxin of the bacterium, Pseudomonas in the treated mother. Anyhow, the toxin or some other factors must have genotoxic effect as indicated in earlier chapters.

Concluding remarks :

The effect of the intraperitoneal injection of log culture of P. aeruginosa - (1) in male parents and their mating to virgin females leading to, (2) F_1 generation and (3) in breeding of F_1 brother-sister of F_1 production F_2 generation and (4) females after showings sign of pregnancy injected with the bacterial culture 1 to 5 days in different sets assayed by various mutagenicity testing protocols proved reasonably that the bacterial culture was clastogenic, mutagenic and teratogenic under experimental condition in mice as compared to the relevant controls. The results have been presented and discussed before in different chapters and summing up of them one by one have been attempted here to have a comprehensive view.

The clastogenic potentiality of the bacterium has been proved primarily by the study of the bone marrow chromosome aberrations in mice when the cells were fixed at different intervals from few minute to several weeks. In all cases the frequency of aberration was significantly high than that of control but some deviation was found when each individual type of aberrations was considered. Among the individual type aberrations, the break type ones like chromatid break, acentric fragment, translocation or association were of genetic significance while other types like gaps, constrictions precocious centric separation of chromosomes were of debatable significance. If the frequency of true break type aberration in treated series was compared with that of control, the former would out-

number the latter. Among gross types, aberrations involving numerical change would more have genetic significance than other types. The frequency of cell with gross numerical changes would also out-number the frequency of the control series. Thus if too much importance on the exact value was not attached because of the inclusion of some arbitrary types, even then it would reasonably be clear that on the whole the effect for the treatment of log culture of Pseudomonas was much higher than that of control. In other words the clastogenic property of Pseudomonas in treated mice was quite evident.

The occurrence of the chromosome aberrations in bone marrow cells encountered as early as 10 minute and as late as 7 week would indicate that the effect was immediate which lingered for a long time. Further, the individual type aberrations were mostly of chromatid type which would indicate that the G_2 phase was most vulnerable. The response was comparable to those of chemically induced aberration as similar situation was generalized by Kihlman (1966). It was not certain how many of the affected chromosomes underwent restitution or else the affected cells died. It seemed that both were effective because more or less the same chromatid type aberrations were encountered at various intervals. It has been assumed that the aberrations were produced not by the direct effect of the bacterium but very likely the treatment induced certain mutagenic change in the some chromosome loci which under normal condition were responsible to maintain the structural integrity. Such a hypothesis was forwarded by Manna (1984). The same has also been

envisaged in the present study because the same types of aberration were encounted in the spleen of embryos and bone marrow cells of treated parents and in F_1 and F_2 generation.

The individual type aberrations specially the chromatid breaks when analysed, showed that they were nonrandomly distributed between and within the chromosome which was revealed not only in the parental generation of treated series but also in the treated line of F_1 generation. This has been explained as due to the presence of some inherent weaker region in the chromosome of mice. Manna (1975, 1980, 1981) made a critical evaluation of the group-wise and region-wise distribution of chromatid breaks induced by good number of chemical and living mutagens in bone marrow chromosome of mice and had suggested the presence of inherent weaker region in chromosomes in mice. He cited evidences from prokaryotype chromosomes as well (Stahl, 1972). In recent years nearly 18 heritable fragile sites in human cancer chromosomes have been detected (LeBeau and Rowley, 1984; Chaganti, 1985). In the present study the occurrence of the nonrandom distribution of chromatid breaks found in F_1 generation of treated line mice would also support that the genetic loci controlling them were inherited.

The differential occurrence of the precocious separation of chromatids of some chromosomes belonging to different groups showed that the maximum incidence took place in group V. The next one was group IV while it was very rare in chromosomes of groups I and II. The differential precocious separation of the chromatids at

metaphase plates in mice was experienced by different workers in our laboratory when mice treated with some chemical and living mutagens. Thus it could be envisaged that the chfomosomes of smaller groups specially of groups V amd IV might have some defect in centromere. This was found more in treated series due to genotoxic effect than that of the control. Surprisingly this trend was also found in F_1 generation. This would indirectly lead to suspect that the phenomenon could be under genetic control. Anomalous chromosomes behaviour due to centromeric condition are known in many organisms while it has been found also in some chromoscmes of mice. Further asynchromous separation of chromosomes of the complement in other mammals has also been claimed (Farook and Farook, 1985).

The clastogenic potentiality of the log culture of P. aeruginosa besides the somatic chromosome aberration study was also supported by the micronuclei test. However, the dose dependent effect could not be established for the present because the higher doses were fatal to the treated mice. The treatment of 1 ml dose revealed that the frequency of micronucleated polychromatic and normochrematic erythrocytes was greatly enhanced than that of control. However, the frequency of micronucleated cells was relatively low than of the chromosome aberrations. This was not unexpected because in MNT only few among many forms of chromosome aberrations could be detectable.

The clastogenic potentiality of the bacterium was further supported by the study of the meiotic chromosome aberration studied at spermatogonial metaphase, diakinesis, metaphase I and metaphase II. The frequency of aberration

in treated mice was significantly high than that of control which was observed at all 18 intervals between 10 minute to 7th week. This confirmed the findings of the bone marrow cells. All the more the aberration study in the meiotic chromosomes also indicated that the effect of the bacterium was not restricted to any particular tissue. Over and above the occurrence of the germinal chromosome aberration induced by the bacterium would have direct implication of its mutagenic potentiality since meiotic chromosomes are included in gamete and one transferred through fertilization to the next generation.

The study of sperm count of the epididymal samples clearly indicated that there was considerable depletion in the bacterial culture treated males than that of control though the genetic significance of the test is subjudice. However, the occurrence of the higher frequency depletion in sperms in epididymal samples in F_1 generation and F_2 generation of the treated lines than that of control lines might tempt to imply some genetical involvement but we refrained from making any positive claim until it is positively proved.

The study of the frequency of sperms with abnormal head morphology in the epididymal samples showed that the frequency in treated males was significantly high than that of control. The occurrence of higher frequency of sperms with abnormal head morphology in treated lines of F_1 and F_2 males than that of control would support the genetic control of sperm head morphology which has been claimed by many workers (see Wyrobeck, 1979).

The treatment of the Pseudomonas log culture to males and females brought changes in the histological structure of the testis and ovary. This also affected the reproductive potentiality for which there were some increase in the frequency of sterility of the mating individuals. These results might imply that the decrease in the reproductive potentiality in treated series could be genetical if not mechanical. However, the study of the histological sections of the germinal glands in F_1 and F_2 generation showed some structural abnormalities along with the reduction in the reproductive potentiality in treated lines more than that of control. This would imply very likely the genetic involvement as histological changes in reproductive gland for the treatment of some insecticide has been claimed to be a parameter of mutagenicity testing protocol (Bal, 1984). Anyhow the study of histological section of uterus in bacteria treated females showed various changes in the structure. This was considered to be a sort of mechanical obstruction to the normal reproduction.

It is well known that the mutagenic potentiality of an agent could be established more definitely if some changes could be shown in F_1 generation against suitable controls. With this in view, several cytogenetic assays were conducted in F_1 generation of which so-called the "dominant lethal test" in mice was very crucial. Anyhow, for the lethal test the male mouse treated with the log culture of Pseudomonas was allowed to mate with different sets of virgin females consecutively for 7 week to cover the time-table of spermatogenesis and spermiogenesis from undifferentiated spermatogonia (Adler, 1982). However,

the data of lethal test had limitation largely due to sterility developed in the treated series discussed before. It was found that the mutagenic index was much higher than that of control for mating in the 1st, 2nd, 3rd and 4th week while no data in the treated line was available for cent percent sterility developed from 5 to 7 week. Therefore, in spite of some limitation, the data indicated the mutagenic potentiality in the treated series. Therefore, though the stage sensitivity of the spermatocytes cycle could not be assessed for the limited data, the combined data of all weeks was significantly higher than control ($P < 0.05$) indicating at least lethals were induced for the treatment of male parent with log culture of Pseudomonas. The effect of the treatment of Pseudomonas was supported further by the study of the chromosome aberration in the liver cells of F_1 embryos at 15 day of gestation and in bone marrow cells of 3 month old F_1 adults. The chromosome aberration study in the liver of several embryos of mothers mated to log culture treated males in 2nd, 3rd, 4th and 7th week revealed that (Table 17) the frequency of chromosome aberration was strikingly higher than that of control. Similarly the study of bone marrow chromosome aberration in 3 month old F_1 adults raised from the mating of treated males with virgin females in 1st, 2nd, 3rd and 4th week revealed very high frequency of chromosome aberrations than that of control (Table 20). Therefore both the studies showed that the living embryos or adults in F_1 generation of the treated line had very high frequency of aberration than that of control which had been explained as due to the inheritance of the mutagenic factor from parent to F_1 generation. Further, the effect

was not tissue specific as liver and bone marrow showed higher frequency of aberrations in F_1 generation. Basing on the hypothesis advocated by Manna (1984) it has been envisaged that there was mutation in some chromosomal loci which under normal condition would have maintained the structure integrity of the chromosome but once mutated, they had lost the control and that had led to the appearance of various types of chromosome aberration as observed in our study. It was also envisaged that the same chromosomal mutation could lead to lethality of the developing embryos if the aberrations were severe or of vital nature. Otherwise they might cause aberrations which could be present in F_1 living individuals. Anyhow, the study of the chromosome aberrations in F_1 generation would support that it was under the control of genetical factor.

The chromosome aberration was found not only in the somatic cell of F_1 embryos and bone marrow cells of adult but also in the testis cells of F_1 males of 3 month old mice of treated line. The study of chromosome aberration in spermatogonial metaphase, diakinesis, metaphase I and metaphase II of the treated line F_1 males of 4 week mating revealed very high frequency of aberrations in all these stages as compared to that of control presented before. It could be generalised that the high frequency of the chromosome aberrations in somatic and germinal cells of F_1 treated line than that of control must have been produced due to some mutagenic factor transmitted from treated male parent to the F_1 generation.

The study of the sperm count and the sperm head morphology of the epididymal samples of those F_1 males in which the meiotic chromosome aberration study was conducted. It revealed that there was significantly high depletion in the sperm count in treated line males than that of control in all weeks. This result perhaps would imply some genetic factor transmitted in F_1 generation which was responsible not only for the higher frequency of the chromosome aberration but also the sperm depletion effect. Similarly the higher frequency of sperms with abnormal head morphology in the epididymal samples of 3 month old F_1 males of four week treated lines than that of control would also imply the same factor was transmitted from treated male to offspring in F_1 generation (Table 32).

The study of the teratogenic effect in F_1 generation from the mating of treated males with virgin females suffered for adequate data. Only very few instances of defects in skeletal structure, brain, size of the individuals were encountered but all were in the treated line and none in the control line. Therefore, they were indicative of the teratogenic effect induced by the treatment of the log culture.

Beside the cytogenetic and teratogenic effect found in the F_1 generation metnioned above there was also some effects in the histological structure of the testis, ovary in F_1 individuals than as compared to controls while no effect was found in the uterus.

The various results obtained in F_1 generation summarised above would testify the mutagenic potentiality of the bacterium, P. aeruginosa. However, the specific factor in P. aeruginosa acting as the mutagen is yet to be determined. The bacterial toxin a (Manna, 1980) or else the cell wall or some component of the bacterium is to be critically evaluated.

The mutagenic potentiality of the bacterium was further evidenced when the result of the different studies carried out in F_2 generation were considered. As the treatment of the bacterium reduced the reproductive potentiality and in some cases induced complete sterility, the data of F_2 generation suffered considerable limitation. Because of the limited availability of specimens it was not possible to varify if there was any lethal effect in F_2 generation during gestation after selfing the F_1 treated line males and females in comparison to that of control line males and females. It was attempted after parturition but the data was also very limited (Table 34). However, the chromosome aberration study in the bone marrow cells of the F_2 individuals obtained from mating of F_1 treated line males and females of 2nd and 4th week revealed higher frequency of aberrations than that of controls (e.g. 37.5% against 9.5% in the control of 2nd week and 35.0% against 8.5% in the control of 4th week) indicating the mutagenic factor was transmitted from F_1 to F_2 generation.

Further, the study of the chromosome aberration in the spermatogonial metaphase, diakinesis, metaphase I and metaphase II from the testis of two male individuals of the

2nd and 4th week treated line revealed also higher frequency of aberrations than that of control. The results of chromosome aberrations in cells of testes of F_2 treated line males also supported the view that the mutagenic factor was transmitted in the F_2 generation from F_1 generation. It was mentioned before that the sperm count of the epididymal sample of 2nd week treated line F_2 males did not show much difference with that of control while the difference was somewhat significant in 4th week line. On the otherhand the study of the frequency of sperm with abnormal head morphology in the epididymal sample of 2nd week and 4th week treated line F_2 male individuals was significantly higher than that of control in each week. Therefore, almost all the tests mentioned above showed that the mutagenic change induced in the parental male was transmitted to F_1 and F_2 generations.

The teratogenic effect intended to be studied by treating the females with log culture from one to five day after observing the vaginal plug against nutrient broth injected control. This was largely not fruitful because 100% sterility was evidenced as when the uteri of the treated females were exposed on 15 day after plugging, on sign of implantation was observed. This was the case of females which were injected with the log culture of the bacterium on 1st day and 2nd day after the appearance of the plug. The sign of implantation was partly seen when the log culture was injected on 3rd day after the plugging. The treatment of the log culture on 4th day and 5th day after plugging yielded some pregnant females relatively more than that of 3rd day. However, the study of the

teratogenic effect was seriously handicapped because almost all the implants were dead. Thus out of the total of 60 implants observed in 12 pregnant mothers treated with Pseudomonas culture on 3rd day, 4th day and 5th day after plugging only 3 were alive and remaining 57 were dead. These 3 had teratogenic effect like retarded growth, enlarged brain and deformed fore-limbs etc. So in spite of this limited data the study indicated the possibility of very high frequency of teratogenic effect induced by log culture of Pseudomonas treated females. The high frequency of pre-implantation loss of the fertilized eggs curtailed the data of the present study.

Summary

The clastogenic, mutagenic and teratogenic potentialities of the bacterium, P. aeruginosa belonging to the family Pseudomonadaceae under the order Pseudomonadales have been assessed in mice system using different testing protocols against nutrient broth injected controls. The effects in the parental generation assessed by various testing methods have been presented in Chapter I and they have been summarized in the following :

Laboratory bred Swiss albino mice, Mus musculus of about 3 month old were used as experimental animals. The log culture of P. aeruginosa containing approximately 17×10^7 cell/ml generally injected intraperitoneally at the rate of 1 ml/100 gm body weight of the individual served as treated series while freshly prepared nutrient broth without putting any bacterium injected at the same rate to normal mice served as controls. Bone marrow cells of mice injected with log culture of P. aeruginosa and other set with nutrient broth were collected separately at various intervals after the injection and the chromosome aberrations were assessed in 100 plates per individual using 2 males and 2 females at each fixation interval. The chromosome aberrations were put under two categories as individual types consisting of break, acentric fragment, translocation, gaps and constrictions, precocious centromeric separation etc. and gross types consisting of numerical change in the form of aneuploidy and polyploidy and other types like stickiness, pycnosis etc. As compared to control, the aberration types were more common in the treated specimens.

The frequency of aberration taking both individual and gross type together showed the net increase in the treated series over control as 41.50% in 1st week, 38.50% at 2nd week, 38.25% at 3rd week, 39.0% at 4th week, 42.50% at 5th week, 57.25% at 6th week, 31.50% at 7th week and 41.14% in data of all intervals combined. The difference in chromosome aberration data of control and treated specimens was statistically highly significant. The aberration frequency in the two sexes was not significantly different. Among the individual type aberrations, the frequency of centromeric dissociation was higher than any other type. The next one was the translocation followed by the chromatid breaks.

An analysis of the cases of centromeric dissociation in different groups of chromosomes in material fixed in 7 weeks of the control and treated samples was made. Both in control and treated specimens, the frequency of centromeric dissociation was relatively high in chromosomes belonging to group V and group IV while it was practically absent in chromosomes belonging to groups I and II. The frequency of centromeric dissociation was relatively very high in chromosomes of the treated specimens and the net increase over control was 15.75% in 1st week, 11.25% in 2nd week, 19.25% in 3rd week 11.50% in 4th week, 24.0% in 5th week, 20.50% in 6th week, 10.25% in 7th week and 17.21% in combined data of all weeks. Chromosomes of smaller group have been suspected to have defective centromere and the frequency was enhanced for the treatment of the log culture of Pseudomonas.

The chromatid breaks were found nonrandomly distributed between and within the five groups of chromosomes when the observed and the expected number were compared. The data showed that chromosomes belonging to group I and to some extent group II were more vulnerable while those belonging to groups III to V were somewhat resistant when the group-wise breakage data were analysed. The region-wise analysis of the chromatid breaks also indicated that distal region of the affected chromosomes was more vulnerable while the proximal region was somewhat resistant as the observed and the expected numbers were compared. This has led to support the earlier view advocated by Manna that mice chromosomes possibly have inherent weaker regions which was supported in recent years by the discovery of some 18 fragile sites in human cancer chromosomes.

The clastogenic potentiality of the P. aeruginosa was supported further by the study of the micronucleated polychromatic and normochromatic erythrocytes as the data were compared with that of the control specimens. The verification of the dose-dependent effect on the production of micronucleated cells using double and triple doses of the log culture of Pseudomonas failed because these doses were fatal to the treated specimens. The frequency of micronucleated erythrocyte was lower than that of the chromosome aberrations which has been explained as all forms of aberrations did not contribute to the micronucleus formation.

The clastogenic potentiality of the P. aeruginosa was further substantiated by the study of the meiotic

chromosome aberrations in different stages of male mice fixed at 18 intervals between 10 min and 7 week against nutrient broth injected controls. The frequency of aberration in each interval was mostly higher than that of control. The net increase in treated series over control in 18 intervals, 10 min, 1 hr, 2 hr, 4 hr, 8 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 1st week, 2nd week, 3rd week, 4th week, 5th week, 6th week and 7th week was in spermatogonial metaphase, 1.37%; 24.25%; 3.08%, 16.86%, 12.71%, -1.60%; 11.66%, 10.71%, -9.52%, 17.59%, 13.58%, 48.10%, 17.33%, -1.22%, 42.31%, 24.67%, 55.39% and 19.09% respectively; in diakinesis 7.26%; 1.96%, 5.72%, 21.78%, 64.57%; 14.06%, 8.38%, 29.57%, 2.26%, 34.86%, 60.27%, 92.11%, 22.22%, 71.57%, 52.83%, 29.59%, 74.14% and 58.47% respectively; in metaphase I 11.33%, 5.34%, 1.34%, 5.66%, 23.34%, 23.67%, 9.66%, 11.67%, 7.00%, 19.84%, 22.14%, 22.75%, 27.67%, 28.67%, 35.00%, 40.52%, 65.93% and 26.42% respectively and in metaphase II 15.40%, 17.05%, 11.05%, 15.72%, 11.78%, 4.74%, 20.83%; 66.92%, 44.23%, 58.25%, 65.92%, 55.25%, 66.36% and 47.99% respectively. If data of the frequency of chromosome aberrations of all intervals were combined, the average increase was 17.11% in spermatogonial metaphase, 33.99% at diakinesis, 19.56% at metaphase I and 28.53% at metaphase II. If the data of all stages of division were combined to have a very large sample, the net increase in the aberration frequency in meiotic chromosomes over control was 14.62%. The occurrence of the higher frequency of chromosome aberration in different stages of meiosis confirmed the clastogenic potentiality of bacterium in treated mice which was evidenced by the study of bone marrow chromosome aberrations and by the occurrence of higher frequency of micronucleated cells.

The effect of Pseudomonas culture was assessed on the sperm count at 18 intervals of the sperm suspension prepared from the left and right epididymes of both treated and control specimens. The difference in the sperm count in the two sides was not so significant in the average of either 3 control or in 3 treated male specimens. In the combined data of the left and right epididymes, the mean values of the treated versus control at 18 different intervals were 13.61 ± 0.47 against 20.41 ± 0.53 at 10 min, 15.88 ± 0.68 against 22.92 ± 0.71 at 1 hr, 16.35 ± 0.43 against 19.28 ± 0.45 at 2 hr, 17.83 ± 0.45 against 21.35 ± 1.36 at 4 hr, 8.42 ± 0.40 against 15.48 ± 0.78 at 8 hr, 16.24 ± 0.47 against 14.03 ± 0.50 at 24 hr, 4.89 ± 0.42 against 18.26 ± 0.66 at 48 hr, 8.14 ± 0.36 against 13.71 ± 0.83 at 72 hr, 7.30 ± 0.54 against 15.63 ± 0.46 at 96 hr, 6.72 ± 0.52 against 18.63 ± 0.38 at 120 hr, 4.61 ± 0.33 against 16.86 ± 0.39 at 144 hr, 6.31 ± 0.58 against 12.64 ± 0.40 at 1st week, 3.68 ± 0.40 against 11.82 ± 0.33 at 2nd week, 5.86 ± 0.68 against 11.78 ± 0.76 at 3rd week, 9.27 ± 1.24 against 11.81 ± 0.81 at 4th week, 7.64 ± 0.54 against 18.55 ± 0.96 at 5th week, 6.11 ± 0.49 against 25.82 ± 0.48 at 6th week and 11.10 ± 0.53 against 16.33 ± 0.86 at 7th week. The data clearly indicated that the treatment of log culture of P. geruginosa induced significant decrease in sperm count and the difference was statistically significant almost in every intervals.

The frequency of sperms with abnormal head morphology was studied from smear of the epididymal sample of the control and treated males. The frequencies of sperms with abnormal head morphology found in the epididymis of treated and control specimens respectively were 1.22% against 0.94% at 10 min, 3.5% against 0.77% at 1 hr, 3.16%

against 1.50% at 2 hr, 3.6% against 6.72% at 4 hr, 3.9% against 1.44% at 8 hr, 5.66% against 1.44% at 24 hr, 9.33% against 1.16% at 48 hr, 10.5% against 1.33% at 72 hr, 10.5% against 2.0% at 96 hr, 12.61% against 2.83% at 120 hr, 12.0% against 1.50% at 144 hr, 5.44% against 1.22% at 1st week, 9.22% against 0.22% at 2nd week, 10.22% against 1.61% at 3rd week, 17.0% against 1.22% at 4th week, 27.5% against 2.05% at 5th week, 28.0% against 1.0% at 6th week, and 22.5% against 3.33% at 7th week. The difference was statistically significant at all intervals except at 10 min, 8 hr, 144 hr, 3rd week and 7th week. If the data of all intervals were combined, the average frequency was 32.58% in treated series against 4.25% in the control series and the difference was statistically highly significant ($P < 0.001$).

The study of the histological section of the testis and ovary of P. aeruginosa culture treated male and female mice respectively showed some changes in the structure of the gland concerned as compared to that of control specimens. This also added evidence on the changes induced by the treatment of log culture of Pseudomonas in two sexes of experimental mice.

The different tests carried out in the parental generation were found to be significantly high in the treated specimens than that of control implying the clastogenic and mutagenic potentiality of the bacterium P. aeruginosa.

The mutagenic potentiality of the bacterium, P. aeruginosa was substantiated in F_1 generation using different testing methods and the results have been presented in Chapter II and they have been summarized in the following :

The lethal test, a crucial one for testing mutagenic potentiality of an agent has been conducted by the mating log culture of Pseudomonas treated male mice with different sets of virgin females each week for consecutive 7 weeks to cover the time table of spermatogenesis and spermiogenesis. The lethal effect in F_1 generation was assessed for the post-implantation loss at 15 day of gestation and at the end of weaning period after parturition of the pregnant mother against control. In the treated series there was some sterility developed possible for the treatment of bacteria to male parent for which not even a single virgin female out of 21 deployed was impregnated by the mating of 7 different treated males in each 5th and 6th week. Beside the complete sterility developed in 5th and 6th week, in other week also good amount of sterility observed for which out of 21 females, only a few were impregnated which limited the data of lethal test. The mutagenic index in treated series was found relatively high in 1st to 4th week as compared to control while no data could be obtained in the remaining weeks for the reason stated before. The mutagenic index in the combined data of during gestation and after parturition in treated and control series respectively were 40.0% against nil in 1st week, 25.0% against 4.17% in 2nd week, 22.73% against nil in 3rd week and 29.09% against nil in 4th week. When the data of all weeks were combined, the mutagenic index of the average was 24.44% in the treated series against 1.58% in control series. Thus in spite of the limited data, the results clearly indicated that the treatment of log culture to male parents induced higher frequency of dead implants as well as sterility as compared to control. According to time table of the spermatogenesis the stages from spermatocyte to the transfer of mature sperms in epididymis were very sensitive but it could

not be said about earlier stages of meiosis because no data were available after 4th week.

The mutagenic potentiality of the log culture of P. aeruginosa also supported by the study of the chromosome aberration in liver cells of embryos at 15 day gestation and bone marrow cells of 3 month old individual of F_1 control and treated lines. The chromosome aberrations in embryos of both control and treated series could be assessed for mating of 2nd week, 3rd week, 4th week and 7th week lines which showed qualitatively more or less the same type of aberrations as found in the treated and control specimens of the parental generation having both individual and gross type aberrations. However, the frequency was much higher in treated line embryos than that of control line. The average frequency of aberration in the liver cells in different embryos of treated and control lines were 13.9% against 7.1% in 2nd week, 15.07% against 5.1% in 3rd week, 23.6% against 4.8% in the 4th week and 28.75% against 4.73% in the 7th week respectively. All weeks taken together, the average frequency was 19.97% in the treated line against 5.4% in the control line indicating clearly that the embryos in the treated line had much higher frequency of chromosome aberration than that of control. Similarly the study of the bone marrow chromosome aberrations in 3 month old F_1 adults were found much higher in treated line than that of control and the frequencies were 34.33% against 5.6% in 1st week, 28.12% against 6.30% in 2nd week, 31.33% against 6.0% in 3rd week, 40.6% against 5.66% in 4th week respectively. If aberration data of all weeks were combined the average frequency was 33.31% against 5.94%. Therefore, the frequency of the chromosome aberrations assessed from liver cells of

embryos during gestation and the bone marrow cells of adult after parturition showed more or less the same trend of having much higher frequency of aberrations than that of control which has been considered to be due to the chromosomal mutation induced in parental generation was transmitted to F_1 generation. It was envisaged that possibly be some loci in chromosomes which normally control the structural integrity of chromosome underwent mutational changes and therefore, the presence of the factor prevented the maintenance of the structural integrity unlike normal state.

The assessment of the centromeric dissociation and their non-random distribution in five groups of chromosomes showing the same trend as found in parental generation which indicated that chromosome belonging to groups IV and V might have defective centromere as they had more centromeric dissociation than in other groups. The frequency was relatively more in the treated line than that of control line. The study of the distribution of chromatid breaks within and between chromosome revealed that chromosomes belonging to group I was more susceptible so also the distal region which supported the idea of inherent weaker region present in chromosomes.

In F_1 generation the frequency of chromosome aberrations at spermatogonial metaphases, diakinesis, metaphase I, and metaphase II in the testes of 3 month old males of different week mating line revealed that the it was higher in treated line than that of control in each weak end in each of meiotic division stage. The frequencies

of total aberrations of spermatogonial metaphase, in treated versus control lines were 46.6% against 8.0% 1st week, 50,87% against 10.81% in 2nd week, 41.33% against 12.76% in 3rd week, 52.2% against 7.08% in the 4th week and the average of all weeks taken together was 49.01% in treated line against 8.54% in the control.

At diakinesis, the frequencies of aberration in treated and control line F_1 males were 25.0% against 8.0% in 1st week, 44.28% against 9,05% in 2nd week, 34.28% against 5.88% in 3rd week, 32.43% against 8.69% in 4th week and all weeks taken together the average was 34,24% against 8,45% respectively.

At metaphase I the frequencies of chromosome aberrations in treated versus control were 30.0% against 7.5% in 1st week, 35.0% against 5.4% in 2nd week, 42.0% against 2.0% in 3rd week 38.25% against 6.8% in 4th week and in the combined data of all weeks, it was 36.87% against 5.8% respectively.

At metaphase II the aberration frequencies in treated versus control were 53,33% against 7.20% in 1st week, 63.04% against 17.34% in 2nd week, 58,82% against 6.24% in 3rd week, 39.0% against 12.11% in 4th week and in the combined data of all weeks, the average frequency was 52.75% against 12.27% respectively. The data were critically analysed and on the whole, it has been generalised that the chromosome aberration frequency was much higher in treated line F_1 males than that of control in all stages of meiosis. It was also found in bone marrow cells. Therefore chromosomes of both somatic and germinal cells were affected more in the treated line indicating the inheritance of the mutagenic factor through chromosomal agency from treated male parent to the F_1 generation.

Like the chromosome aberration study, the study of sperm count from the epididymal suspension of sperms revealed higher frequency of sperm depletion than that of control and in the combined data of mean values obtained from 25 trials of left and right side epididymal suspensions, it was 6.66 against 22.26 in 1st week, 10.29 against 21.17 in 2nd week, 6.68 against 16.23 in 3rd week and 14.85 against 22.78 in 4th week indicating that the depletion in sperm count in treated males was much higher than that of control as found in parental generation. The contrinuation of the higher depletion in sperm count in the treated line F_1 generation has been considered to be due to the inheritance of the mutagenic factor affecting the sperm production.

The study of sperm with abnormal head morphology in F_1 treated line and control line males was found much higher in frequency in the treated line than that of control. The average frequencies in treated versus control line were 11.66% against 0.83% in 1st week, 13.58% against 2.5% in 2nd week, 10.33% against 0.66% in 3rd week, 17.62% against 1.08% in 4th week indicating that the trend of higher frequency of sperm with abnormal head morphology was continued in F_1 treated line like parental generation. This has also been taken as a supportive evidence of the inheritance of mutagenic factor from the parental generation to F_1 generation in the treated line.

The study of the teratogenic effect was also attempted on the living embryos collected while testing the lethal effect and in some organs of the 3 month old F_1 individuals but higher percentage of sterility yielded the limited data.

Out of only 68 living embryos obtained from 13 F_1 treated line mothers only 1 showed the rib abnormality while it was totally absent in 131 embryos examined in control line. Further out of 19 F_1 adults examined 3 were dwarf in size and 8 had morphological changes in the brain. Further the testes and uterus of the dwarf individuals were ill developed. On the other hand, an examination of 35 individual of control line did not show any such teratogenic effect.

The histological study of testis, ovary and uterus of 3 month old F_1 treated and control line individuals revealed some structural differences in section of the testis and ovary of the treated line F_1 individuals like that of individual treated with log culture of P. aeruginosa. On the other hand the section of the testis and ovary of F_1 control line had practically no difference from that of normal structure. The histological structure of uterus of control and treated line females showed no marked changes.

The mutagenic potentiality of the treatment of log culture of Pseudomonas in treated male parent was followed to some extent in F_2 generation and the results have been presented in Chapter III as summarized in the following :

The data of the lethal test carried out by brother-sister mating of F_1 treated line against control line were very limited because not many individuals for mating were available and in some week they were completely absent. Therefore, no lethal testing was possible at 15 day gestation and the very limited data obtained after parturition yielded only 1 pregnant female having 4 embryos all were alive in 1st week, so also the case of another mother of 4th week.

The higher frequency of sterility developed in the F_1 individuals prevented extension of the data. It was very uncertain if there was any lethal effect in F_2 generation because of the very meagre data though there was some indication shown by the chromosome aberration study in F_2 individuals.

The study of the bone marrow chromosome aberration in 4 F_2 adults of 3 month old of 2nd week treated line yielded the frequency of 37.5% against 7.6% in the control line. Similarly in the 4 F_2 individuals of the 4th week treated line the average aberration frequency was 35.75% against 8.5% in the control line which indicated that the living implants carried the mutagenic factor transmitted from the F_1 parents.

The study of spermatogonial metaphase chromosome aberrations in the testes of 2 individuals of the treated lines and 3 of control line of 2nd week mating yielded in spermatogonial metaphase 46.34% aberration against 11.80% in the control and in the 2 male individuals of 4th week treated line and 3 individuals of control line, the average frequency was 57.7% against 14.59%. In diakinesis the average aberration frequency was 36.23% in the treated line against 18.94% in the 2nd week the control line and 40.57% against 20.0% in the 4th week control line. In metaphase I the average aberration frequency was 18.0% in the treated line against 7.66% of 2nd week control line and in the 4th week it was 17.0% in the treated line against 9.0% in the control line. In metaphase II the average aberration frequency was 36.23% in the treated line against 18.94% in the 2nd week control line and 40.57% in the treated line against 20.0%

in the 4th week control line. Therefore, the frequency of meiotic chromosome aberration in different stages of meiosis was much higher than that of controls indicating that the mutagenic factor was inherited from F_1 to F_2 generation.

The study of the sperm count in 2 males, each of the 2nd week and 4th week treated line and 3 males each in 2nd week and 4th week control line showed that it was not very different between 2nd week treated and control lines while in 4th week the mean frequency was 17.28 in the treated line against 23.06 in the control line indicating some amount of sperm depletion in 4th week treated line.

The study of the frequency of sperm with abnormal head morphology was found to be much higher than that of control. In 2nd week line it was 9.83% in treated against 1.1% in the control line and in 4th week it was 5.50% against 1.50% in the control line indicating that the mutagenic factor was inherited in F_2 generation which caused the higher frequency of sperm with abnormal head morphology in treated line.

The histological study of the testis and ovary of the F_2 males and females of the treated and control line showed some amount of difference but it was not so glaring as found in the parental generation and in F_1 generation.

The results of the different studies of parental, F_1 and F_2 generation have been discussed in detail while presenting the data and it has been concluded that the treatment of log culture of P. aeruginosa in mice has shown clastogenic, mutagenic and teratogenic effect which continued to F_2 generation assessed at present.

The teratogenic effect was presented specially in Chapter IV. It was planned to study effect by injecting the females with log culture of Pseudomonas after normal mating and showing signs of pregnancy by the appearance of the vaginal plug against control. The log-culture in treated series and the nutrient broth in control series were injected in the different sets of female mice showing vaginal plug at an interval of 1 day, 2 day, 3 day, 4 day and 5 day. The treatment of the log culture to all mated females 1 day and 2 day after the appearance of vaginal plug did not have any sign of implantation examined on 15th day after injection. This has been assumed to be due to the effect of the bacterial culture on the fertilized eggs which made them inviable before reaching the uterus. The treatment in 3rd, 4th and 5th day after observing the vaginal plug had partial effect on the pre-implantation loss and few of them got the chance of implantation but all were dead except for three embryos. These 3 embryos some way or the other were deformed for the enlarged brain and loss of digit in the fore-limbs. Therefore, it was assumed that the teratogenic effect was high but the limitation of the data did not allow us to make any positive claim.

REFERENCES

Adler, I.D. 1970. The problem of Caffeine mutagenicity. In Chemical Mutagenesis in Mammals and Man. Edt. F. Vogel and G. Rohrborn. Springer-Verleg. Berlin. Heidelberg. New York. pp. 383-403.

Adler, I.D. 1974. Comparative cytogenetic study after treatment of mouse spermatogonia with mitomycin-C. Mutat. Res. 23(3):369-379.

Adler, I.D. 1982. Male germ cell cytogenetics. In Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu,Allanheld, Osmun Publishers, USA. pp. 249-276.

*Ahlstrom, C.G. 1951. Acta Pathol: Microbiol. Suppl. 91:52.

Ahlstrom, C.G. 1965. Acta Pathol Microbiol. Suppl. 91:52.

Ahlstron, C.G. Kato, R. and Levan, A. 1964. Rouse sarcoma in Chinese hamster. Science 144:1232-1233.

Alaksandrowicj, Z., Dobrowobski, J. and Lisiewicz, J. 1975. Effect of selenium on immunosuppressive and teratogenic properties of aflatoxin B_1. Rev. Esp. Oncol. 22(2):239-241.

Ames, B.N. 1970. The detection of chemical mutagen with enteric Bacteria. In : Chemical mutagens. Ed. A. Hollaender. Plenum Press, N.Y. Chap. 9. 1:267-282.

Arnold, C.G. 1970. The induction of Extrakaryotic Mutations. Chemical Mutagenesis in Mammals and Man. Edt. F. Vogel and G. Rohrborn. Springer-Verlag. Berlin - Heidelberg. New York 285-293.

Auerbach, C. 1967. The chemical production of mutation. The effect of chemical mutagens on cells and their genetic material in discussed. Science. 158:1141-1147.

Auerbach, C. and Robson, J.M. 1944. Mutation chemically induced : Production of mutation by allyl isothiocyanite. Nature.154:81.

Aula, P. 1965. Virus associated chromosome breakage. A cytogenetic study of chicken pox, measles and mumps patients and of cell cultures infected with measles virus. Ann. Acad. Sci. Fenn. Series A. IV. Biologica. 89:78.

Aula, P. and Nichols, W.W. 1968. Lysosomes and virus-induced chromosome breakage. Exp. Cell. Res. 51:595-601.

Aula, P., Nichols, W.W. and Levan, A. 1968. Virus induced chromosome changes. Ann. N.Y. Acad. Sci. 156:737-747.

Aula, P. and Saksela, E. 1966. Morphology of the chromosome damage induced by sendai - virus. Heredites. (Lund). 55:362-366.

Au, Willam and Hsu, T.C. 1982. Assays for chromosome aberration using mammalian cells in culture. In cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld, Osmun. Publisher, USA. pp. 203-220.

Awerbuch, T.E. and Stark, A.A. 1979. Plat diffusion assay as a rapid method for dosimetry of mutagens. Appl. Environ. Microbiol. 38(6):1127-1131.

Aya, T. and Makino, S. 1966. Notes on chromosome abnormalities in cultured leucocytes from serum hepatitis patients. Proc. Japan Acad. 42:648-653.

Aya, T., Makino, S. and Yamada, T. 1967. Chromosome aberrations induced in cultured human leukocytes by herpes simplex virus infection. Proc. Jap. Acad. 43:239-244.

Bacq, Z.M. and Alexander, P. 1961. Fundementals of Radiobiology (2nd Edl.). Pergamon Press, Oxford.

Bal, Harpal, S. 1984. Effect of Methoxychlor on reproductive systems of the rat (41861). Proceeding of society for Experimental Biology and Medicine. 176:187-196.

Banerjee, G. 1982. Cytogenetic effects of a chemosterilant, D-glucoseamine hydrochloride in mice. Ph.D. thesis. University of Kalyani.

Barinsky, L.F. and Dementiev, I.V. 1968. Virological and cytogenetic studies on the involvement of bone marrow of mice in some hepatoencephalotropic viral infections. Acta. Virol. 12:464-467.

Barthelmess, A. 1970. Mutagenic substances in human environment. In Chemical Mutagenesis in Mammals and Man. Edt. Vogel, F. and Rohborn, G. Springeer-Verlag. Berlin-Heidelberg, New York. p. 69-147.

Bartsch, H.D., Hubermehl, K.O. and Diefenthal, W. 1967. Chromosomal damage after infection with poliomyelities virus. Exp. Cell. Res. 48:671-675.

Bartsch, H.D. 1970. Virus induced chromosomal alterations in mammals and man. Chemical mutagenesis in mammals and man. Ed. F. Vogel and G. Rohrborn, Springer-Verlag, New York. Art. 26:420-432.

Bateman, A.Y. 1958. Mutagenic sensitivity of maturity germ cells in the male mouse. Heredity. 12:213-232.

Bateman, A.Y. and Epstein, S.S. 1971. Dominant lethal mutations in mammals. Chemical Mutagens : Principles and Methods for their detection. Vol. 2 Edl. By A. Hollaender. Plenum Press, New York. pp. 541-568.

Bauch, R. 1947. Tryptoflavin als typhus der chromosomen gifts. Naturwiss. 34:356-357.

Beck, F. and Lloyd, Y.B. 1966. The teratogenic effects of Azo dyes. In : Advances in Teratology. Edt. D.H.M. Woollam. Logos Press, Academic Press. 1:131-195.

Bender, M.A., Griggs, H.G. and Badford, J.S. 1974. Mechanism of chromosomal aberration production. III Chemical and ionizing radiation. Mutat. Res. 23:197-212.

Bennett, D. 1975. The T-locus of the mouse. Cell. 6:441-454.

Biesele, J.J. 1958. Mitotic poisons and the cancer problem. Elsevier. Publ. Co.

Bloom, Stephen, E. 1982. Detection of sister chromatid exchanges in vivo using avian embryos. In Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld, Osmun Publishers, USA pp.137-160.

Boiron, M., Tanzer, Y., Thomas, M. and Hampe, A. 1966. Early diffuse chromosome alterations in monkey kidney cells infected in vitro with herpes simplex virus. Nature, 209:737-738.

Bone, J.G., Bone, A., Moorhead, P.S. and Plotkin, S.A. 1964. Alternations chromosomiques induited par le virus de la rubeola dans les cullules embryonuaires diploides humanaines cultivees in vitro. C.R. Acad. Sci. Paris. 259:687.

Bone, J.G., Bone, A., Montagnier, L., and Vigier, P. 1968. Evolution chromosomique d'um clone de cellules humaines transformees par le virus du sarcoma de Rous. C.R. Acad. Sci. (Paris), Ser. D. 266:178-181.

*Brachet, J. 1954. Experientia. 10:492.

Brathwaite, C.W.D. and Cunningham, H.G.A. 1982. Inhibition of selerotium relfsii by Pseudomonas aeruginosa. Can. J. Bot. 60(3):237-238.

Brewen, J.S., Payne, H.S. and Preston, R.J. 1976. X-ray induced chromosome aberrations in mouse dictyate oocytes. I. Time and dose relationships. Mutat. Res. 35:111-120.

Brewen, J.G. and Preston, R.J. 1982. Cytogenetic analysis of mammalian oocytes in mutagenecity studies. In : Cytogenetic Assay of Environmental Mutagens. Edt. T.C. Hsu. Allanheld, Osmun. USA. pp. 277-287.

Briggs, J.C. and Wilson, J.G. 1959. Comparison of the teratogenic effects of trypan blue and low temperature in the medaka fish Oryzias latipes. Q. Jl. Fla. Acad. Sci. 22:54-68.

Brown, G.C. 1966. Recent advances in the viral actrology of congenital anomalies. In : Advances in Teratology. Edt. D.H.M. Woollam. Logos Press. Academic Press. 1:55-81.

Brown, M.H., Szezech, G.M. and Purmalis, B.P. 1976. Teratogenic and toxic effects of Ochratoxin - A in rats. Toxicol. Appl. Pharmacol. 37(2):331-338.

Brown, P., Gregoriou, M. and Tata, R. 1979. Relationship between mutant amidases of Pseudomonas aeruginosa and hydroxy urea as an inhibitor. Mol. Gen. Genet. 165(2):213-220.

Buckton, K.E. and Evans, H.J. 1982. Human peripheral blood lymphocyte culture : An in vitro assay for the cytogenetic effects of environmental mutagens. In cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu , Allanheld, Osmum Publisher, USA. pp. 183-202.

Buening, M.K., Fortner, J.G., Kappas, A. and Conney, A.E. 1978. 78-Benzoflavone, stimulates the metabolic activation of aflatoxin B_1 to mutagens by human liver. Biochem. Biophyes. Res. Commun. 82(1):348-355.

Canteli, K., Saksela, E. and Aula, P. 1966. Virological studies on chromosome damage of HeLa cells induced by myxoviruses. Ann. Med. Exp. Fenn. 44:255-259.

Catcheside, D.G. 1948. Genetic effects of radiations. Adv. Genet. 2:271-358.

Cattanach, B.M. 1959. The sensitivity of the mouse testis to the mutagenic action of triethylenemelamine. Z. Vererbungsl. 90:1-6.

Cattanach, B.M. 1982. The heritable translocation test in mice. In. Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld. Osmun Publisher. USA. pp. 289-323.

Cattanach, B.M., Pollard, C.I.E. and Isaacson, J.H. 1968. Ethyl methanenesulfonate induced chromosome breakage in the mouse. Mutation Res. 5:297-307.

Cattanach, B.M. 1971. Specific locus mutations in mice. In : Chemical mutagens : Principles and methods for their detection. Ed. A. Hollaender, Plenum Press, New York. 2:535-539.

Chaganti, R.S.K. 1985. Cytogenetic basis of human cancer. J. Genet. 64(1):

Chakrabarti, S. 1974. Effect of Stephylococcus aureus on the dividing bone marrow cells of Mus musculus. Ph.D. Thesis, University of Kalyani.

Chakraborti, S. and Manna, G.K. 1984. Effect of spores of Aspergillus flavus on bone marrow chromosomes of mice, Mus musculus. Section of Zoology. Entomology and Fishery. Proc. 71st Session of Ind. Sci. Cong. Ranchi. Part 3.

Chakraborti, S. and Manna, G.K. 1985. Aspergillus flavus spore induced chromosome aberrations in mice. Section of Botany. Proc. 72nd Ind. Sci. Cong. Lucknow.Part. 3.

Chang, T.H., Moorhead, P.S., Boul, J.G., Plotkin, S.A. and Hoskins, J.M. 1966. Chromosome studies of human cells infected in vitro and in vivo with rubella virus. Proc. Soc. Exp. Biol. (N.Y.). 122:236-243.

Chu, E.H.Y. 1970. Point Mutations in Mammalian cell cultures as measures for Mutagenicity testing. In Chemical Mutagenesis in Mammals and Man. Edt. F. Vogel and G. Rohrborn. Springer-Verleg. Berlin. Heidelberg. New York. pp: 247-250.

Chumakov, M.P., Mustafina, A.N., Chumakova, Ya., Karmysheva, N.M. and Shestopalova-Reinfold, V.N. 1964. Cultivation of simian virus SV-40 in continuous human diploid cells. Acta. Virol. 8:217.

Cohen, M.M. and Shaw, M.W. 1965. Specific effects of viruses and antimetabolites on mammalian chromosomes. In : The chromosome. Ed. G. Verganian. Boston. Publ. of Tissue Culture Assoc. Inc. pp. 50-66.

Corston, J. Mc D. and Mirchandani, G. 1964. Investigation of virus infection in pregnancy. Amer. of Obstet. Gynec. 88:291-297.

Crippa, M. 1964. The mouse karyotype in somatic cells cultured in vitro chromosoma. 15:301-311.

Cury, M. and Montenogro. 1969. Minimal teratogenic doses of actinomycin-D and mitomycin-C on chick embryo. Rev. Med. Chila. 97(4):233-237.

D'Amato, F. 1954. Consideraziani sulla inhibizione *selettiva dei vari stadi della mitosi.* Giom. Ital. Chemoterap. 1129-1134.

Darlington, C.D. 1953. Physical and chemical breakage of chromosomes. Pubbl. Satz. Zool. Napoli. 22:22-31.

Das, S. Kr. 1976. Effect of pathogenic bacteria and their toxins and vaccine on bone marrow chromosome of mice. Ph.D. Thesis, K.U.

Datta, P.K., Frigger, H. and Schleiermacher, E. 1970. The effect of chemical mutagens on the mitotic chromosomes of mouse, in vivo. Chemical mutagenesis in mammals and man. Edt. Vogel and G. Rohrborn Springer-Verlag. Berlin-Heidelberg. New York. 194-213.

Dennis, C., Curle, M., Ray, M. and Persaud, T.V.N. 1983. Methylmercury toxicity : in vivo evaluation of teratogenesis and cytogenetic changes. Anat. Anz. Jena. 153:169-182.

Desmond, Murdina, N., Wilson, Geraldine, S. Verniaud, Willie, M., Melnick, Joseph, L., and Rawls, William, E. 1970. The early growth and development of infants with congenital rubella. In : Advances in Teratology. Ed. D.H.M. Woollam. A Logos Press Book. pp. 39-63.

Dewey, W.C. and Humphrey, R.M. 1962. Relative radio-sensitivity on different phases in the life cycle of L-P 59 mouse fibroblasts and ascite tumor cells. Radiol. Res. 16:503-530.

Dey, M. and Manna, G.K. 1978. Effect of the bacteria Pseudomonas aeruginosa on the chromosomes of adults mice and their progeny. 3rd All India. Cong. Cytol. and Genet. Hissar. S, 8-1:108-109.

Dey, M. and Manna, G.K. 1981. Effects of the bacteri Pseudomonas aeruginosa on the chromosomes of adult mice and their progeny. Proc. 3rd All Ind. Cong. Cytol. and Genet. Hissar, 1978. In : Perspectives of Cytology and Genetics, Ed. G.K. Manna and U. Sinha. 3:374-378.

Dey, M. 1981. Effect of the bacterium, Pseudomonas aeruginosa on chromosomes of treated adult parent and progeny of mice. Ph.D. Thesis. University of Kalyani, Kalyani.

Diaz, M. and Pavan, C. 1965. Changes in chromosomes induced by microorganisms infection. Proc. Nat. Acad. Sci. 54:1321-1327.

Dolimpo, D.A., Jacobson, C. and Legator, M. 1968. Effect of aflatoxin on human leukocyte. Proc. Soc. Exp. Biol. (N.Y.). 127:559.

Dulbecco, R. 1965. Interaction of viruses with the genetic material of the host cells. Reproduction : Molecular, subcellular and celluar. Edt. Licke M. Acad. Press, N.Y.

Dustin. P. Jr. 1952. Reu belge pathol. et. med. Exptl. 22:55.

Egbunike, G.N. 1979. The effect of microdoses of aflatoxin B, on sperm production rats, epididymal sperm abnormality and fertility in the rat Zentralbl vete = Rinaermed Reihea. 26(1):66-72.

Egisti, O.J. and Duotin, P. Jr. 1955. Colchicine in Agriculture, Medicine, Biological Chemistry, Amer. Iowa, State College Press.

Ehling, U.H. 1970. Evaluation of Presumed Dominant Skeletal Mutations. In Chemical Mutagenesis in Mammals and Man. Edt. F.Vogel and G. Rohrborn. Springer-Verlag. Berlin. Heidelberg. New York. 162-166.

El-Alfi, O.S., Smith, P.M. and Biesele, J.J. 1965. Chromosomal breaks in human leukocyte cultures induced by an agent in the plasma of infections hepatitis patients. Hereditas (Lund). 52:285-294.

Epstein, S.S. and Shafner, H. 1968. Chemical mutagens in the human environment. Nature. 219:385.

Evans, H.J. 1962. Chromosome aberrations induced by ionizing radiation. Int. Rev. Cytol. 13:221.

Evans, H.J. 1976. Cytological methods for detecting chemical mutagens. In : Hollaender (Ed.) Chemical Mutagens. Principles and methods for their detection plenum. Press. N.Y. 4:1-29.

Eykyn, S.J. 1985. Major common problems treatment of Pseudomonas aeruginosa infection. Journal of Applied Medicine. 11:157-161.

Fahmy, M.J. and Fahmy, D.H.S. 1978. Aflatoxin B_1 2, 3, - Dichloride as a model of the active metabolite of aflatoxin B_1 in mutagenesis and carcinogenesis. Intern. Cong. of Genet. Contribution paper. Session. Abstr. Part II. Sect. 21-32, Moscow.

Farook, S A F and Farook, T T 1985 Centromere separation and C-heterochromatin National Seminar On Current Trends in Chromosome Dynamics pp. 12-13

Fishbein, L., Flamm, W.G. and Falk, H.L. 1970. Chemical mutagens - Environmental effects on biological systems. Academic Press, New York and London. pp. 25-26.

Fogh, J. and Fogh, H. 1967. Irreversibility of major chromosome change in mycoplasma modified line of human amnion cells. Proc. Soc. Exp. Biol. (N.Y.). 126:67-74.

Freese, E. 1963. Molecular mechanisms of mutations. In J.H. Tayler molecular genetics, Part I. p. 207-269. New York and London, Academic Press.

Fujimoto, T., Fuyuta, M., Kiyofuji, E. and Hirata, S. 1979. Prevention by Tropronin (2-Mercaptopropionyl glycine) of Methylmercuric chloride induced teratogenic and fetotoxic effects in mice. Teratology, Philadelpha. 20:297-302.

Fuykta, M., Fuyimoto, T. and Kiyofuji, E. 1979. Teratogenic effects of a single oral administration of Methylmercuric chloride in mice. Acta anat. Basel. 164:356-362.

Gallagher, R.T. and Wilson, J.B. 1979. Aflatrem, the tremorgenic mycotoxin from Aspergillus flavus. Mycopathologia. 66(3):183-186.

Gaulden, Mary Esther and Liang, Jan. C. 1982. Insect cells of testing clastogenic Agents. In: Cytogenetic Assays of Environmental Mutagens. Ed. T.C. Hsu. Oxford and IBH Publishing Co. pp. 107-136.

Gavrilov, V.I. , Yershov, F.I., Blyumkin, V.N., Kvokov, I.I., Levina, D.S. and Zmiyeva, R.G. 1965. Peculiarities of morphogenesis of the CA-SV 40-63-1 cell line. Vop. Virus 10:223.

Gaydos, J., Michael, Lee, Carrick, Jr. and Richards, Berk (1976). Experimental studies on mice challenged subcutaneous with Pseudomonas aeruginosa. Proc. Soc. Exp. Biol. Med. 149(4):908-914.

Gebhart, E. 1970. The treatment of human chromosomes in vitro : Result. In : Chemical mutagenesis in mammals and man. Ed. F. Vogel. and G. Rohrborn, Springer-Verlag. Berlin - Heidelburg. N.Y. pp. 367-382.

Genereso, W.M., Russell, W.L., Huff, S.W., Stout, S.K. and Gosslee, D.G. 1974. Effects of dose on the induction of dominant lethal mutation and heritable translocations with EMS in male mice. Genetics. 77:741-752.

Ghosh, T.K. 1985. Mutagenic Effect of the Bacterium, Staphylococcus aureus tested in Experimental mice. Ph.D. Thesis, Kalyani University.

Ghosh, T.K. and Manna, G.K. 1984. Clastogenic potentiality of bacterium, Staphylococcus aureus on the male germinal chromosomes of albino mice. In : Perspectives in Cytology and Genetics. Ed. G.K. Manna and U. Sinha. Vol. 5 (In press).

Giles, N.H. 1954. Radiation induced chromosome aberrations in Tradescantia, in "Radiation Biology". Ed. A. Hollaender. Vol. (Part 2). McGraw Hill Books. Inc. New York. p. 713-761.

Gilliavod, N. and Leonard, A. 1974. Dose relationship for translocations induced by X-irradiation in mouse oocytes. Mutat. Res. 25:425-426.

Goodspeed, T.H. and Olson, A.R. 1928. The production of variation in Nicotiana species by X-ray treatment of sex cells. Proc. Nat. Acad. Sci. U.S. 14:66-69.

Goswami, S. 1985. Cytogenetic assay of the effect of non-pathogenic bacteria Sarcina lutea in adult, parent and progeny of mice. Ph.D. Thesis, K.U.

Goswami, S. and Manna, G.K. 1984. Micronuclei test (MNT) for detecting mutagenic property of nonpathogenic bacterium, Sarcina lutea in treated mice. In : Perspectives in Cytology and Genetics. Ed. G.K. Manna, and U. Sinha. Vol. 5 (In Press).

Goze, A., Sarasin, A., Moule, Y. and Devoret, R. 1975. Induction and mutagenesis of phosphase in Escherichia coli K_{12} by metabolites of aflatoxin B_1. Mutat. Res. 21(1):1-8.

Green, S., Legator, M. and Jacobson, C. 1967. Utilization of a cell line derived from rat kangaroo for cytogenetic studies. Mammalian Chro. News. Lett. 8(1):36.

Greenberg, M.P., Pelliteri and Barton, J. 1957. Frequency of defects in infants whose mothers had rubella during pregnancy. J. Amer. Med. Ass. 165:675-678.

Grover, S., Solanki, B.K., Khandelwal, M.K., and Jaiswal, R.B. 1968. Chromosomal abrormalities in measles. Indian Pedial. 5:326-329.

Gruneberg, H. 1954. Variation within inbred strains of mice. Nature (Lond.): 173:674-676.

Gupta, S. 1976. Effect of four pathogenic bacteria on the chromosomes and mitotic index of mice and their alterations by antibiotics. Ph.D. Thesis, University of Kalyani, Kalyani.

Gupta, P.K. 1985a. Genetic control of chromosome pairing in Hordeum polyploids. Nat. Seminer on chromosome Dynamics, Panjab University. 21-23 Nov. Abs. pp.3.

Gupta, P.K. 1985b. The genetic system in male affecting chromosome pairing in intergeneric hybrids. Nat. Seminer on chromosome dynamics. Panjab University. Nov. 21-23. Abs. pp. 3.

Halkka, O. 1967. Chromosome breakage associated with infections. Hereditas (Lund.). 58:248-252.

Halkka, O. and Heinonen, L. 1968. Chromosome breakages associated with infection. II Stained Sections. Hereditas. 58:253-261.

Halkka, O., Meynadier, G., Vago, C. and Brummer-Korvenkontio. 1970. Rickettsial induction of chromosome aberrations. Hereditas (Lund.). 64:126-223.

Hamper, B. and Ellison, S.A. 1961. Chromosomal aberrations induced by an animal virus. Nature (Lond.). 192:145-147.

Hampar, B. and Ellison, S.A. 1963. Cellular alterations in the MCH line of Chinese hamster cells following injection with herpes simplex virus. Proc. Nat. Acad. Sci. 49:474-480.

Harnshaw, James B. 1970. Developmental abnormalities associated with congenital cytomegalovirus infection. In : Advances in Teratology. Edt. D.H.M. Woollam. Logos Press. 4:64-93.

Harnden, D.G. 1964. Cytogenetic studies on patients with virus infections and subjects vaccinated against yellow fever. Amer. J. Human. Genet. 16:204-213.

Harris, S.B., Wilson, J.G. and Printz. 1972. Embryotoxicity of Methylmercuric chloride in Golden hamster. Teratology. Philadelphia. 6:139-142.

Hazra, M.K. 1980. The bacterium, Staphylococcus induced mammalian chromosome aberration and their alteration by antibiotic, Penicillin. Ph.D. Thesis. University of Kalyani.

Hazra, M.K. and Manna, G.K. 1980. Bacterial culture injected chromosome aberrations in the Syrian hamster. Sci. and Culture. 46(3):144-145.

Hazra, M.K. and Manna, G.K. 1981. Bone marrow chromosome aberrations in four mammalian species injected with Staphylococcus aureus. Proc. 3rd All Ind. Cong. Cytol. Genet., Hissar. 1978. In : Perspectives in Cytol. Genet. Ed. G.K. Manna and U. Sinha. 3:417-420.

Heddle, J.A. 1973. A rapid in vivo test for chromosome damage. Mutat. Res. 18:187-190.

Heddle, J.A. 1981. Micronuclei in vivo and in vitro as short term test. Proc. IIIrd Intern. Conf. Environ. Mutagens. Japan (Abs.). pp. 28.

*Henrici, A.T. 1939. J. Immunol. 36:319.

Hertwig, P. 1935. Sterilitatserscheinungen bei rontgenbestrahlten Mausen. Z. Indukt. Abstamm. U. Verebsl. 70:517-523.

Hicks, Samuel, P. and D'Amato, Constance, J. 1966. Effects of ionizing radiations on mammalian development. Advances in Teratology. Edt. Woollam, D.H.M. 1:195-250.

*Hill, A.B., Doll, R., Galloway, T.M. and Hughes, J.P.W. 1958. Virus diseases in pregnancy and congenital defects. Brit. J. Prev. Soc. Med. 12:1-7.

Hillman, N. and Nadijcka, M. 1978a. A study of spermatozoan defects in wild-type and T;t-bearing mice. J. Embryol. Exp. Morphol. 44:243-262.

Hillman, N. and Nadijcka, M. 1978b. A comparative study of spermiogenesis of wild type and T/t bearing mice. J. Embryol. Exp. Morphol. 44:253-280.

Hittelman, Walter N. 1982. Premature Chromosome condensation for the detection of mutagenic activity. In cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu, Allanheld, Osmun Publishers, USA. pp. 353-384.

Hollaender, A. 1954. Edt. "Radiation Biology". McGraw Hill, New York.

Hollaender, A. 1971a. Edt. Chemical Mutagens : Principles and Methods for their detection. Vol. 1. Plenum, New York.

Hollaender, A. 1971b. Edt. Chemical Mutagens. Principles and Methods for their detection. Vol. 2. Plenum, New York.

Hollaender, A. 1973. Edt. chemical mutagens. : Principles and Methods for their detection. Vol. 3. Plenum, New York.

Hollaender, A. 1976. Edt. Chemical Mutagens : Principles and Methods for their detection. Vol. 4. Plenum, New York.

Hollaender, A. 1978. Edt. Chemical Mutagens : Principles and methods for their detection. Vol. 5. Plenum, New York.

Hollaender, A. and de Serres, F.J. 1978. Edt. chemical mutagens : Principles and Methods for their detection. Vol. 6. Plenum, New York.

*Holowach, J., Thurston, D. L. and Becker, B. 1957. Congenital defects in infants following mumps during pregnancy; a review of the literature and a report of chorioretinitis due to fetal infection. J. Pediat. 50:689-694.

Holowiecka, D. and Donuata, R. 1969. Studies on the cytotoxic effect of Corynobacterium diphtheriae on fetal guineapig cell cultures. Arch. Immunol. Ther. Expl. 17(6):72.

Homma, M., Ohira, M. and Ishida, N. 1968. Specific chromosome aberrations in cells persistantly infected with type -2 hemedsorption virus. Virology. 34:60-68.

Hooftman, Rai, N. and De Raat, W.K. 1982. Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudiminnow umbra pygmaea by ethyl methanesulphonate. Mutat. Res. 104:147-152.

*Houson, F. and Edle, T. 1954. Patent USA 2652356. Chem. Abstr. 48:14.

Hsu, T.C. 1982. Cytogenetic Assays of Environmental Mutagens Allanheld. Osmum. Publishers.

Huang, C.C. 1967. Induction of high incidence of damage to the X-chromosomes of Rattus (Mastomys) natatensis by base analogues, viruses and carcinogens. Chromosoma (Berl.). 23:162-179.

Huang, C.C. 1971. Effect on chromosomes of human haemopoitic cell line by injection with herpes type virus, herpes simplex virus and cytomegatovirus. Mamm, Chrom. Newsletter. 12(1): 7.

Hugenholtz, A.P. and Bruce, W.R. 1977. Transmission of radiation induced elevations in abnormally shaped murine sperm. In : Proc. 8th Ann. Meet. Environ. Mutagen. Soc. Denever. Colorado (Abst.). pp. 75.

Hugenholtz, A.P. and Bruce, W.R. 1977b. "Ash-1" a mutation in murine sperm head shape. In : Proc. 4th NICHD testis workshop, Toronto, Canada, May. 25-27 (abs.).

Hung, C.C. 1967. Induction of high incidence of damage to the X-chromosomes of Rattus (Mastomys) patalensis by base analogues, viruses and carcinogens. Chromosoma (Berl). 23:162-179.

Hunt, D.M. and Johnson, D.R. 1971. Abnormal spermatogenesis in two pink eyed steriles mutation in the mouse. J. Embryol. Exp. Morph. 26:111-121.

*Inouye, M. and Murakami, U. 1975. Teratogenic effect of orally administered Methylmercuric chloride in rats and mice. Cong. Anomalies. Kyoto. 15:1-9.

Jacobs, M.B. and Gerstein, M.J. 1960. Handbook of Microbiology van Nostrand Reinhold comp. Litton Educational Publishing Inc. New York, Cincinnati, Toronto, London, Melbourne.

Jacobsen, Lars. 1970. Radiation induced foetal damage : A quantitative analysis of seasonal influence and possible threshold effect following low dose X-irradiation. In : Advances in Teratology. Edt. D.H.M. Woollam. Logos Press. 4:95-124.

Johnson, D.R. and Hunt, D.M. 1971. Hop-sterile, a mutant gene affecting sperm tail development in the mouse. J. Embryol. Exp. Morph. 25:223-236.

Joklik, W.K. and Wilbett, H.P. 1976. Zinsser Microbiology. Appleton-Century - crofts/New York. Prentice/Hall. International. INC. Englewood Cliffs, pp. 1-1165.

Kato, R. 1967. Localization of spontaneous and RSV-induced breakage in specific regions of the chromosomes of the Chinese hamster. Hereditas (Lund.). 58:220-247.

Kato, H. and Sandberg, A.A. 1968a. Chromosome pulverisation in Chinese hamster cells induced by sendi virus. J. Nat. Cancer. Inst. 41:1117-1123.

Kato, H. and Sandberg, A.A. 1968b. Cellular phase of chromosome pulverisation induced by sendai virus. J. Nat. Cancer. Inst. 41:1125-1131.

Keast, D. 1966. Karyotype studies on lymphoma (2731/L) resulting from Reo-virus type-3 infection of neonatal mice. Exp. Cell. Res. 44:295-302.

Kerkis, Ju. J.A., Sablina, O.V., Radzable, S.I. and Bocarov, E.F. 1967. An investigation of chromosomal abnormalities in peripheral blood leukocytes of patients affected by acute viral hepatitis. Genetica (Lenengrads). 5:85-94.

Kihlman, B.A. 1966. "Actidn ofchemicals on dividing cells". Prentice-Hall Inc. Englewood. Cliffs. New Jersey.

Kihlman, B.A. 1982. Root tips of Vicia faba as a material for studying the induction of chromosomal aberrations and sister chromatid exchanges. In Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld Osmun. Publishers. USA. pp. 81-106.

Kindsin, R.B. 1976. Chromosomal aberration induced by T. strain mycoplasma. J. Med. Genet. 8(2):181-187.

Klamerth, O.L. 1970. Biochemical analysis of genetically different and altered DNA methods and limitations. Chemical Mutagenesis in Mammals and Man. Edt. F. Vogel and G. Rohrborn. Springer-Verleg. Berlin. Heidelberg. New York. 279-284.

Kligerman, A.D. 1982. The use of cytogenetics to study genotoxic agents in fishes. In Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld. Osmun. Publisher, USA, pp. 161-182.

Klimova, N.E. 1972. Characteristics of toxic and teratogenic effects of Olivomycin on growing chick embryo. Ant. Biotiki. 17(7):587-593.

Kortee, A. 1980. Chromosomal analysis in bone marrow cells of Chinese hämster after treatment with mycotoxins. Mutat. Res. 78(1):41-50.

Korte, A. Renate, S.E. and Guenter, O. 1979. The influence of ethanol treatment on cytogenetic effect in bone marrow cells of Chinese hamsters by cyclophosphamide, aflatoxin, B_1 and patulin. Toxicology. 12(1):53-62.

Krzanowska, H. 1972. Influence of Y-chromosome of fertility in mice. In : The genetics of spermatozoan. Ed. R.A. Beatty and S. Gluecksohn. Waelsch. Proc. Int. Symp. Edinburgh. pp. 370-386.

Kuhlmann, W. 1970. Cytology and timing of Meiotic stages in female Germ cells of Mammals and Man. In Chemical Mutagenesis in Mammals and Man. Edt. Vogel, F. and Rohrborn, F. Springer-Verlag. Berlin. Heidelberg. New York. pp. 180-193.

Kundu, B. 1983. Bone marrow chromosome aberration in mice induced by the spores of the Fungus, Aspergillus niger. Section - Zoology, Entomology and Fisheries. 70th Session of Ind. Sci. Cong. Tirupati. pp.

Kundu, B. and Manna, G.K. 1984a. A further study in the induction of bone marrow chromosome aberration in mice by the spores of the fungus. Aspergillus niger. Proc. 71st Ind. Sci. Cong. Ranchi. Part 3. Sec-9. Page 11-12.

Kundu, B. and Manna, G.K. 1984b. Spores of a fungus, Aspergillus niger as clastogen in chromosomes of treated mice. Proc. 5th All India Congr. Cytol. and Genet. Bhubaneswar. In Perspectives in Cytology and Genetics. Ed. G.K. Manna and U. Sinha. Vol. 5 (In press).

Kundu, B. and Manna, G.K. 1985. A further evidence of clastogenic potentialities of the spores of Aspergillus niger, by micronuclei test in treated mice. Sec. 6. Botany. Proc. 72nd Ind. Sci. Cong. Lucknow. pp. 141.

Kuroki, Y., Makino, S., Aya, T. and Nagayama, T. 1966. Chromosome abnormalities in cultured leucocytes from rubella patients. Jap. J. Hum. Genet. 11:17-27.

Kuroki, T., Malaveilla, C., Drevon, C., Piccole, C., Macleod, M. and Selkirk, J.K. 1970. Critical importance of microsome concentration in mutagenesis assay with V79 Chinese hamster cells. Mutat. Res. 63(2):259-272.

Kuroki, T., Malaveille, C., Drevon, C., Piccole, C., Macleod, M. and Selkirk, J.K. 1979. Mutat. Res. 63(2): 259-279.

Lasßikka, L., Virsu, M.L., Halkka, O., Errikesson, K. and Estola, T. 1976. Gonimitosis in rats affected by mycoplasma or macrobiols. Met. Biol. 54:146-149.

Latt, S.A., Schreck, R.R., Loveyoy, K.S. and Shuler, C.F. 1982. Sister chromatid exchange analysis : Methodology, Applications and interpretation. In Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld, Osmun. Publishers. USA pp. 29-80.

Lea, D.E. 1962. Actions of radiations on living cells. Second ed. Cambridge University Press, Lond. New York.

Leblond, C.P. and Clermont, Y. 1952. Definition of the stages of the cycle of the seminiferous epithelium in rat. Ann. N.Y. Acad. Sci. 55:548-573.

Lee, H.Y., Yorge, L.C. and Martin, A.L. 1972. Teratogenic effects of Phleomycin in early chick embry. Teratology. 6(2):201-205.

Lehman, J.M. and Defendi, V. 1970. Early chromosome damage in Chinese hamster cells by SV-40 virus. Mamma. Chrom. Newsletter. 11:69.

Leonard, A., Deknudt, G., Linden, G. 1975. Mutagenicity tests with aflatoxins in the mouse. Mutat. Res. 28:137-139.

*Levine, et al. 1968. Altered nucleic acid metabolism in human cell cultures infected with mycoplasma. Proc. Nat. Acad. Sci. 60:583-589.

Libikova, H. and Motajova, J. 1967. Persistent infection of human diploid cell strain with tick-borne encephalitis virus. Acta Virol. 11:380.

Lilly, L.Y. 1965. In : Induction of chromosome aberrations by aflatoxin. Nature (Lond.). 207:433.

Macpherson, I. 1966. Mycoplasma in tissue culture. J. Cell. Sci. I:145-168.

Manna, G.K. 1969. Some aspects of chromosome cytology. Presidential address, Section of Zoology. Proc. 56th Ind. Sci. Congr. 2:185-214.

Manna, G.K. 1971. Bone marrow chromosome aberrations in mice induced by Physical, chemicals and living mutagens. Symp. On Chromosome aberration. Proc. 1st All India. Cong. Cytol. and Genet. Chandigarh. Jour. Cytol. and Genetics, Congress. Vol. 144-150.

Manna, G.K. 1973. Chromosome aberrations induced by living mutagens. Presidential address. Biol. Sci. 43rd Ann. Sess. Nat. Acad. Sciences, India. 1-15.

Manna, G.K. 1975. Induced breakages in chromosome-mechanical and/or chemical. Symp. on structural and functional aspects of chromosomes. BARC, March. 25-26, 194-204.

Manna, G.K. 1980a. The living mutagen. Proc. Nat. Acad. Sci., India. In : Golden Jubilee Commomeration Volume. Nat. Acad. Sci. Allahabad. Edt. Srivastava, U.S. Naya Prakash Publishers. Calcutta 573-608.

Manna, G.K. 1980b. Antibiotics in different roles in the induced chromosome aberrations. Intern. Seminer on Management of Environment, BARC, Bombay. Ed. B. Patel. pp. 145-164.

Manna, G.K. 1981a. Non-random aberrations and their implications on the probable existence of weaker regions in chromosome. Proc. 3rd All India Cong. Cytol. Genet. , Hissar, 1978. In : Perspectives in Cytology and Genetics, Vol. 3. Ed. G.K. Manna and U. Sinha. Hindasia Publishers, New Delhi, pp. 131-135.

Manna, G.K. 1981b. Cytogenetic effects of antibiotics on man and mammals. Indian Review of life sciences. 1:189-223 (Eds. D.N. Sen and P.K. Sen).

Manna, G.K. 1982. The new horizon of mutagenesis. Curr. Sci. 51(23):1087-1093.

Manna, G.K. 1984. Mouse bone marrow as a means of testing clastogenic agents. Cancer genetics and cytogenetics. Ed. Avery Sandberg, New York, USA (In Press).

Manna, G.K. 1984. On the possible involvement of genes in the induction of chromosome aberrations. Proc. 4th All Ind. Cong. Cytol. Genet., Bhagalpur, 1981. In : Perspectives in Cytol. Genet. Ed. G.K. Manna and U. Sinha. 4:25-32.

Manna, G.K. 1985. The living mutagens. In instructional workshop on "Perspectives in Chromosomal Research", Calcutta University, Calcutta, December 10-24. pp. 55-72

Manna, G.K., Banerjee, G. and Gupta, S. 1985. Micronuclei test of different genotoxic agents in the peripheral erythrocytes of the exotic fish, Sarotherodon mossambica. Nucleus (In Press).

Manna, G.K. and Bardhan, S. 1973a. Effect of two antibiotics on the chromosomes and mitotic frequency in the bone marrow cells of mice. Jerusalem Chromosome Conference. Sept. 1972. Chromosome To-day Ed. K.R. Lewis and J. Wahrman. 4:277-282.

Manna, G.K. and Bardhan, S. 1973b. Penicillin induced bone marrow chromosome aberrations in mice. Ind. Jour. Zool. 1:1-11.

Manna, G.K. and Bhowmick, S. 1969. Induction of chromosomal aberrations in mice, Mus musculus by the bacterium Staphylococcus aureus. Proc. 56th Ind. Sci. Cong. Pt. 23:454.

Manna, G.K. and Biswas, S. 1986. Micronucleus test in four freshwater species of fishes injected with the log-culture of bacterium, Pseudomonas aeruginosa. 73rd Session of All Ind. Sci. Congress Association. Section Zoology, Entomology and Fishery, Delhi. pp:137.

Manna, G.K. and Chakrabarti, S. 1970. Report on the study of the chromosome aberration by bacterial culture, filtrate and the bacterium, Staphylococcus aureus. Nucleus.13(2):167-176.

Manna, G.K. and Chakrabarti, S. 1975. Bacteria induced chromosome aberrations in mice and their repair. Symp. on structural functional aspects of chromosomes. BARC March 25-26. 175-193.

Manna, G.K. and Das, R.K. 1972. Chromosome aberrations in mice induced by aluminium chloride. Chrom. inf. Service (Japan). 13:9-11.

Manna, G.K. and Das, R.K. 1973. Induction of chromosome aberrations by a sedative, barbitol in the bone marrow cells of mice. Ind. J. Med. Res. 61(10): 1568-1573.

Manna, G.K. and Das, S.K. 1974. Chromosome aberrations in mice injected with the bacterium, Salmonella typhi, its culture and filtrate. Nucleus. 17(2):71-75.

Manna, G.K. and Dey, M. 1979. Chromosome aberrations in the F_1 and F_2 offspring derived from the parent mice treated with bacterium, Pseudomonas aeruginosa. Proc. 49th Ann. Sess. Nat. Acad. Sci., Nagpur. India. pp. 4.

Manna, G.K. and Ghosh, T.K. 1984. Chromosome aberrations in regenerating liver and bone marrow cells of partially hepatectomised mice treated with Staphylococcal samples and their reduction by penicillin injection. Nucleus.26(3):153-159.

Manna, G.K. and Goswami, S. 1982. Bone marrow chromosome aberration in mice treated with culture of the soil bacterium, Sarcina lutea. Nat. Acad. Sci. Letter 5(6):209-211.

Manna, G.K. and Gupta, S. 1976. Bone marrow chromosome aberrations in mice induced by the log phase culture of the bacterium Neisseria meningitidis and their alternations by the treatment of penicillin. Proc. 63rd Ind. Sci. Cong. 3:150.

Manna, G.K. and Gupta, S. 1977. Streptococcus induced chromosome aberrations and their alterations by penicillin in mice. Nucleus. 21(1):179-185.

Manna, G.K. and Gupta, S. 1979. Meningococcus associated chromosome aberrations with their alteration by penicillin and mitotic inhibition in mice. Nucleus. 22:12-22.

Manna, G.K. and Hazra, M.K. 1981. The bacterium, Styphylococcus aureus induced chromosome aberrations with their protection by penicillin and mitotic inhibition in Syrian hamster. Proc. Ind. Acad. Sci. 90(2):129-143.

Manna, G.K. and Mazumder, S.C. 1967. On the quantitive aspects of X-ray induced sex chromosome aberration in the spermatocyte of grasshoppers. Nucleus. 10(2):128-139.

Manna, G.K., and Mitra, A.B. 1971. Effect of three antibiotics on mitotic index and chromosomes of bone marrow cells in mice. Nucleus. 14(2):161-168.

Manna, G.K. and Pal, J. 1982. Fish pathogenic bacteria induced chromosome aberrations in bone marrow cells of treated mice. Nat. Acad. Sci. Lett. 5(2): 273-275.

Manna, G.K. and Patel, S. 1981. Aspergillus spore induced chromosome aberrations and its effect on the progeny of mice. Nat. Acad. Sci. Lett. 4(11): 455-458.

Manna, G.K.and Patel, S. 1984. Lethality in F_1 and F_2 generation induced from mating of Aspergillus fumigatus spores treated male parent with normal female mice. In perspectives in Cytology and Genetics. Vol. 5. Ed. G.K. Manna and U. Sinha. (In press).

Manna, G.K. and Roy, P.P. 1984. Induction of dominant lethals and chromosome aberrations in the F_1 progeny of the chloramphenicol treated male mice. 4th All India Congress of Cytology and Genetics. Perspectives in Cytology and Genetics. Vol. IV. Ed. G.K. Manna and U. Sinha. pp. 127-134.

Manson, M.M., Logan, W.P.D. and Loy, R.M. 1960. Rubella and other virus infections during pregnancy. Great Britain, Ministry of Health, London : Her Majesty's Stationery Office (No. 101, Reports on Public Health and Medical Subjects).

Manna, G.K. and Sadhukhan, A. 1985. Use of cells of gill and kidney of Tilapia fish in micronucleus test. (MNT), Current Science.(In Press).

Marquez-Monter, H., Higuera Ballesteros, F.J. Alfaro-Kofman, S., Monte Mayor, E.G. and Gonzalez-Angulo, A. 1967. Chromosomes in human rabies. Lancet. 1967:11-102.

Matsaniotis, N., Naounis, F., Kiossoglon, K.A. and Anagnostakis, D.E. 1968. Chromosomes in infections hepatitis. Delt. Paidiat. Klin. Panep. Athin. 15:14-26.

Matsul, S., Weinfeld, H. and Sandberg, A.A. 1972. Dependence of chromosome pulverization in virus-fused cells on events in the G_2 period. J. Nat. Cancer Inst. 47:401-411.

Matter, B. and Schmid, W. 1971. Trenimon-induced chromosomal damage in bone marrow cells of six mammalian species, evaluated by the micronucleus test. Mutat. Res. 12:417-425.

Matzinger, P.K. and Ong, T.M. 1976. Mutation induction of rodent liver microsomal metabolites of aflatoxins B_1 and G_1 in Neurospora crassa. Mutat. Res. 37(1):27-32.

Mazumder, S.C. 1967. Spontaneous and induced chromosome aberrations in grasshoppers. Ph.D. Thesis. University of Kalyani.

Mazzone, H.M. and Yerganina, G. 1963. Gross and chromosomal cytology of virus infected Chinese hamster cells. Exp. Cell. Res. 30:591-592.

Mc Clintock, B. 1951. Chromosome organisation and genetic expression. Cold Spring. Harbor. Symp. Quant. Biol. 16:13-47.

Mc Clintock, B. 1955. Intercellular systems controlling gene action and mutation, Brookhaven Symposium. Biol. 8:58-74.

Mella, B. and Lang, D.J. 1967. Leukocyte mitosis suppression in vitro with acute infectious hepatitis. Science. 155:80-81.

Meyer, J.M. and Hornsperger, J.M. 1978. Role of pyoverdine, the iron-binding fluorescent pigment of Pseudomonas fluorescens, an iron transport. J. Gem. Microbiol. 107:329-331.

Michajlova, G.R. and Gorsunova, L.P. 1969a. Chromosome disorders in bone marrow cells of mice immunized with different antiviral vaccines. Vop. Virus. 14:326-331.

Michajlova, G.R. and Gorsunova, L.P. 1969b. Studies on chromosomes in bone marrow cells of mice immunized with polio vaccine. Genetika (Moskva). 5:48-56.

Michajlova, G.P. and Gorsunova, L.P. 1975. Study of chromosomes in the bone marrow cells of mice inoculated successively with various vaccine. Tsitol. Genet. 9:460-461.

Michajlova, G.P. and Gorsunova, L.P. 1979. Studies on the chromosomes in bone marrow cells of mice immunized with polio-vaccine. Genetika. 14:251-256.

Michelle, M. and Rowley, Janet, D. 1984. Heritable fragile site in Cancer. Nature. 308:607-608.

Miles, C.P. and O'Neill, F.J. 1969. Chromosome abnormalities induced in human cells by herpes virus type-2. Amer. Asso. Cancer Res. 10:230-232.

Misaghi, I.J., Grogan, R.G., Spearman, L.C. and Stowell, L.J. 1981. Antifungal activity of fluorescent pigment produced by fluorescent Pseudomonas. Phytopathology. 71:106.

Mitchell, I.D.E.G. 1978. Microbial assays for mutagenicity. A modified liquid culture method compared with the agar plate system for precision and sensitivity. Mutat. Res. 54(1):1-16.

Moorhead, P.S. and Saksela, E. 1965. The sequence of chromosome aberrations, during SV-40 transformation of human diploid cells strain. Hereditas (Lund.). 52:271-284.

Muller, H.J. 1927. Artificial transmutation of the gene. Science. 66:84-87

Muller, H.J. 1954a. In "Radiation Biology" (A. Hollaender, ed.). 1(1):351. McGraw-Hill, New York.

Muller, H.J. 1954b. In"Radiation Biology". (A. Hollaender, ed.). Vol. 1, Pt. 1, p. 475. McGraw-Hill, New York.

Nachtigal, M., Aderca, I., Matache, A. and Timovici, M. 1966. Chronic infections with RNA viruses in cell cultures III. Chromosomal changes in normal and chronically infected cell cultures. Rev. Roum. Inframicrobiol. 3:43-52.

Nichols, W.W. 1963. Relationship of viruses, chromosomes and carcinogenesis. Hereditas. 50:53-59.

Nichols, W.W. 1966. Chromosome breakage and virus. Heriditas. 55:1-27.

*Nichols, W.W. 1968. Somatic mutation in mammalian cells. Proc. Internat. Cong. Genetics. Tokio.

Nichols, W.W. 1978. Genetic effects of virus transformations. Institute for Medical Research, Camden. N.Y., USA. Proc. 14th Int. Cong. Genet. Plenary Sess. and Symp. Abstract. Moscow. pp. 83.

Nichols, W.W. and Levan, A. 1965. Measles associated chromosome breakage. Arch. Ges. Virusforsch. 16:168-174.

Nichols, W.W., Levan, A., Hall, B. and Ostergren, G. 1962. Measles associated chromosome breakage. Preliminary Communication. Heriditas. 48:367-370.

Nichols, W.W., Levan, A., Kihlman, B.A. 1964. Chromosome breakage associated with viruses and DNA inhibitors. In : Cytogenetics of cells in culture. Ed. R.J.C. Harris. Academic Press, London. N.Y. pp. 255-271.

Nichols, W.W., Levan, A., Aula, P. and Norrby, E. 1965. Chromosome damage associated with the measles virus *in vitro*. Hereditas. 54:101-118.

Oakberg, E.F. 1956. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Amer. J. Anat. 99:391-409.

*Oakberg, E.F. 1957. Duration of spermatogenesis in the mouse. Nature (Lond.). 180:1497.

Oakberg, E.F. 1960. Irradiation damage to animals and its effects on their reproductive capacity. J. Dairy Science. 43:54-67.

Oehlkers, F. 1943. Die anslosung von chromosomen mutation in der Meiosis durch einerirkung von Chemikalien. 2. Induktive Abstammungs. U. Vererbungslehre. 81:313-341.

O' Neill, F.Y. and Rapp, F. 1971. Chromosome abnormalities in human cells inoculated with EB virus. Mamma. Chrm. Newsletter. 12:28.

Oxford, A. and Haistrick, H. 1942. Antebacterial substances from moulds. IV. Spinulosin and fumigatin, metabolic products of Penicillium spinulosum Thom. and Raper Aspergillus fumigatus. Fres. Chem. and Industr. 61:128.

Painter, Robert. B. 1982. DNA Rapair . In : Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld. Osmun Publisher. pp. 11-27.

Pal, J., Pal, B.C. and Banerjee, R. 1978. Epithelial Carcinoma in Anabas testudineus. J. Fish. Biol. 13:693-694.

Pal, J. and Pal, B.C. 1984. Induction of tumours by bacterial culture, Perspectives in Cytology and Genetics. Vol. V. Edt. G.K. Manna and U. Sinha (In Press).

Parida, B.B., Stich, W. Stich, H.F. and Kato, K. 1984. Some short-term screening tests for carcinogens in rats and man. Proc. 5th All India Cong. Cytol. Genet. Bhubaneswar, 8-10 Oct., 1984. In Perspectives in Cytol. and Genet. Vol. 5. Eds. G.K. Manna and U. Sinha (In press).

Park, J.E. and Park, K. 1977. In : Text book of preventive and social medicine. Sixth edt. Banarsidas Bhanot. Publ., Jabalpur, India.

Patel, S. 1982. Effects of the Fugus Aspergillus on chromosomes of the parent and progeny of mice. Ph.D. Thesis. Kalyani University.

Patel, S. and Manna, G.K. 1982. Effect of pathogenic fungus on the bone marrow chromosomes of mice. Proc. 69th Ind. Sci. Cong. 3:9.

Patel, S. and Manna, G.K. 1984. Bone marrow chromosome aberrations in mice injected with Aspergillus spores. Proc. 4th All Ind. Cong. Cytol. Genet. Bhagalpur, 1981. In : Perspectives of Cytology and Genetics. Ed. G.K. Manna and U. Sinha. 4:151-160.

Paton, A.M. 1960. The role of Pseudomonas in plant disease. Journal of Applied Bacteriology. 23:526-532.

Paton, G.R., Jocobs, J.P. and Parkins, F.T. 1965. Chromosome changes in human diploid calls cultures infected with mycoplasma. Nature. 207:43-45.

Pavan, C. and Basile, R. 1965. Chromosome changes induced by infections in tissue Rhypchosciara angelae. Science. 151:1556-1558.

Payne, H.S. and Jones, K.P. 1975. Technique for mass isolation and culture of mouse ova for cytogenetic analysis of the first cleavage mitosis. Mutat. Res. 33:247-249.

Ponten, J. and Lithner, F. 1966. Absence of specific chromosomal alternations in bovine lung fibroblasts exposed to RSV. Int. J. Cancer. 1:589-598.

Postgate, J.R. 1982. The fundamentals of nitrogen fixation, Cambridge University Press, Cambridige, London, New York.

*Potter, E.L. 1961. The effect on the fetus of viral disease in the mother. Clin. Obstet Gynee. 4:327-340.

Preston, R.J. and Brewen, J.G. 1982. The use of the host-Mediated Assay for Cytogenetic. In : Cytogenetic Assays of Environmental Mutagens. Edt. T.C. Hsu. Allanheld. Osmun. Publisher. pp. 231-236.

Rao, P.N. and Johnson, R.T. 1970. Mammalian cell fusion. I. Studies on the regulation of DNA synthesis and mitosis. Nature (London). 225:159-164.

Ray-Chaudhuri, S.P. 1961. Induction of chromosome aberrations in the spermatocytes of grasshopper. Nucleus. 4:47-66.

Reiss, J. 1971. Chromosomen aberration in den Wurzelspitzen von Allium cepa durch Aflatoxin B_1. Experientia. 27(8):971.

Reiss, J. 1977. Effects of mycotoxins on the development of epiphyllos budo of kalanchea daigremonteans. Z. Pflanzen. Physiol. 82(5):446-449.

Renda, Y. 1968. Viruses and their effect on human chromosomes. Turk. J. Rediat. 10:13-22.

Rohrborn, G. 1970. The dominant lethals : method and cytogenetic examination of early cleavage stages. In chemical Mutagenesis in Mammals and Man. Ed. Vogel, F. and Rohrborn, G. Springer-Verlag, Berlin-Heidelburg. New York. pp. 141-161.

Rous, P. 1910. A transmissable avian neoplasm (Sarcoma of common fowl). J. Exp. Med. 12:696-706.

Rous, P. 1911. A sarcoma of the fowl transmissable by an agent separable from the tumor cells. J. Exp. Med. 13:397-411.

Roy, P.P. 1982. Cytogenetic effect of the Antibiotic, Chloramphenicol on adult, male parent and progeny of mice. Ph.D. Thesis, University of Kalyani.

Roy, P.P. and Manna, G.K. 1981. Cytogenetical effects of the antibiotic chloramphenicol on mammalian parent and progeny. J. Cytol. Genet. 16:15-24.

Rugh, Roberts (1962). Early embryology of the mouse. In Laboratory manual of vertebrate embryology. Ed. Roberts Rugh. Allied Pacific Private Ltd. Bombay. India. Exercise.24:182-198.

Sablina, O.V. and Bocarov, E.F. 1968. Chromosomal aberrations in the HeLa and HEP-2. Tissue culture cells induced by herpes simplex virus. Genetika (Moskva). 4:128-134.

Sadhukhan, G. and Manna, G.K. 1984. Clastogenic effect of nitrogen fixing Xanthobactor culture on treated mice. In : Perspectives in cytology and Genetics. Ed. G.K. Manna and U. Sinha. Vol.5 (In Press).

*Salkinder, M.G. and Gear, J.H.S. 1964. Virus and chromosomes interaction. Meeting of South African Society of Pathology.

Sarihyan, D. and Singh, J.R. 1985. Centromere separation sequence in Rattus norvagicus Nat. Seminar on current trend in chromosome dynamics. Nov. 21-23, Panjab University, Chandigarh. Abs. pp. 11.

Savage, J.R.K. 1970. Sites of radiation induced chromosome exchange- In : Current Topics in Radiation Research. Ed. M. Ebert and A. Howard. North Holland. Austerdam. 6:129-194.

Sax, K. 1940. An analysis of X-ray induced chromosome aberrations in Tradescantia. Genetics. 25:41-68.

Sax, K. 1941. Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spring. Harbor. Symp. Quant. Biol. 9:93-101.

Sax, K. 1957. The effect of ionizing radiation on chromosomes. Quart. Rev. Biol. 32:15.

Scharmann, W., Jacob, F. and Postendoerfer, J. 1977. The cytotoxic action of leucocidin from Pseudomonas aeruginosa on human polymorphonuclear leucocytes. J. Gen. Microbiol. 93(2):304-309.

Schimmer, O. and Werner, R. 1974. Mutagenic effect of aflatoxin B_1 on nuclear and extranuclear DNA in Chlamydomonas reinfardic. Mutat. Res. Sect. Environ. Mutagen. Relat. Subj. 26(5):423-425.

Schmid, W. 1973. Chemical mutagen testing on in vivo somatic mammalian cells. Agents. Actions. 3:77-85.

Schmid, W. 1975a. The micronucleus test. Mutat. Res. 31:9-15.

Schmid, W. 1975b. Chemical mutagen testing on in vivo somatic mammalian cells. Agents Actions. 3:77-85.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. In : Chemical Mutagens : Principles and methods for their detection. Ed. A. Hollaender, Plenum Press, 4:31-53.

Schmid, W. 1982. The Micronucleus Test : An in vivo bone marrow method. In : Cytogenetic Assays of Environmental Mutagen : Ed. T.C. Hsu, Allanheld, Osmun. Publishers. pp. 221-229.

Schoeller, L. and Wolf, U. 1970. Possibilities and limitations of chromosome treatment in vitro for the problem of chemical Mutagenesis. In Chemical Mutagenesis. in Mammals and Man. Edt. F. Vogel and G. Rohrborn. Springer-Verleg. Berlin. Heidelberg. New York. pp. 232-240.

Schroeder, T.M. 1966. Cytogenetische und cytologische Befunde bei enzymopenischen Panmyelopathien und Pancytopenien. (Familiare Panmyelopathie Fanconi, Glulathionreduktase-mangel Anamie, magaloblastare Vitamin B_{12} Folsauremangel Anamie). Humangenetik. 2:287-316.

Schroeder, T.M. 1970. Investigation of Bone marrow smears. In : Chemical Mutagenesis in Mammals and Man. Springer-Verlag. Heidelberg. pp. 214-219.

Schuler, D., Hervei, S. Gacs, Kirchner, M. and Szathmany, J. 1968. Finflud von. Pockenimpfviren auf mesenchliche chromosomen in vivo and in vitro. Human Genetik. 6:55-60.

Searle, A.G. 1974. Mutation induction in mice. Advances in Radiation Biology. Vol. 4. Acad. Press.

Searle, A.G. and Beechy, C.V. 1974. Sperm count, egg fertilization and dominent lethality after X-irradiation of mice. Mutat. Res. 22(1):63-72.

Sensakovic, J.W., Pksquale, F.B. 1975. Biological activity of fragments derived from the extra-cellular saime glycolipo protein of Pseudomonas aeruginosa. Inffet. Immun. 12(4):808-812.

Sharma, A. 1981. Impact of the development of science and Technology on environment. Presidential Address. 68th Session. Indian Science Congress. Asso. Calcutta. p. 1-43.

Sharma, A. 1984. Environmental chemical Mutagenesis. Golden Jubilee Publication. Ind. Sci. Acad. New Delhi. Perspective Report Series. 6:1-53.

Sharma, Gita and Polasa, Hanmanlu. 1978. Cytogenetic effects of influenza virus infection on male germ cells of mice. Hum. Genet. 45:179-187.

Sharma, Gita and Polasa, H. 1978b. Induction of dominant lethality in mice by influenza virus. Indian J. Med. Res. 68:1-6.

Sharma, A.K. and Sharma, A. 1960. Spontaneous and chemical induced chromosome breaks. 1st Rev. Cytol. 10:101-136.

Singh, S. and Singh, J.D. 1976. Mitomycin C induced malformations in chick-embryos. J. Anat. Soc. India. 25(3):107-114.

Singleton, W.R. 1965. Elementary Genetics. D. Ven Nostrand Comp. Inc. Princeton, N.Y. Affiliated. East West Press Pvt. Ltd., New Delhi.

Snell, G.B. 1933. Genetic changes in mice induced by X-rays. Amer. Naturalist. 67:24.

Sneu, G.B. 1934. The production of translocations and mutations in mice by means of X-rays. Amer. Naturalist. 68:178.

Smell, G.B. 1935. The induction by X-rays of hereditary changes in mice. Genetics. 20:545-567.

Soares, E.R. 1972. Estimating the frequency of induced dominant lethals in mice by uterine dissection after weaning progeny. Mutat. Res. 16:425-427.

Sobels, F.H. 1974. The advantage of Drosophila for mutation studies. Mutat. Res. 26:277.

Sofuni, T., Makino, S. and Kobayashi, H. 1967. A study of chromosomes in Friend virus induced leukemias. Proc. Jap. Acad. 43:389-394.

Sparrow, A.H. 1951. Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytoghemical changes. Ann. N.Y. Acad. Sci. 51:1505-1540.

Spyker, J.M. and Smithberg, M. 1971. Effects of methylmercury on prenatal and postnatal development in mice. Teratology, Philadelpha. 4:242-243.

Spykar, J.M. and Smithberg, M. 1972. Effects of methylmercury on prenatal development in mice. Teratology. Philadelpha. 5:181-190.

Stadler, L.Y. 1928. Mutation in Barley induced by X-rays and radium. Science. 68:186.

Stall, F.W. 1972. The mechanism of inheritance. Prentice Hall. USA.

Stenman, S. and Saksela, E. 1969. Susceptibility of human chromosome of pulverization induced by myxoviruses. Hereditas. (Lund.). 62:323-338.

Stich, H.F., Hsu, T.C. and Rapp, F. 1964a. Viruses and mammalian chromosome. 1. Localization of chromosome aberrations with herpes simplex virus. Virology. 22:439-455.

Stich, H.F., Hsu, T.C., Hoosier, G.L. and Trentin, J.J. 1964b. Viruses and mammalian chromosomes II. Chromosome aberrations by human adenovirus type. 12.Exp. Cell. Res. 34:400-403.

Stoltz, D.B., Stich, H.F., Yohn, D.S. 1967. Viruses and mammalian chromosomes. VII. The persistence of chromosomal instability in regenerating transplanted and cultured neoplasms induced by adenovirus type 12 in Syrian hamsters. Cancer Res. 27:587-598.

Szanto, Y., Motajova, J. and Pristasova, S. 1967. Persistant infection of heteroploid and diploid cells with plasma variants of NDV. Acta. Virol. 11:363-371.

Thom, C. and Raper, K.B. 1945. In : A manual of the Aspergilli williams and Wilkins. Comp. Baltimore, Md., pp. 148.

Thust, R. and Kneist, S. 1979. Activity of citrinin metabolized by rat and human microsome fractions in clastogenicity and sister chromatid exchange assays on Chinese hamster V-79 E cells. Mutat. Res. 67(4):321-330.

Tolstova, T.L., Kulikova, K.S., Bogdanova, N.S., Pershin, G.N. and Mironova, L. 1966. Propagation of influenza virus in diploid cell strains and BHK-21 cells. Acta. Virol. 10:315-321.

Tsuchide, R. and Rich, M.A. 1964. Chromosome aberrations in viral leukemogenesis. I. Friend and Rauscher Leukemia. J. Nat. Cancer. Inst. 33:33-49.

*Tuchmann-Duplessis H. 1972. Teratogenic drug screening. Present procedures and requirements. Teratology. 5:271-286.

Vogel, F. and Rohrborn, G. 1970. Chemical mutagenesis in Mammals and Man. Springer-Verlag. Berlin, Heidelberg. New York.

Van Demark, N.L. and Free, M.J. 1970. Temperature effects. In : The testes : Influencing factors. Vol. 3. Ed. A.D. Johnson, W.R. Gomes, N.L. Van Demark. Academic Press, New York and London. pp. 223-312.

Von Rosen, G. 1964. Hereditas. 51:89.

Vogel, F. and Sobels, F.H. 1976. The function of Drosophila in genetic toxicology testing. In Chemical Mutagenesis, Principles and Methods of their detection. Edt. A. Hollaender, Plenum Press. 14:93-142.

Vogt, M. and Dubbecco,R. 1963. Steps in the neoplastic transformation of hamster embryo cells by polyoma virus. Proc. Nat. Acad. Sci. (Wash.). 49:171-179.

Waksman, S., Harning, H. and Spencer, E. 1943. Two antagonistic fungi, Aspergillus fumigatus and Aspergillus clavatus and their antibiotic substances. J. Bact. 45:233.

Walton, A. 1927. The relation between density of sperm suspension and fertility as determined by artificial insemination of rabbits. Proc. Royl. Soc. B. 101:303-315.

Weinstein, D. and Moorhead, P.S. 1965. Karyology of permanent human cell line W-18 VA 2 iginated by SV-40 transformation. J. Cell. Comp. Physiol. 65:85-92.

Wickes, I.G. 1954. Foetal defects following insulin chemotherapy in early pregnency. Brit. Med. J. 2:1029.

Wilson, G.B. 1960. The study of drug effects at the cytological level. Int. Rev. Cytol. 9:293-304.

Wilson, G.B. 1965. The assay of antimitotics. Chromosoma. 16:133-143.

Wolff, S. 1963. Radiation induced chromosome aberration. Columbia University Press, New York.

Wolman, S.R., Hirschhorn, K. and Todaro, J. 1964. Early chromosomal changes in SV 40 infected human fibroblast cultures. Cytogenetics. 3:45-61.

Wong, J.J., Singh, R. and Hsieh, P.D.H. 1977. Mutagenicity of fungal metabolites related to aflatoxin biosynthesis. Mutat. Res. 44(3):447-450.

Wyrobek, A.J. and Bruce, W.R. 1975. Chemical induction of sperm abnormalities in mice. Proc. Nat. Acad. Sci. USA. 72(11):4425-4429.

Wyrobek, A.J., Heddle, J.A. and Bruce, W.R. 1975. Chromosomal abnormalities and the morphology of mouse sperm heads. Can. J. Genet. Cytol. 17(4):675-681.

Wyrobek, A.J., Meistrich, M.L., Frurrer, R. and Bruce, W.R. 1976. Physical characteristics of mouse sperm nuclei. Biophys. J. 16:811-825.

Wyrobek, A.J. 1977. Sperm shape abnormalities as an indicator of mutagenic damage pp. 519-527. In : Testes in normal and infertile men. Edited Troen, P. and Nankin, H.R. Ravan Press, New York.

Wyrobek, A.J. 1979. Changes in mammalian sperm morphology after X-rays and chemical exposures. Genetics. 91(1, suppl.):105-119.

Yerganian, G. 1966. Relative growth and oncogenic potential of normal, malignant and virus - transformed euploid cells of dwarf species hamster. In : Recent result in cancer research. 6 (Edt). W.H. Kirsten, Springer-Verlag, N.Y. pp. 112-122.

Yerganina, G., Cho, S.S., H.T. and Nell, M.N. 1965. Euploidy and chromosome aberrations in normal malignant and tumor virus transformed cells of Chinese and armenion hamster. In : Symp. Mutational Process. Prague. pp. 349-360.

Yoshida, M.C., Makino, S. and Kinjo, T. 1967. Notes on chromosome abnormalities in dog blood and kidney cell in vitro after infection with infectious Canine hepatitis virus. Proc. Japan Acad. 43:662-667.

Zurffausen, H. 1967. Induction of specific chromosomal aberrations by adenovirus type 12 in human embryonic kidney cells. J. Virol. (Baltimore). 1:1174-1185.

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