Chem.-Biol. Interactions, 6 (1973) 375–392

CHROMOSOME DAMAGE INDUCED BY CHEMICALS

N. P. BISHUN, D. C. WILLIAMS, J. MILLS, N. LLOYD, R. W. RAVEN AND D. V. PARKE*

*Research Department, Marie Curie Memorial Foundation, The Chart, Oxted, Surrey, and *Department of Biochemistry, University of Surrey, Guildford, Surrey (Great Britain)*

(Received January 10th, 1973)
(Accepted February 9th, 1973)

SUMMARY

During the past half century there has been a substantial increase in the number and extent of usage of drugs, food additives, pesticides and other environmental chemicals. As a result, mutation-induced susceptibility to disease, once largely self-eradicating, is now being conserved and even propagated. In addition, we have over the same time period, added to our internal and external environments, numerous synthetic chemicals, the cytogenetic properties of some of which have been reviewed, and to which man's metabolism has not necessarily been conditioned and adapted, and which may have the potential to augment the number of mutations of each succeeding generation.

We need to know far more about what we are doing to ourselves and our planet, and the foetuses of our unborn children, and to the genetic heritage of these children, by the permissive use of the chemical wonders of our age. It would surely be impracticable that one should wait for time to reveal harmful mutations before we try to research out and eliminate the chemical mutagens.

Chromosomal studies and evaluation of mutagenic potential of all new and established drugs, food additives and environmental chemicals should therefore be integral aspects of the current practices of safety evaluation of these materials, and the same stringent principles should be applied to the numerous chemicals met with as solvents and intermediates in industrial processes. Persons exposed to high risk, such as those working in chemical industries, or patients on continuous chemotherapy, should be offered routine chromosomal monitoring, and the appearance of adverse effects and abnormalities notified and correlated.

INTRODUCTION

It is well established that radiation and radioactive substances can cause

Abbreviations: FSH, follicle-stimulating hormone; ppm, parts per million.

damage to genetic material and consequently the need for appropriate measures for detection and control are now recommended and widely accepted[1]. Present information would indicate that many of the chemicals to which we are exposed may be just as dangerous, in terms of genetic defects, as radiation and may constitute a far greater risk. Such chemicals include medicinal agents, food additives, industrial chemicals, pesticides and environmental pollutants. Induction of mutagenesis has long been a subject evoking considerable scientific and medical interest as well as public emotionalism. However, recent technical advances, notably in tissue culture and other chromosomal techniques, have increased the number of cytogenetic investigations of these chemicals and have permitted a more critical evaluation of their overall mutagenic potential to be made.

Chromosomal damage induced by chemicals is similar, in the type of aberrations, to that induced by radiation and viruses[2]. As the latter has been directly linked with the development of a variety of neoplastic conditions, it seems not unreasonable to suppose that once chromosomal damage has been inflicted, whatever the agency, the potential exists for the development of some form of malignancy, assuming that the damaged cells survive and proliferate[3,4]. In an *in vivo* system it would be possible to monitor the survival of chromosomal aberrations, through periodic studies over prolonged periods, in order to assess the survival time of the inflicted cells. It might perhaps then be possible to correlate chromosomal damage, 'incubation time' and the development and the progression of neoplasia, associated with all of these agents.

The problem of evaluating and limiting the use of potential mutagenic chemicals has been neglected largely because of the inability to develop valid test systems that are relevant to man. It is, however, relatively easy to test the effectiveness of suspected mutagens in producing chromosome breaks by conducting cytological observations on cultured cells. Lymphocyte cell cultures can readily be obtained from the peripheral blood of animals or man suspected of exposure to a mutagen[1], and diploid cell lines from human skin fibroblasts are also available.

Many drugs used in cancer therapy are known to cause gross chromosomal damage and since the majority of cancers for which these cytotoxic agents are employed cause fatal disease, the risk of mutagenesis due to the drugs is justified[1]. In contrast, where drugs are used for the treatment of psychiatric disorders and other non-fatal diseases, the justification of exposure of patients to medicines that are known to be mutagenic may be questioned, particularly if less mutagenic ones, as therapeutically effective, are available. Hence, it becomes of paramount importance to study and evaluate, for chromosomal damage and mutagenicity, those commonly used drugs and other chemicals to which man is constantly exposed and to document and correlate such information.

METHODOLOGY

The need for developing improved systems for determining mutagenicity is now well appreciated[5], but before results can be realistically applied to man, tech-

niques utilising mammalian systems and preferably also human cell cultures should be employed. These mammalian systems should include *in vivo* and *in vitro* cytogenetic testing, dominant lethal tests and the host-mediated assay[5] (WHO Report, 1971). Cytogenetic studies in man involve the relatively simple procedure in which chromosomal damage resulting from the exposure of human subjects to toxic chemicals can be observed periodically by culturing circulating lymphocytes[6], or alternatively human lymphocyte cultures may be exposed to the chemicals *in vitro*. When a more comprehensive examination is necessary the chemical is administered to laboratory animals, such as rats and mice, in which case germ cells as well as somatic cells can be examined. The host-mediated assay facilitates the study, in a rapidly dividing unicellular population, of the mutagenic effects of not only the chemicals administered but also of their mammalian and microbial metabolites. This is a flexible technique which allows mutation frequency to be measured in a variety of micro-organisms and mammalian hosts. The dominant lethal test system, is a simple technique which gives an indication of the genetic changes that are incompatible with the survival of the conceptus and is mainly carried out in mammalian systems. A mutagenic index is then calculated to relate pre-implantation loss and early deaths in the treated to a non-treated control group.

Several factors mediate against the extrapolation of mutagenic data from simple unicellular systems to mammalian organisms, and from the latter to man. The pharmacokinetics of absorption, distribution and excretion of the mutagenic chemical administered, the nature and amounts of its metabolites, and the mutagenic activity of these metabolites, may all profoundly affect the extent and character of mutation and chromosomal damage. Microbial systems will provide information only on the extent and nature of the lesion produced by the mutagen itself and by its microbial metabolites, and as these latter are likely to differ considerably, in amounts even if not in chemical nature, from metabolites produced in mammalian systems, studies *in vivo* in laboratory animals are essential to any evaluation of the mutagenic potential of chemicals which is to be meaningful to man. In this respect, the host-mediated assay, in which micro-organisms are exposed to both the original chemical and to its mammalian and microbial metabolites, is of particular usefulness, especially as human cell-line cultures, because of loss of biochemical differentiation, may not metabolize the administered mutagenic chemicals in the manner in which they are metabolized *in vivo*. The carcinogenic chemicals, cycasin and nitrosamines, which were shown not to be mutagenic *per se* to micro-organisms have now been shown to be mutagenic, as well as carcinogenic, in higher organisms by virtue of their metabolites[4,7].

In addition to tissue culture systems, bone marrow, regenerating liver (which necessitates hepatectomies), and dividing cells from the testis are other useful animal systems normally utilised. However, they all have their limitations; the bone marrow cell population is heterogeneous and may have varying sensitivities with respect to individual cell types, liver which also contains a heterogeneous cell population is, moreover, more exposed to numerous xenobiotics, and the testis does not yield suitable metaphases in sufficiently high numbers.

The report of the WHO scientific group recommended that all mutagenic tests of drugs should be done primarily on mammals, although systems utilising submammalian species such as viruses, micro-organisms, Drosophila, fishes, birds, *etc.*, should also be studied. The report stresses that submammalian test systems and *in vitro* cell cultures, even human, should only be regarded as ancillary procedures although the results obtained from them may help considerably in explaining the basic molecular mechanisms involved in mutation. In selecting the test system to be used for evaluating chromosome breakage potential of a drug, it is necessary to be aware of the shortcomings of the different systems involved. The ideal method, of course, would be to examine cells of human subjects before and after exposure of the subjects to chemicals, but in so many cases this is impractible so the experimentalist is forced to resort either to tissue cultures or animal systems. The compromise solution would be to complement studies in the experimental animal with human tissue culture systems, since substances which might produce a detectable increase in mutation frequency in a human cell culture system may be innocuous to mammals *in vivo* because of metabolic transformations in the whole animal, or because the chromosomal effect may be intrinsic to the cell culture test system. The fact that compounds may be effective only *in vivo*, *e.g.* cyclophosphamide, perhaps because only metabolites are mutagenic, is sufficient reason for not dispensing with the animal system, especially the host-mediated assay. On the other hand, because of species differences in metabolic deactivation of potentially mutagenic drugs, the human cell culture system may detect cell damage which might not become manifest in any one species of experimental animal, especially if this species had a highly effective metabolic defense system, and thus a positive response may not necessarily constitute a false alarm.

In the final assessment of the results one must be cautious when extrapolating the results obtained from experimental animals and *in vivo* culture systems to human situations. Furthermore, one must be aware that a negative result obtained from a certain dose level and period of exposure does not necessarily indicate that the potential mutagenic agent is harmless at and below this threshold. There is sufficient evidence available to suggest that point mutations may continue to occur at very low dose levels[8], and it is strongly suggested that point mutations may be highly correlated with visible chromosomal damage[9]. The present review, therefore, deals with some of the cytogenetic information available on various commonly used chemicals which for diverse reasons, and by various means, eventually reach the tissue of human organs *in vivo*.

MEDICINES

Anticancer drugs

Almost all of these drugs caused chromosomal damage. Nevertheless, in view of the high probability of the eventual fatal outcome of this disease, it may often be considered justified to institute this type of therapy. However, unless these drugs are carefully investigated and their biological effects documented one can never be certain

of the actual benefits or risk to the patients receiving this type of drug. The list of anticancer drugs is extensive and a few members of some of the more important classes are now considered.

Triaziquone (2,3,5-tris[ethyleneimino]benzoquinone; Trenimon). This is a strong alkylating agent used in the treatment of cancer which gives rise to side-effects of leucopenia and thrombocytopenia. This drug has been reported to cause chromosome aberrations *in vivo*[10] in Chinese and Syrian hamsters, mouse, rat and guinea pig. In a report by ARAKAKI AND SCHMID[11], who used fibroblast cells of Chinese hamster and human lymphocytes, it was shown that much higher concentrations of drug were required in the human lymphocyte system than in the hamster cells to cause chromosomal damage; it was also found that control hamster cells showed a higher incidence of spontaneous chromosome breakage than human controls. Using Chinese hamster bone marrow as an *in vivo* system SCHMID *et al.*[8] showed that Trenimon predominantly induced chromosome breaks in preference to other types of chromosome damage; abnormal metaphases were found at 6–8 h after intraperitoneal injection of Trenimon (2×0.25 mg/kg) and the damage gradually decreased as the time after the final injection increased.

Triethylenemelamine (2,4,6-tris[ethylenimino]-*s*-triazine; TEM or Tretamine), is an alkylating, antineoplastic drug with side-effects of bone-marrow depression, which can induce a significant increase in chromosome aberrations when added to a culture of human male leucocytes. This is a powerful mutagen and at a concentration of $5 \cdot 10^{-6}$ *M* it can induce 79% abnormal cells[12].

Apholate (2,2,4,4,6,6-hexabis[ethylenimino]hexahydro-1,3,5,2,4,6-triazatriphosphorine), can also induce chromosomal aberrations and at a concentration of $1 \cdot 10^{-4}$ *M* it produces 89% of abnormal cells.

Cyclophosphamide. This is an alkylating antineoplastic and immunosuppressive agent used in the treatment of lymphomas and certain leukaemias which has also been used in the treatment of severe skin conditions such as psoriasis. It does not cause chromosomal damage in human leucocyte cultures[12] when added directly, but plasma taken from rats previously injected with the drug and added to the cultures produced many chromosomal aberrations including chromatid and isochromatid breaks, minute fragments and chromatid interchanges. This is indicative that cyclophosphamide is effective *in vivo* but not *in vitro*[13]. It has also been shown to cause chromosome breaks in Chinese hamster bone marrow cells *in vivo*, with the number of breaks decreasing with time after the cessation of treatment. This drug has been reported to have chromosome breaking properties on cancer cells *in vitro*, and as a result it has been postulated that excess phosphatase in the cancer cell is responsible for activating the cyclophosphamide *in vitro*[14].

Floxuridine (fluorodeoxyuridine), is an antiviral as well as an antineoplastic drug. It causes chromosomal damage of the kind similar to cyclophosphamide but fewer chromatid exchanges are found with this drug[12].

Methotrexate (amethopterin). This is also an antineoplastic drug, which can produce chromosomal damage when added directly to human peripheral blood cell cultures (personal observations). Furthermore, JENSEN *et al.*[15] have shown that bone

marrow cells of patients treated with this drug show a significant increase in chromosomal aberrations consisting of chromatid breaks and acentric fragments. Methotrexate is commonly used for the treatment of acute leukaemia, and has also been used in the treatment of severe psoriasis.

6-Mercaptopurine, is another antineoplastic agent which has been shown to cause chromosome breakage in animal tissue cultures. Breakages and an increase in aneuploidy and tetraploidy have been found in a skin biopsy from a person under treatment with the drug[16].

Azathioprine. This is an S-substituted nitroimidazolyl derivative of 6-mercaptopurine and is widely used in the treatment of auto-immune disease and psoriasis. This drug has been found to cause widespread chromosomal damage in human bone marrow cells without causing an increase in aneuploidy[15].

Prednisone. This is an anti-inflammatory and anti-leukaemia drug and does not produce any alteration in chromosome number or structure[15] but after prolonged treatment of patients can cause an impairment of the DNA synthesis mechanism[14].

Cytosine arabinoside, is an analogue of the natural nucleoside, cytidine, which is used for the treatment of disseminated malignant disease, and severe herpes viral infections and has the ability to cause severe chromosomal damage to cultures of human leucocytes[17a]. It has also been shown to cause chromosomal abnormalities in patients under treatment, with morphological changes being seen in the bone marrow cells as early as 6 h after administration of the drug[17]. These changes are known to increase in severity in 5 days, but within 2 weeks after discontinuation of therapy the changes disappear. Some of the other important chromosomal abnormalities include breaks, despiralised and sticky chromosomes, and extensive fragmentation.

Daunomycin. This is a nitrogenous glycosidic antibiotic of the rhodomycin group which since 1963 has been used regularly for the treatment of childhood leukaemias[18]. It has a narrow effective dose range which causes chromosomal damage for above this range mitosis is inhibited. The frequency of aberrations varies from subject to subject and appears to depend on the genetic background of those under investigation. Using human leucocytes, VIG *et al.*[19] ascertained that 0.015 μg/ml of daunomycin produced chromosome breaking which was specific in nature and non-random along the chromosomes. There was also no change in the specificity of the drug either with respect to sex or to the addition of arginine (0.01%). Daunomycin has a specific chromosome breaking effect when cells are treated in the G1 phase and it is not possible to relate the chromosome breaking to the early or late DNA synthesis of the chromosomes.

Mitomycin C, is an antineoplastic antibiotic derived from Streptomyces which is known to disrupt DNA synthesis and to result in cross-linking of the complementary strands of DNA. It has been shown to result in extensive chromosome damage and possible translocations[21,22].

Drugs of abuse

Lysergic acid diethylamide (LSD). In 1957 COHEN *et al.*[23] reported that minute amounts of LSD caused extensive damage to the chromosomes of human leucocytes

in vitro and studies *in vivo* have confirmed these findings. Investigations of children whose mothers had taken LSD during pregnancy indicated that the drug could cross the placental barrier and so affect the chromosomes in the blood and tissues of the foetus with the potential danger of teratogenesis. Injection of LSD into pregnant hamsters[24] led to severe malformations and stunting in the offspring and there are at least two reports of congenital malformation in human infants, both of whose parents had taken LSD[24], but considerable controversy exists, and SATO *et al.* found no effect of a 100 μg/kg single oral dose on rat embryo or bone-marrow cells[25]. The differences in results may be due to differences in level and frequency of dosage, and in the case of studies with human addicts, to the simultaneous administration of other psychotogenic drugs. COHEN *et al.*[23] found that the frequency of chromatid gaps was significantly higher in those patients which had been treated with LSD than in the control group. Damage to chromosomes in the germ cells had been considered as perhaps the most important hazard of LSD. HSU[26] and others found chromosome abnormalities in a newborn female whose parents had both been taking LSD before conception. This abnormality was similar to D-trisomy and it is possible that LSD damaged the maternal germ cell before conception, inducing chromosomal rearrangements. However, the mother took many other drugs during pregnancy including marihuana, barbiturates and methedrine.

GILMOUR and others[27] have looked at the chromosomes of patients receiving some other psychoactive drugs and he divided these into five categories namely those taking: *(a)* marihuana in small amounts, *(b)* marihuana in large amounts, *(c)* phenothiazines, *(d)* amphetamines and *(e)* heroin. Each of the groups of drug users, except those taking small amounts of marihuana, showed an increase in the occurrence of chromosomal damage as compared with the controls. Dicentrics or an increase in chromatid exchanges were not found but chromatid breaks and centromere breaks were evident.

Nicotine. The active carcinogenic agent of cigarette smoke, which may also be mutagenic, has been postulated to be either cotinine or nicotine-1-oxide, metabolites of nicotine. An extensive amount of work has also been undertaken in an attempt to assess the active principle in cigarette smoke which is responsible for women giving birth to children of low birth weight[28].

We have studied the effects of adding nicotine to human peripheral blood cultures and also after injections into rats. The results show that, at high concentrations, the drug is toxic to the human peripheral blood cultures but does not induce any chromosomal abnormalities. However, experiments with rats *in vivo* showed a high degree of chromosomal abnormalities which included an increased frequency of aneuploid cells and metacentric chromosomes, thus confirming that the mutagenic agent may be a metabolite of nicotine[29].

Other drugs

Oral contraceptive steroids. CARR[30] examined the chromosomes of the abortuses of women who had "taken the pill" and found a striking increase in triploidy in the abortuses from women conceiving within 6 months of discontinuing oral contracep-

tives. This was thought to be the result of temporary hormonal imbalance in these women, such imbalance affecting cell division in the ovum. It was further suggested that if cell division could be suppressed while still allowing normal chromosome division to occur it could result in either triploidy or tetraploidy. Triploidy would result at the time of the first or second meiotic divisions, and tetraploidy would occur if the zygote were affected at the first cleavage division. With these considerations in mind SINGH AND CARR[31] tested the effects of a series of hormones in an *in vitro* system but found that none of the hormones except FSH caused mitotic inhibition. However, cells in tissue cultures are not comparable to germ cells because formation of the latter involves partitioning of the chromosomes to produce a haploid number. Nevertheless, the mechanism of the second meiotic division is similar to that of mitosis in somatic cells. They also failed to find any increase in polyploid cells in leucocytes cultured *in vitro* when they were treated with various hormones, which probably implies that there was no direct effect of these agents on cell division. It therefore seemed likely that the increase in triploidy from the women taking oral contraceptives was due to an indirect effect on germ cell maturation or on the timing of fertilisation.

In a recent study undertaken in the Marie Curie Laboratories, leucocytes from the blood of babies and their mothers, some of whom had taken an oral contraceptive and some who had not, were cultured for 3 days, harvested, preparations made and 25 chromosome spreads counted. Altogether 940 subjects (mothers and babies) were studied in the survey, and nearly two thousand cells were counted from mothers who had "been on the pill"; two thousand more were from control mothers and a thousand more from the babies' blood cultures (test and control). A comparison of abnormalities in the cells showed that 2.16% have more than 46 chromosomes in test mothers compared to 2.45% in control mothers; and in babies there was 1.79% abnormal cells in test babies compared to 1.97% in control babies. Thus no significant differences were found. Other abnormalities studied were structural alterations including gaps, breaks and rearrangements of chromosomes, and again, no significant differences were found between the test and control groups.

Acetylsalicyclic acid. JARVIK AND KATO[32] investigating the chromosome breaking effects of aspirin, have shown that this drug produced a significantly high percentage of breaks in human leucocyte cultures, but this work has not been substantiated by MAUER and his colleagues[33]. Obviously with a commonly used drug like aspirin, a great deal more work is required.

Diazepam. This tranquilizer/muscle relaxant has been studied by NIELSEN *et al.*[34], who performed chromosome analyses on the leucocytes of 23 patients using the drug and of 8 controls, by further exposure of the cell cultures to the drug over 0.5–36 months. One patient showed chromosomal breakage in 15.3% of the cells examined, but 6 months after having stopped diazepam the breakage rate returned to the control level. These results disagree with those of COHEN *et al.*[35] who found no increase in chromosomal damage *in vivo*. Similarly STAIGER[36] who exposed leucocytes to concentrations of 0.5–1% of the drug, found no evidence to suggest that diazepam possesses chromosome breaking properties.

Chlorpromazine. In a study of the chromosomes of leucocyte cultures from

patients treated with chlorpromazine, or the other psychotropic drugs, perphenazine and lysergide, significant inductions of chromosomal abnormalities were seen. Patients treated with chlorpromazine (13), perphenazine (15) or lysergide (19) showed significant increases in the induction of gaps and breaks and in the frequency of hypodiploid cells. The frequency of gaps and breaks showed a correlation with the duration of treatment and amount of drug administered for perphenazine but not for chlorpromazine. Moreover, with perphenazine and lysergide, but not chlorpromazine, there were significantly higher total frequencies of chromosome fragments, dicentric chromosomes and ring chromosomes[34].

Chlorimipramine. WILKIE AND DELHANTY[37] added 10 μg/ml of chlorimipramine to cell cultures of human skin fibroblasts and found this substance leads to the arrest of cell division in metaphase, prevents oxygen uptake and eventually causes cell death. The effect of this drug appears to vary with the cell strain used, and S.V.-40 transformed cells are considered to be very sensitive to this compound. However, in *in vivo* studies carried out in the Marie Curie Laboratories no chromosomal abnormalities have been observed on treating rats with a derivative of this compound.

Chloramphenicol. This antibiotic, once widely used in the treatment of a wide variety of bacterial infections in man and domestic animals, is now restricted in use to the treatment of *B. typhosum* infections because of its high toxicity. Bone marrow toxicity of this antibiotic is of particular significance and toxic damage may extend to development of marrow aplasia and acute leukaemia. Previous observations[38] have shown the presence of nuclear and cytoplasmic vacuolations in haemopoietic cells of patients on prolonged chloramphenicol therapy. More recently, chromosome breaks and fragmentations have been discovered by MITUS AND COLEMAN[39] who carried out experiments to determine whether similar lesions could be produced *in vitro* by this drug. Addition of chloramphenicol (10 μg/ml) to short-term cultures of leucocytes produced gaps, breaks, fragments, secondary constrictions, and ring chromosomes, with significant differences between controls and tests. The nature of the biochemical effect of this drug on bacteria has been studied and levels of 10 μg/ml inhibit protein synthesis by interfering with the attachment of mRNA to ribosomes; a similar effect is not apparent in mammalian cells[37]. The presence of the ring chromosome is of interest because it may indicate that chromosomal damage by chloramphenicol is liable to produce rearrangements. The development of new abnormal cell lines after exposure to this once widely used antibiotic may be of future significance in understanding the evolution of neoplasia.

Colchicine. This is an alkaloid still widely used in the treatment of gout and has been extensively studied for its chromosomal damaging effects. Addition of colchicine (10^{-6} *M*) to cultures of 11-day-old chick embryo breast muscle resulted in fragmentation of the elongated myotubules into rounded cytoplasmic sacs containing various numbers of nuclei[16]. Comparison of the dose-response relationship between myotubule fragmentation and metaphase arrest suggests that the underlying mechanism may be similar in both cases. The effects of colchicine on the mitotic spindles of mammalian cells can be readily reversed by washing the cells free of drug and then growing them in fresh media. This has been shown by KATO AND YOSHIDA[40] using a Chinese hamster

cell line with a modal chromosome number of 22; colchicine was added to the cultures and cells were washed free of drug 30 min, 2 and 4 h after addition of colchicine. Deviation from the modal chromosome number became greater with increasing duration of the colchicine block and it thus appeared that the damage to the spindle fibres was not completely repaired. From studies of the stability of "colchicine-reversal" cells with abnormal chromosome numbers it was found that cells with lower chromosome numbers than controls disappeared rapidly from the population as incubation time increased and those with a larger chromosome number persisted rather steadily until the end of the cultivation periods. These observations suggest that the viability of the cells which suffered chromosome losses was impaired and their survival time greatly curtailed.

We have also carried out studies on the induction of chromosomal abnormalities by reversal of colchicine inhibition[41] using human peripheral blood leucocyte cultures[12]. The test cultures had 71% of cells with 45 chromosomes or less and there was also a marked increase in endo-reduplication (the chromosomes reduplicate themselves and can be seen lying side by side). Our observations also support those of KATO AND YOSHIDA[40] in that the colchicine inhibition effect is not completely abolished by washing the cells.

Phenylbutazone. In a study of fifty patients who had been taking phenylbutazone, a widely used anti-rheumatoid drug, for at least 3 months it was found that the frequency of chromosomal damage in lymphocytes was significantly greater (2.0%) than in controls (0.5%); the frequency of dicentric chromosomes was also increased[42]. Evidence was obtained that the drug does not act directly on DNA, and it is likely that individual susceptibility occurs. MULLER AND STRASSE[43] investigated the effects of oral administration of this drug (400 mg/kg) on Chinese hamster bone marrow cells; results showed only 1% of chromosome breakage, with an average of 4.5 chromatid gaps per animal in the test group as compared with 3.8 in the controls. In this species phenylbutazone only infrequently caused chromatid breaks and chromatid gaps. In view of the possible widespread usage of this drug and the equivocal findings further long-term studies ought to be undertaken with phenylbutazone, oxyphenbutazone and related drugs.

Lithium carbonate, is used in the treatment of manic depression. Cultures of leucocytes from patients taking lithium carbonate failed to show a significant difference from controls in either breaks or hypodiploidy[3]. However, these results do not agree with the findings of FRIEDRICH AND NIELSEN[44] who observed chromosomal breaks in a patient who had been on the drug for only 2 months.

FOOD CHEMICALS

Caffeine occurs in coffee and tea and so a great number of people are exposed to regular dosage with caffeine. It has been suggested that because of its purine nature it may have a mutagenic potential, and there is a large amount of conflicting evidence of its effects on chromosomes. THAYER and colleagues[45] exposed HeLa cells to caffeine and found no significant increase in aberrations at a dose of 20 μg/ml over 29 to

48 cell divisions. Other workers (LEE SUNG[46] and personal observations) found that caffeine caused aberrations both in HeLa cells and other plant and animal cells. LEE SUNG[46] carried out studies on the effects of caffeine on human leucocytes and human embryonic cells in cultures containing 0.005–0.10% of caffeine. A significant increase in chromosomal abnormalities especially chromatid gaps, isochromatid gaps, chromatid breaks and acentric fragments were found, but no exchanges were seen. KIHLMAN and others[47] treated *Vicia faba* root tips, tree onions and Chinese hamster cells for 2 h with $2 \cdot 10^{-2}$ *M* caffeine and obtained various chromosomal aberrations. "Sub-chromatid" and chromatid exchanges were most apparent in the plant cells but chromatid breaks were more abundant in the Chinese hamster cells.

Studies were also made by WALKER AND REID[48] who administered caffeine to mouse L-cells, previously exposed to UV light, and found that the caffeine potentiated the lethal action of UV light. They showed that the action of methyl methanesulphonate and other alkylating agents on mouse L-cells was increased after subsequent incubation in 2 m*M* caffeine for 48 h. HeLa cells cultured for 4 weeks in a medium supplemented with caffeine at concentrations some 10 times the maximum levels of caffeine ingested by man, showed a significant increase in chromosomal breaks and rearrangements[49].

KIHLMAN *et al.*[47] also exposed the same cells to 8-ethoxycaffeine and found that this substance was much more active than caffeine. After exposure to concentrations of 10^{-2} *M* for 45 min many aberrations similar to those produced by caffeine were seen in all cell types, and a high proportion of isolocus breaks focalised in the nuclear constriction in the *Vicia faba* root tip cells were also found.

6-Methylcoumarin. KIHLMAN and others[47] further found that 6-methylcoumarin in *Vicia faba* root tip cells, onion tree cells and Chinese hamster produces the same sort of chromosomal aberrations as 8-ethoxycaffeine and caffeine.

Cyclamate and other sweeteners. The artificial sweetener, sodium cyclamate, in relatively high concentrations produces chromosome breakages in plant cells and in human leucocytes. SAX AND SAX[50] and STONE *et al.*[51] showed that chromosomal breaks in cultures of human leucocyte cultures supplemented with cyclamate (250–500 μg/ml) ranged from 0–35% of the cells, averaging 11%, whereas for the control cultures this was only 0–12% cells; with lower concentrations of cyclamate (50–100 μg/ml) there was no clear effect on chromosome breaks.

Some work has also been carried out by CATTANACH[52] with cyclohexylamine, a metabolite of cyclamate; at concentrations of 50–100 mg/kg cyclohexylamine does not induce dominant lethal mutations or chromosomal translocations in rats.

STONE[51] studied the supplementation of leucocyte cultures with saccharin and showed that even at the high concentration of 500 μg/ml there was no evidence for chromosome breakage. Again, in a second series of experiments using human skin monolayer cultures and varying concentrations of cyclamate, no increase in chromosome breaks was found.

INDUSTRIAL CHEMICALS

Hydroxylamine

BROGGER[53] investigating the effect of this chemical (25–100 μg/ml) on human leucocyte cultures found that it induced chromosomal damage and that the extent of the damage was proportional to the dose and length of exposure. A wide range of chromosomal damage was observed and the aberrations included isolocus constrictions, gaps and breaks.

Trimethylphosphate

This chemical is used in industry as a methylating agent and also as a solvent for paints. Work done by ADLER *et al.*[54] has shown that this chemical produces the greatest number of chromosome breaks in rat bone marrow cells after 48 h. After 12 h chromosomal gaps were prevalent but with longer periods of treatment cells with chromatid exchanges became very frequent.

Ethanol

Ethanol at concentrations of 0.2–2% has been reported to cause breaks in the chromosomes of *Vicia faba* and onion roots[55]. At a concentration of 1.2% in human fibroblast cultures treated for 48 h, the number of chromosome breaks increased about five-fold. However, human leucocyte cultures exposed to 0.3% ethanol for 48 h showed no significant increase in the number of breaks compared to controls, but when the dose is increased to 1.2% the frequency of breaks increased with a corresponding increase in the frequency of gaps.

Benzene

Benzene is a solvent which despite its known toxicity to the bone marrow still has widespread use in industry, particularly the rubber industry. The first case of leukaemia attributed to benzol vapour was reported in 1928 and many cases have been reported subsequently. Benzene is metabolized in the body to phenol *via* the arene oxide, which is probably the radiomimetic toxic entity.

TOUGH AND COURT BROWN[56] carried out a survey in 1964 and 1965 on men exposed to benzene for periods up to 20 years, and examination of blood cultures from these men showed that there was an unusual number of cells with chromosomal aberrations. FORNI AND MOREO[57] in 1967 investigated a woman who had been working with benzene for 22 years and suffering initially from anaemia later developed acute myeloblastic leukaemia. Cultures of peripheral blood cells from this subject showed a high rate of chromosomal aberrations and many cells with 47 chromosomes were found in bone marrow cells. They suggested that cells with different chromosome aberrations induced by benzene may give rise to abnormal clones which may in turn cause leukaemia. FORNI AND MOREO[57] in 1969 carried out chromosomal studies on a patient suffering from acute erythroleukaemia after having been exposed to benzene for 7 years; 2 chromosomes from group G were absent and 2 small marker chromosomes were present in all metaphases observed. TOUGH *et al.*[59] in 1970

examined the chromosomes of individuals exposed to atmospheric benzene and they also found a higher percentage than normal of cells with "unstable" chromosomal aberrations. PHILIPS AND JENSEN[58] looked at the effects of benzene on rat bone marrow cells and found that benzene (2 ml/kg) given subcutaneously produced chromatid breaks within 12 h but recovery had occurred 36 h after injection and no breaks were then seen.

OTHER ENVIRONMENTAL AGENTS

Pesticides

Captan. This is a general fungicide used for soil treatment, mildews *etc.*, which is highly mutagenic in *E. coli* and other cell cultures. It is also a potent teratogen in the chick embryo[9].

DDT. This is the most common and one of the most effective of all pesticides, and is widely used in most parts of the world in spite of its acknowledged toxicity to mammals. It has been shown recently to be mutagenic in rats and in human cell cultures *in vitro*[9]. More recently it has been shown also to cause chromosomal damage to cultured mammalian cells[60].

2,4-Dichlorophenoxyacetic acid (2,4-D). 2,4-D is a plant hormone with herbicidal properties which does not appear to have any toxic effect on cultures of pea callus below a concentration of 50 mg/l[61]. As the concentration is increased the rate of polyploid dividing cells decreased with the age of the culture, and at a higher concentration mitosis in the cells was impaired.

Gases

Chlorine is used to purify water supplies and in various manufacturing processes[62]. Addition of chlorine to human lymphocyte cultures at concentrations above 20 ppm produced an increase in the number of chromosome breaks, gaps, translocations and dicentrics[62], but no effects were observed at concentrations below this. This concentration is higher than that found in most water supplies although the chlorine concentration of water supplies may vary considerably.

Ozone. This is a gas used in certain industrial processes and is found naturally in the upper atmosphere. Inhalation of ozone by Chinese hamsters caused chromosomal aberrations[63] in bone marrow cells and subsequent X-irradiation produced an increased number of breaks. Further investigations showed that ozone can be either synergistic or antagonistic with other mutagenic agents. Ozone also causes breaks in monolayer cultures of human cells, and in view of the fact that there is constant exposure to ozone more extensive work is necessary.

CONCLUSION

The human organism because of its ubiquity, can be said to have adapted itself successfully to its environment. Man is found in almost all of the diverse climatic conditions which exist on this planet, and his ability to constantly adapt to his

changing environment has been due mainly to variations in physical traits such as pigmentation of skin and hair, and to chemical changes in enzyme activities and physiological functions. In the past decade, rapid and dramatic changes of the environment, imposed by the numerous products of modern chemical technology, have further tested man's ability to adapt and survive. Day by day, everyone, almost everywhere is exposed to some pollutant in the air he breathes, the food he eats, or the water he drinks. The polar ice, like the fat depots in our own bodies, contains its quota of DDT and other environmental contaminants and the fact that some of these environmental agents can and do induce mutations and chromosomal aberrations has been known to geneticists for the past three decades. The public today is exposed to an enormous variety and number of chemicals whose long-term, low-dose effects are seldom known and only poorly understood. With regard to their potential genetic or chromosomal effect we are best informed about such potentially toxic substances as the nitrogen mustards, and other cytotoxic agents which are not widely encountered, but we are poorly informed about the effects of less toxic and more commonly used compounds, such as aspirin, caffeine and oral contraceptives.

A number of chemical agents have been shown to alter the chemical makeup of genes and chromosomes; the alterations may be in the DNA molecule or they may involve alterations of the linear structure along the chromosomes, this being a result of chromosome breakage and rearrangement. Chemicals may also interfere with cell division and may thus alter the chromosomal numbers. Although the molecular mechanism of chromosome damage is not precisely known, incorporation of nucleotide analogues into, and alkylation of DNA, intercalation and cross-linking of DNA, free-radial formation by ionizing radiation and by radiomimetic chemicals, lysosomal damage with release of DNA- and RNA-hydrolases, and impaired synthesis of protein, enzymes and coenzymes, have been postulated. In reality, probably all of these mechanisms, and others, may be involved with different agents acting by different mechanisms.

The systematic screening of chemicals for mutagenicity has played a significant role in establishing the fundamental points of the chromosomal theory of carcinogenesis[1]. Aflatoxin has been shown to be teratogenic and carcinogenic in addition to being mutagenic[9]. Cyclamate has been shown to produce bladder cancers in addition to producing cytogenetic abnormalities[4]; and Captan has been characterised as a highly mutagenic agent[9], as well as being teratogenic. DDT is mutagenic, produces tumours in animals and also produces chromosomal abnormalities[60].

According to the chromosomal theory of carcinogenesis, chromosomal aberrations are the common pathway through which carcinogenic factors induce cancer and MILLER AND MILLER[64] have concluded that many and perhaps all carcinogens are mutagens, although not all mutagens are necessarily carcinogenic. The review has demonstrated that many exogenous chemicals including medicines, food additives, pesticides and industrial and environmental chemicals can cause chromosomal damage. It is then logical to suppose that because of the chromosomal damage caused by these chemicals they may be a causal factor in the evolution of the neoplastic growth.

Recently, studies have been undertaken to establish correlations between the

chromosomal damage produced by carcinogenic chemicals and the molecular aberrations that may be involved in the induction of the carcinogenic process. In a study of the mutagenic properties of *N*-acetyl-2-aminofluorene and its metabolites in Drosophila, in relation to the molecular mechanisms of carcinogenesis, FAHMY AND FAHMY[65,66] have shown that a subtle correlation exists between the mutagenic selectivities and the carcinogenic potential of this series of compounds. The carcinogenic parent amine, its *N*-hydroxylamines and the acetoxy derivative were all initially specific for the bobbed *(bb)* loci, which is in accordance with the concept that the rRNA genes might be significant in the initiation of cancer. Mutation at any of the RNA-forming cistrons would result in the production of aberrant RNA molecules which would then manifest altered efficiencies in the transcriptional and translational phases of protein synthesis and thus lead to the metabolic consequences characteristic of the neoplastic cell. Although much similar information, concerning the molecular effects of carcinogenic chemicals that induce mutations, is available almost nothing is known of the molecular effects of environmental chemicals on chromosomal material. However, it does not follow that a chemical which is mutagenic and produces chromosomal abnormalities is necessarily carcinogenic, although there may be similarities in those interactions with DNA which lead either to chromosomal aberrations, mutagenicity or carcinogenicity. In short, far from finding a close correlation between mutagenesis and the development of cancer, one may conclude that almost any chemically reactive compound, if tested by a sufficiently wide range of methods is likely to prove mutagenic to some systems. Some agents which are mutagenic to bacteria and viruses may not be mutagenic to man and although laboratory mammals provide rather better test systems, the results obtained must on all occasions be extrapolated to man with great caution. No doubt an enormous amount of work needs to be done regarding the relationship of chromosome breaking and the development of neoplasia.

The knowledge that certain psychoactive drugs, *e.g.* LSD, may lead to leukaemia and neonatal deformities has exemplified the need for careful screening of drugs for possible chromosome-breaking potentialities[1]. Several of the commonly used psychoactive drugs have been shown to produce chromosomal breaks and re-arrangements, abnormalities that may lead to malignant growth. An even greater potential hazard is the possible chromosomal damage of the germ cells and this has been shown to occur with LSD[67]. These mutations will not necessarily lead to clinical manifestation in the carrier, and perhaps not even to effects in all the offspring, but may remain latent with the potential for morphological or metabolic aberrations becoming only fully apparent in subequent generations.

The past half century has seen substantial increases in both the life-expectancy of man and in the number and extent of usage of drugs, food additives, pesticides and other environmental chemicals. The successful treatment, both by drugs and diet therapy, of many diseases of infection and metabolic origin has resulted in an increase in longevity with a simultaneous decrease in the process of natural selection. Mutation-induced susceptibility to disease, once largely self-eradicating, is now being conserved and even propagated. In addition, we have over the same time period, added to our

internal and external environments, numerous synthetic chemicals to which man's metabolism has not necessarily been conditioned and adapted, and which may have the potential to augment the increasing legacy of mutations of each succeeding generation. Chromosomal studies and evaluation of mutagenic potential of all new and established drugs, food additives and environmental chemicals should therefore be integral aspects of the current practices of safety evaluation of these materials, and the same stringent principles should be applied to the numerous chemicals met with as solvents and intermediates in industrial processes. Persons exposed to high risk, such as those working in chemical industries, or patients on continuous chemotherapy, should be offered routine chromosomal monitoring, and the appearance of adverse effects and abnormalities notified and correlated.

The extent of the induction of mutations by radiation and viruses is reasonably known whereas the contribution provided by drugs and environmental chemicals can only be conjectured. A recent estimate of the ill-health that is of genetic origin has been given by LEDERBERG[68] as 25% of our health-burden. In our advanced civilization the financial cost of this health burden is already reaching maximum permissible limits, so that there are pressing economic and social reasons, as well as medical needs, for limiting, and hopefully reducing, the mutagenic contribution from environmental chemicals.

REFERENCES

1 N. P. BISHUN, Cytogenetics in cancer in R. W. RAVEN (Ed.), *Modern Trends in Oncology*, Butterworth, London, in press.
2 W. W. NICHOLS, Chromosome breakage and virus, *Hereditas*, 55 (1966) 1–27.
3 L. F. JARVIK, N. P. BISHUN, H. BLEWEISS, T. KATO AND E. MORALISHVILLI, Chromosome examinations in patients on lithium carbonates, *Arch. Gen. Psychol.*, 24 (1971) 166–168.
4 M. S. LEGATOR, Chemical mutagens, *Ann. Rev. Med.*, 23 (1972) 413–428.
5 Evaluation and testing of drugs for mutagenicity: Principles and problems, *W. H. O. Scientific Report*, No. 482, Geneva, 1971.
6 K. HIRSCHHORN AND M. M. COHEN, Drug-induced chromosomal aberrations, *Ann. N.Y. Acad. Sci.*, 151 (1968) 977–987.
7 M. S. LEGATOR AND H. V. MALLING, in A. HOLLAENDER (Ed.), *Environmental Chemical Mutagens*, Plenum, New York, 1970 pp. 569–589.
8 W. SCHMID, D. T. ARAKAKI, N. A. BRESLAU AND J. C. CULBERTSON, The Chinese hamster bone marrow as an "*in vivo*" test system, I. Cytogenetic results on basic aspects of the methodology obtained with alkylating agents, *Humangenetik*, 11 (1971) 103–118.
9 L. FISHBEIN, Pesticidal, industrial, food additive and drug mutagens, in H. E. SUTTON AND M. I. HARRIS (Eds.), *Mutagenic Effects of Environmental Contaminants*, Academic Press, London, 1972, p. 129.
10 B. MATTER AND W. SCHMID, Trenimon-induced chromosomal damage in bone marrow cells of six mammalian species, evaluated by the micronucleus, *Mutation Res.*, 12 (1971) 417–425.
11 D. T. ARAKAKI AND W. SCHMID, The Chinese hamster bone marrow as an "*in vivo*" test system, II. Correlation with "*in vitro*" results on Chinese hamster fibroblasts and human fibroblasts and lymphocytes, *Humangenetik*, 11 (1971) 119–131.
12 K. E. HAMPEL, B. KOBER, D. ROSCH, H. GERHARTZ AND K. H. MEINIG, The action of cytostatic agents on the chromosomes of human leukocytes "*in vitro*", *Blood*, 27 (1966) 816–823.
13 W. SCHMID AND G. R. STAIGER, Chromosome studies on bone marrow from Chinese hamsters treated with benzodiazepine tranquillizers and cyclophosphamide, *Mutation Res.*, 7 (1969) 99–108.
14 N. P. BISHUN, Suppression of DNA synthesis in phytohaemagglutinin stimulated lymphocytes by prednisone treatment, *Experientia*, 24 (1968) 718.

15 M. K. JENSEN, Chromosome studies in patients treated with azathioprine and amethopterin, *Acta Med. Scand.*, 182 (1967) 445–455.
16 R. BISCHOFF AND H. HOLTZER, The effect of mitotic inhibitors on myogenesis "*in vitro*", *J. Cell Biol.*, 36 (1967) 111–127.
17 W. R. BELL, J. J. WHANG, P. P. CARBONE, G. BRECHER AND J. B. BLOCK, Cytogenetic and morphologic abnormalities in human bone marrow cells during cytosine arabinoside therapy, *Blood*, 27 (1966) 771–781.
17a B. A. KIHLMAN, W. W. NICHOLS AND A. LEVAN, The effect of deoxyadenosine and cytosine arabinoside on the chromosomes of human leukocytes "*in vitro*", *Hereditas*, 50 (1963) 139–143.
18 J. WHANG-PENG, B. G. LEVENTHAL, J. W. ADAMSON AND S. PERRY, The effect of daunomycin on human cells "*in vivo*" and "*in vitro*", *Cancer*, 23 (1969) 113–121.
19 B. K. VIG, L. D. SAMUEL AND S. B. KONTRAS, Specificity of daunomycin in causing chromosome aberrations in human leukocytes, *Chromosoma*, 29 (1970) 62–73.
20 B. K. VIG, S. B. KONTRAS AND A. M. AUBELE, Sensitivity of G1 phase of the mitotic cycle to chromosome aberrations induced by daunomycin, *Mutation Res.*, 7 (1969) 91–97.
21 M. W. SHAW AND M. M. COHEN, Chromosome exchanges in human leukocytes induced by mitomycin C, *Genetics*, 51 (1965) 181–190.
22 K. HIRSCHHORN AND M. M. COHEN, Drug-induced chromosomal aberrations, *Ann. N.Y. Acad. Sci.*, 151 (1968) 977–987.
23 M. M. COHEN, K. HIRSCHHORN AND W. A. FROSCH, "*In vivo*" and "*in vitro*" chromosomal damage induced by LSD-25, *New Engl. J. Med.*, 277 (1967) 1043–1049.
24 W. F. GEBER, Congenital malformations induced by mescaline, lysergic acid diethylamide, and homolysergic acid in the hamster, *Science*, 158 (1967) 265–267.
25 H. SATO, E. PERGAMENT AND V. NAIR, LSD in pregnancy: Chromosomal effects, *Life Sci.*, 10 (1971) 773–779.
26 L. Y. HSU, L. STRAUSS AND K. HIRSCHHORN, Chromosome abnormality in offspring of L.S.D. user, *J. Am. Med. Ass.*, 211 (1970) 987–990.
27 D. G. GILMOUR, A. BLOOM, K. LELE, E. ROBBINS AND C. MAXIMILLIAN, Chromosomal aberrations in users of psychoactive drugs, *Arch. Gen. Psychiat.*, 24 (1971) 268–272.
28 J. F. MURPHY AND R. MULCATTY, The effect of age, parity and cigarette smoking on baby weight, *Am. J. Obstet. Gynecol.*, III, No. 1 (1971) 22–25.
29 N. P. BISHUN, N. LLOYD, R. W. RAVEN AND D. C. WILLIAMS, The "*in vitro*" and "*in vivo*" cytogenetic effects of nicotine, *Acta Biol.*, 23 (2) (1972).
30 D. H. CARR, Chromosome studies in selected spontaneous abortions: conception after oral contraceptives, *Canad. Med. Ass. J.*, 103 (1970) 343–348.
31 O. S. SINGH AND D. H. CARR, A study of the effects of certain hormones on human cells in culture, *Canad. Med. Ass. J.*, 103/4 (1970) 349–350.
32 L. F. JARVIK AND T. KATO, Is lysergide a teratogen?, *Lancet*, 1 (1968) 250.
33 I. MAUER, D. WEINSTEIN AND H. M. SOLOMON, Acetylsalicylic acid: no chromosome damage in human leukocytes, *Science*, 169 (1970) 198–201.
34 J. NEILSEN, U. FRIEDRICH AND T. TSUBOI, Chromosome abnormalities in patients treated with chlorpromazine, perphenazine and lysergide, *Brit. Med. J.*, 3 (1969) 634–636.
35 M. M. COHEN, K. HIRSCHHORN AND W. A. FROSCH, Cytogenetic effects of tranquilizing drugs "*in vivo*" and "*in vitro*", *J. Am. Med. Ass.*, 207 (1969) 2425–2426.
36 G. R. STAIGER, Studies on the chromosomes of human lymphocytes treated with diazepam *in vitro*, *Mutation Res.*, 10 (1970) 635–644.
37 D. WILKIE AND J. DELHANTY, Effects of chlorimipramine on human cells in tissue culture, *Brit. J. Exptl. Pathol.*, 51 (1970) 507–511.
38 T. D. BROCK, Chloramphenicol, *Bacteriol. Rev.*, 25 (1961) 32–48.
39 W. J. MITUS AND N. COLEMAN, "*In vitro*" effect of chloramphenicol on chromosomes, *Blood*, 35 (1970) 689–694.
40 H. KATO AND T. H. YOSHIDA, Non disfunction of chromosomes in a synchronised cell population initiated by reversal of colcemid inhibition, *Exptl. Cell Res.*, 60 (1970) 459–464.
41 N. P. BISHUN, J. MILLS AND D. WILLIAMS, Induction of chromosome abnormalities by reversal of colcemid inhibition in leucocyte cultures, *Experientia*, 27 (1971) 943–944.
42 A. C. STEVENSON, J. BEDFORD, A. G. S. HILL AND H. F. H. HILL, Chromosomal studies in patients taking phenylbutazone, *Ann. Rheum. Dis.*, 30 (1971) 487–500.
43 D. MULLER AND F. F. STRASSER, Comparative studies on the Chinese hamster bone marrow after treatment with phenylbutazone and cyclophosphamide, *Mutation Res.*, 13 (1971) 377–382.

44 U. FRIEDRICH AND J. NIELSEN, Lithium and chromosome abnormalities, *Lancet*, 2 (1969) 435–436.
45 P. S. THAYER, P. HIMMELFARB, R. H. LISS AND B. L. CARLSON, Continuous exposure of HeLa cells to caffeine, *Mutation Res.*, 12 (1971) 197–203.
46 LEE SUNG, Chromosome aberrations induced in cultured human cells by caffeine, *Jap. J. Genet.*, 46 (1971) 337–344.
47 B. A. KIHLMAN, S. STURELID, K. NORLEN AND D. TIDRIKS, Caffeine, caffeine derivatives and chromosomal aberrations. II. Different responses of Allium root tips and Chinese hamster cells to treatments with caffeine, 8-ethoxycaffeine and 6 methylcoumarins, *Hereditas*, 69 (1971) 35–50.
48 I. G. WALKER AND B. D. REID, The response of mammalian cells to alkylating agents, *Mutation Res.* 12 (1971) 101–104.
49 N. P. BISHUN, D. C. WILLIAMS AND R. W. RAVEN, Chromosomal damage to HeLa cells grown continuously in caffeine, *Mutation Res.*, 17 (1973) 145–146.
50 K. SAX AND H. J. SAX, Possible mutagenic hazards of some food additives, beverages and insecticides, *Jap. J. Genet.*, 43 (1968) 89–94.
51 D. STONE, E. LAMSON, Y. S. CHANG AND K. W. PICKERING, Cytogenetic effects of cyclamates on human cells "*in vitro*", *Science*, 164 (1969) 568–569.
52 B. M. CATTENACH AND C. E. POLLARD, Mutagenesis tests with cyclohexylamine in the mouse, *Mutation Res.*, 12 (1971) 472–474.
53 A. BROGGER, Chromosome damage in human cells induced by hydroxylamine, *Hereditas*, 69 (1971) 19–24.
54 I. D. ADLER, G. RAMARAO AND S. S. EPSTEIN, "*In vivo*" cytogenetic effects of trimethylphosphate and of TEPA on bone marrow cells of male rats, *Mutation Res.*, 13 (1971) 263–273.
55 A. A. BREGMAN, Cytogenetic effects of ethanol in human leucocyte culture, *EMS Newsletter*, No. 4, March 1971.
56 J. M. TOUGH AND W. M. COURT BROWN, Chromosome aberrations and exposure to ambient benzene, *Lancet*, (1965) 684.
57 A. FORNI AND L. MOREO, Chromosome studies in a case of benzene-induced erythroleukaemia, *European J. Cancer*, 5 (1969) 459–463.
58 P. PHILIP AND M. K. JENSEN, Benzene induced chromosome abnormalities in rat bone marrow cells, *Acta Pathol. Microbiol. Scand.* [*A*], 78 (1970) 489–490.
59 I. M. TOUGH, P. G. SMITH, W. M. COURT BROWN AND D. G. HARNDEN, Chromosome studies on workers exposed to atmospheric benzene, *European J. Cancer*, 6 (1970) 49–55.
60 K. A. PALMER, S. GREEN AND M. S. LEGATOR, Cytogenetic effects of DDT and derivations of DDT in a cultural mammalian cell line, *Toxicol. and Appl. Pharmacol.*, 22 (1972) 355–364.
61 H. KALLAK AND L. YARVEKYLG, On the cytogenetic effects of 2,4-D on pea callus in culture, *Acta Biol. Acad. Sci. Hung.*, 22 (1971) 67–73.
62 G. H. MICKEY AND H. HOLDEN, Chromosomal effects of chlorine on mammalian cells "*in vitro*", *EMS Newsletter*, No. 4 (March 1971).
63 R. E. ZELAC, H. L. CROMROY, W. R. BOLCH, B. G. DUNAVANT AND H. A. BEVIS, Inhaled ozone as a mutagen, II. Effect on the frequency of chromosome aberrations observed in irradiated Chinese hamsters, *Environ. Res.*, 4 (1971) 325–342.
64 E. C. MILLER AND J. A. MILLER, The mutagenicity of chemical carcinogens: correlations, problems and interpretations, in A. HOLLAENDER (Ed.), *Chemical Mutagens*, Plenum, New York, 1970, pp. 83–119.
65 O. G. FAHMY AND M. J. FAHMY, Mutagenic properties of *N*-acetyl-2-amino fluorene metabolites in relation to the molecular mechanisms of carcinogenesis, *Intern. J. Cancer*, 9 (1972) 284–298.
66 O. G. FAHMY AND M. J. FAHMY, Mutagenic selectivity for the RNA-forming genes in relation to the carcinogenicity of alkylating agents and polycyclic aromatics, *Cancer Res.*, 32 (1972) 550–557.
67 M. M. COHEN AND A. B. MUKHERJEE, Meiotic chromosome damage induced by LSD-25, *Nature*, 219 (1968) 1072–1074.
68 J. LEDERBERG, in S. S. EPSTEIN AND M. S. LEGATOR (Eds.), *The Mutagenicity of Pesticides: Concepts and Evalution*, Massachusetts Inst. Technol., Boston, 1971.