THE EFFECTS OF 1-β-D-ARABINOFURANOSYLCYTOSINE AND ADRIAMYCIN ON THE CHROMOSOMES OF CULTURED HUMAN LYMPHOCYTES.

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ABSTRACT (Taken from Summary p.87)

A study of chromosome aberrations induced by 1-β-D-arabinofuranosylcytosine (Ara C) and Adriamycin (AM) in the chromosomes of cultured human lymphocytes was made.

There were significant increases in the frequency of aberrations with increasing concentrations of both Ara C (2.5, 5.0 and 10.0 μg/ml) and AM (0.01, 0.05, 0.10 and 0.15 μg/ml). The frequency of aberrations induced by both drugs also showed a 'levelling off' above particular concentrations. For Ara C the effect of increasing treatment time was also studied. The frequency of aberrations increased significantly with increasing treatment times (2, 3 and 4 hrs) although no 'levelling off' in the number of aberrations was observed.

The relationship between the frequency of the different types of aberrations induced by Ara C and AM was studied. AM allowed for a study of the relative frequency of chromosome versus chromatid aberrations and fragment versus exchange aberrations. There were always more fragments than exchanges, and always more chromatid aberrations than chromosome aberrations. Aberrations induced by Ara C were all of the chromatid fragment type.

A study was made of the distribution of inter- and intra-chromosomal aberrations in relation to light and dark G banded chromosomes. Both drugs induced more aberrations in the light G bands than the dark G bands. Both drugs showed distinct clustering of aberrations in some regions of the chromosomes (hotspots), although the location of AM induced hotspots was different from the location of those induced by Ara C. The distribution of AM induced chromatid aberrations was different from the distribution of the chromosome aberrations, as were the distributions of the fragment and exchange aberrations. The different types of aberrations also differed in the number of AM induced aberrations per unit length between the p and q arms. There were more aberrations per unit length in the p arm than in the q arm for exchanges, whereas for fragments and chromosome aberrations the reverse was true. For chromatid aberrations, there was no significant difference in the number of aberrations per unit length between the p and q arms.

Inter-individual differences in the frequency of AM induced aberrations were observed in the AM dosage experiments. Also there was a suggestion that the distribution of Ara C induced aberrations was different for different donors.

AM increased the frequency of sister chromatid exchanges. Comparable results were not sought for Ara C because after cells were exposed to Ara C they did not pass through an S phase of the cell cycle, as is the case for cells exposed to AM.

The relevance of the present in vitro studies to cancer chemotherapy is briefly discussed.

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INTRODUCTION

(a) General

Many chemical agents, including chemotherapeutic drugs, are known to cause chromosome damage in cultured cells of humans and other organisms (Kihlman, 1966; Shaw, 1970). Knowledge gained by studying the interaction of chemical agents with chromosomes of cultured human cells should provide information on the extent and location of the various types of aberrations that these chemical agents produce. It is not necessarily the chromosomal aberrations *per se* but the mechanisms by which they arise, that are of prime importance; these may ultimately lead to information concerning chromosome structure and function.

The most frequently used human cytogenetic test system for the investigation of exogenous agents involves the use of the peripheral lymphocyte. The advantages of this cell type derive from its ease of access and relatively simple culture technique, yielding large numbers of mitotic cells suitable for study. Using this cell type an investigation was made here of the effects of 1-β-D-arabinofuranosyl-cytosine (Ara C) and Adriamycin (AM) on breakage in human chromosomes.

(b) Chromosome Aberrations

The types of structural change that can occur within or between chromosomes and chromatids are of two types, namely the simple break and the exchange. A simple break results in a chromosome deletion and a chromosome fragment. If two breaks occur in reasonably close proximity to each other, they may interact to form an exchange. Aberrations may be further subdivided into chromosome or chromatid aberrations, depending upon the unit of breakage involved. Chromosome aberrations involve either a break or an exchange at the same locus of sister chromatids, whereas chromatid breaks or exchanges involve only a break at one of the sister chromatids. Chromosome breaks are the result of a lesion that has occurred before DNA replication. At replication, the lesion is reproduced in both chromatids.

Similar appearing lesions which occur after DNA replication are called isochromatid breaks. Exposure of cells to mutagens in G1 results in chromosome type aberrations, whereas those produced in G2 are of the chromatid type. Cells treated in the S phase show both chromosome and chromatid breaks.

There are two main hypotheses used to explain the relative frequency and mechanism of the different types of chromosomal aberrations. The first is the "Breakage First Hypothesis" (Evans, 1962; Lea, 1955). The basis of this hypothesis comes from studies on chromosome damage produced by X-irradiation (Staedler, 1931; Sax, 1940). The immediate effect of X-irradiation is interpreted in this hypothesis as being breakage, after which there are three possibilities: restitution can occur, or the breaks can remain open or the breaks can interact to produce an exchange. The breaks are single hit events, and exchanges are two hit events. The main evidence for this hypothesis comes from the observation that the frequency of breaks increases linearly with the dose of the radiation, while the frequency of exchanges increases with the square of the dose (Sax, 1939, 1940, 1941; Lea, 1955).

The second hypothesis, the "Exchange Hypothesis" (Revell, 1959, 1966), suggests that the immediate effect of X-irradiation is the production of a "primary event" which, though not specified, is not a break, and normally would heal unless two "primary events" occur close together. If this occurs then a second stage, called exchange initiation, is reached, during which exchanges between the two chromatids can take place. The main evidence for this theory comes from many studies in which the frequency of breaks has been reported to increase with the square of the dose (e.g. Brewen and Brock, 1968; Fox, 1967; Revell, 1966). If breaks are the result of an exchange between two "primary events", then a dose squared relationship is expected. The similarity between the exchange hypothesis and the meiotic crossing-over process has been noted by several authors (Östergren and Wakonig, 1955). Normally the crossing over process takes place under cellular control at pachytene of meiosis. It is possible that mutagens may create a situation in the mitotic cell which leads to a similar kind of process. Kihlman (1966)

points out that since the induced exchanges are presumably not under cellular control, it is not surprising that they are asymmetrical and frequently occur between heterologous chromosomes.

It has not been determined which theory is more correct. although it should be noted that both hypotheses have been based on information following X-irradiation; hence it does not follow that aberrations produced by chemicals should fit either hypothesis. It is also possible that one hypothesis may explain the mechanism of chromosome breakage in some instances while the other hypothesis may be applicable in others.

Bender, Griggs and Bedford (1974) formulated a general theory to explain aberrations produced by chemicals. The theory is based largely on the conclusions of Kihlman (1966). Bender et al. (1974) divided chemicals into four categories depending on their effects and stage of the cell cycle at which they operate, as follows:

(1) Compounds producing gaps and deletions in late S and G_2 cells.

These compounds are inhibitors of the biosynthesis of DNA and DNA precursors. The thymidine analogue 5-fluorodeoxyuridine is perhaps the best known (Kihlman, 1955). Ara C is also thought to belong to this group. The mode of action of 5-fluorodeoxyuridine has been explained by the failure of the chromosome to complete DNA synthesis, owing to the lack of one or more nucleotide precursors, with gaps being left in the newly synthesized polynucleotide strand as a consequence. Aberrations could be produced in the G_2 nucleus by a mechanism described by Ahnstrom and Natarajan (1966), who postulated that a precursor deficiency (caused by failure of the biosynthesis of one or more of the nucleotide triphosphates in late S) might somehow result in the expression of a nuclease activity in the G_2 nucleus. The main effect of compounds in this class is the production of single polynucleotide strand breaks, either directly through failure to complete polynucleotide chain assembly, or indirectly, perhaps through stimulation of a nuclease activity.

(2) Compounds producing chromatid aberrations of all types in late S and G_2.

Compounds in this category produce chromatid aberrations of all types, including isochromatid and exchange types, when the cells are treated in late S and the G_2 phases of the cell cycle. They can also produce sub-chromatid aberrations in cells treated in prophase, and also some chromosome aberrations in cells treated while in their G_1 phase. This is, in fact, exactly the pattern of aberration production associated with ionizing radiations. Compounds of this class produce double strand DNA breaks. It is thought that some are able to produce double-scissions directly, while others produce single strand gaps that are particularly subject to attack by a single strand nuclease. 8-ethoxycaffeine (Kihlman, 1955) and streptonigrin (Kihlman, 1964), are examples of compounds in this category. It is also thought that Ara C may fit into this class, as well as the first class mentioned above.

(3) Compounds producing chromatid aberrations of all types, but only in cells treated in G_1 and early S.

Most compounds producing chromosomal aberrations fall into this class. Compounds of this class are known to react directly with DNA and DNA precursors and produce only aberrations of the chromatid type (Evans and Scott, 1969). They produce a chemical alteration of polynucleotide chains (but not a break) which, if still present when the cell reaches the S phase, prevents local completion of the synthesis on a new polynucleotide chain. Compounds in this category are thought to act only in S because they cause gaps in the newly synthesized strand opposite the lesions they produced in the old DNA. Since the DNA is single stranded at this point it is subject to attack by a single strand nuclease, thus producing double-strand breaks. These scissions constitute chromatid breaks unless repaired by recombinational, or post replication, repair processes. Examples of compounds in this class are the alkylating agents (Evans and Scott, 1969 for example). It is also thought that AM may fit into this class.

(4) Compounds producing chromatid aberrations of all types due to repair inhibition.

Compounds that inhibit normal enzymatic DNA repair fit into this category. Three forms of repair may be distinguished: excision repair, recombinational or post replication repair, and single strand break repair. Kihlman (1966) has listed a number of compounds that may fit into this class as, e.g., theophylline and theobromine, which are known inhibitors of recombinational DNA repair and also known to induce chromosomal aberrations.

(c) Sister Chromatid Exchange

As well as inducing chromosome breaks, many chemical agents, irradiation, and some genetic disorders have been shown to increase the rate of sister chromatid exchange (SCE). SCE's involve an exchange between sister chromatids essentially at identical loci. The first observation of SCE was made by Taylor (1958) who differentially labelled the sister chromatids using tritiated thymidine. Since then methods which give better resolution of differentially stained sister chromatids have been developed. Zakharov and Egolina (1972) found that when Chinese hamster cells were treated with 5' bromodeoxyuridine (BUdR) for two rounds of DNA replication and then stained with Giemsa, the two sister chromatids are stained differentially. The chromatids which are bifiliarly substituted with BUdR stain weakly compared to unifiliarly substituted chromatids, which are darkly stained. Since then other methods of differential staining of chromatids have been developed (Latt, 1973; Kato, 1974; Perry and Wolff, 1974; Korenberg and Freedlander, 1974).

SCE's have proved to be sensitive indicators of carcinogenic and mutagenic agents. Perry and Evans (1975) treated Chinese hamster cells with 14 known or suspected mutagens or carcinogens and found that all of the direct acting mutagens gave significantly increased SCE's.

There is considerable evidence that the mechanism involved in the production of chromatid breaks is different from that causing SCE's, including the fact that certain genetic conditions, such as Fanconi's anemia and ataxia

telangiectasia, which predispose cells to an increased frequency of chromosome breaks, produce no enchancement of SCE's (Galloway and Evans, 1975; Sperling, Wegner, Riehm and Obe, 1975). Little is known about the lesions that lead to SCE's. Lesions that lead to SCE's are thought to be induced when the cells are in S. The lesions induced in G_2 do not give rise to exchanges until after the cell has passed through an S phase (Wolff, Bodycot, and Painter, 1974). Whatever the nature of the lesions in the chromosomes that ultimately lead to the formation of SCE's, it is thought that the lesions will be quite different from those that lead to chromosome aberrations. SCE's, for instance, are induced at high frequencies by chemicals that induce very low frequencies of aberrations (Latt, 1974; Perry and Evans, 1975); and they are not markedly increased by low doses of ionizing radiation, whereas chromosome aberrations are (Perry and Evans, 1975; Wolff et al., 1974).

It seems likely that SCE's can arise spontaneously (Kato, 1974). Wolff, Rodin, and Cleaver (1977) have postulated that although the majority of SCE's are genetically neutral, because equal amounts of sister chromatids are exchanged, some unequal exchanges could occur leading to, for example, a deletion or an insertion. The exact biological significance of sister chromatid exchange is still unknown.

(d) Chromosome Banding

Chromosome banding now allows one to pin-point more accurately the location of breaks along a chromosome. The distribution of breaks associated with various chemical and radiation treatments, as well as "spontaneous" breaks associated with some genetic disorders, has suggested a predominance of breaks in the light staining regions of quinacrine (Q) and Giemsa (G) banded chromosomes (Aula and von Koskull, 1976; Honeycombe, 1978; Savage, Watson and Bigger, 1973, and Seabright, 1973). The reason why breaks preferentially occur in light bands is not fully understood. Q and dark G bands are thought to be late replicating and rich in adenine and thymidine while interbands are early

replicating and guanine and cytosine rich (Comings et al., 1973). Holmberg and Jonasson (1973) hypothesized that DNA may be protected differentially in certain chromosome regions by proteins, which may also be responsible for the banding patterns; and that R banded regions may be less contracted than others and, therefore, constitute a larger target.

(e) Distribution of Chromosome Breaks

The distribution of chromosome breaks over the chromosome complement has been studied in both banded and unbanded preparations. Most studies show a localization of breaks (hotspots) in regions of particular chromosomes or chromosome groups (e.g. San Roman and Bobrow, 1973; Morad, Jonasson and Lindsten, 1973; Funes-Cravioto, Yokavienko, Kuleshov and Zhurkov, 1974; and Honeycombe, 1978). The localization of specific chromosomal breakage may be useful in determining the structure of chromosomes, particularly if the action of the external agent under study is well understood.

Different agents generally induce aberrations in different regions of the chromosomes. Even studies using identical agents can induce different non-random location of breaks. For example, Casperson, Haylund, Lindell and Zech (1972) reported that X-irradiation induced more breaks in the centromeric and distal portions of the chromosomes, whereas Seabright (1973) found a paucity of breaks in the distal regions of the chromosomes. The difference in the distribution of chromosome aberrations using the same agent may be explained by the different timing of treatment, different concentrations, different donors, or even, as Sutherland (1977) reported, a difference in culture medium.

(f) Inter-individual Responses to External Agents

Inter-individual response, in respect of either the distribution of breaks over the chromosome complement, or differences in breakage rate, caused by an external agent warrant careful consideration.

Fragile sites on particular chromosomes are known in some cases to be inherited (Fergusson-Smith, 1973; and Giraud, Aymé, Mattei and Mattei, 1976). Age differences have

also been associated with inter-individual responses to various agents (Liniecki, Bajerska, Andryszek, 1971; Bochkov and Kuleshov, 1972). Bochkov and Kuleshov (1972) studied the effect of degranol on lymphocyte chromosomes and demonstrated that the distribution of chromosome breaks varied irregularly with the age of the donor. However, in the same study age differences between individuals had no effect on the distribution when thio-tepa, rather than degranol, was used. Similarly, no differences in the distribution of breaks between people could be demonstrated in studies of patients suffering from anemia or measles (von Koskull and Aula, 1977) nor with treatments with mitomycin C (Morad et al., 1973), thio-tepa and degranol (Funes-Cravioto et al., 1974) or X-irradiation (Seabright, 1973). Differential effects in the distribution of breaks between people, therefore, is very much an open and interesting question.

(g) Ara C and AM

(1) Ara C

Ara C is a synthetic nucleoside analogue of 2'-deoxycytidine in which the deoxyribose of 2'-deoxycytidine is replaced by arabinose. Ara C is rapidly transported into the cell, where it is phosphorylated into its active form, Ara CTP (Momparler, Brent, Labitan, Krygier, 1971; Schrecker, 1968).

The method by which Ara C causes chromosome damage is not fully understood. Brewen and Christie (1967) studied the effects of Ara C on lymphocyte cultures and suggested that the induction of aberrations was closely related to a cell's nucleoside pool and nuclease activity, in a way very similar to that proposed by Ahnstrom and Natarajan (1966). That is, breakage in G_2 (or G_1) is due to a decrease in the triphosphate pool, for example, favouring the breakdown of DNA by DNA polymerase. Such a decrease would result from blockage of dCDP and dCTP production by Ara C.

A number of authors have studied the effects of Ara C on chromosomes in lymphocyte cultures (Kihlman, Nichols, Levan, 1963; Brewen, 1965; Brewen and Christie, 1967; Brehaut and Fitzgerald, 1968; Ayraud, Cantnelle and Lloyd, 1976). Brewen (1965) found that Ara C affected cells that were in G_2, and that cells in the S phase were either slowed or stopped in their progress to metaphase. Others, using different tissue cultures, have also shown that the cell cycle can be virtually stopped at the S phase in the presence of Ara C, and so long as the concentration is not cytotoxic, removal of Ara C will allow the cells to proceed through the S phase to G_2 (Graham and Whitmore, 1970; Jones, Baker and Benedict, 1976; Karon, Henry, Weissman and Meyer, 1966). Brewen and Christie (1967) reported that Ara C produced chromosome damage in the G_1 and S phase of the cell cycle and this was later confirmed by Benedict, Harris and Karon (1970).

In the present study advantage was taken of the cells inability to pass through the S phase in the presence of Ara C. By using Ara C a few hours before harvest one may be assurred that all aberrations are of the chromatid rather than the chromosome type, thus preventing confusion between iso-chromatid and chromosome type breaks.

The distribution of chromosome breaks over the chromosome complement was studied by Ayraud et al. (1976). They reported that nearly all breaks were located in the light regions of R banded preparations, i.e. the equivalent dark bands of Q and G banded preparations. They also reported that although the distribution of breaks was consistent for the five individuals studied, the frequency of breaks at certain sites was significantly higher than at others. These included 1p31, 3p14, 5p14, 5q21, 7p31 and 9q1. Ara C has been shown to increase SCE's in Chinese hamster cells (Benedict and Jones, 1979). Wolff et al. (1974) demonstrated that ultra-violet irradiation can promote conditions conducive to SCE formation at any stage of the cell cycle, but this results in SCE's only when the cells pass through the S phase. It is not known whether Ara C has the same effect. If it has then one would theoretically not expect an elevation of the SCE frequency after Ara C treatment, since all cells that were scored at metaphase were in late S or G_2 at the time of treatment (because of the effect of Ara C on the cell cycle;

see above). This expectation was tested in the present study.

(2) AM

AM is an anthracycline antibiotic isolated from Streptomyces peucetius var. caesius. The cell kinetics of AM are not well known although it has been shown that its biological activity is related to its ability to bind specifically with DNA by intercalation between adjacent base pairs of the DNA, resulting in the inhibition of nucleic acid synthesis (Di Marco, 1975; Di Marco, Zanino, Silvestrini, Gambarucci and Gambetta, 1971; Pigram, Fuller and Hamilton, 1972; Yamamoto, Acton and Henry, 1972; Schwartz, 1976; Goodman, Lee and Bachur, 1977).

Aberrations induced by AM are of both chromatid and chromosome types and include fragments as well as exchanges. The effect of AM appears to be cell cycle specific. Using Chinese hamster cells, Hittleman and Rao (1975) have shown that AM does not delay the progression of G_1 cells into the S phase of the cell cycle but does prolong the duration of the S and G_2 phases, and this effect was dose dependent. The prolongation of the G_2 phase was greater than the prolongation of the S phase. AM is capable of inducing chromosome damage at all stages of the cell cycle, although cells treated during G_1 appear to have relatively fewer chromosome aberrations than those treated in S and G_2 (Vig, 1971, 1973). Hittleman and Rao (1975) also showed that cells exposed to AM during S are more sensitive to chromosome aberrations than those treated in G_2.

A number of authors have studied the effect of AM on cultured human lymphocytes (Vig, 1971, 1973; Massimo, Dagan-Bricarelliand Fassati-Guglielmoni, 1970; Nevstad, 1978). Vig (1971), using unbanded preparations, reported that the distribution of exchange points along the chromosome was non-random. Chromosomes 21 and 22 had more exchanges per unit length than expected and chromosome 3 and the Y chromosome showed a paucity of exchanges.

Nevstad (1978) studied the effect of AM on the frequency of SCE's in cultured human lymphocytes. He reported that at very low doses of AM a significant increase in SCE's could be detected when only a few chromosomal aberrations could be detected.

(h) Aims of this Study

Following preliminary investigations aimed at ascertaining suitable concentrations of Ara C for use in inducing chromosome breaks and for obtaining some knowledge of the effect of the drug on the cell cycle, the following aims were formulated.

(i) To record the effect of different concentrations of Ara C and AM on the frequency of chromosome breakage in order to study the dose kinetics of these two drugs.

(ii) To record the effects of different treatment times on the frequency of Ara C induced aberrations in order to provide information on the sensitivity of the chromosomes to breakage at different periods of time during late S and the G_2 phases of the cell cycle. A similar approach was not extended to AM since the stage of the cell cycle in which Ara C induced aberrations arise could not be accurately identified.

(iii) To compare the frequency of SCE's with the frequency of chromosomal aberrations for different concentrations of AM. A similar comparison could not be made for Ara C because no increase in the frequency of SCE's above the controls was expected with Ara C since the cells studied did not pass through an S phase.

(iv) To record the frequencies of the different types of aberrations induced by Ara C and AM. For AM this allowed for a study of the frequency of chromatid versus chromosome aberrations and the frequency of fragment versus exchange aberrations. Aberrations induced by Ara C were all of the chromatid fragment type, which allowed for a study of this one class of aberration without confusion from other classes.

(v) To record the distribution of Ara C and AM induced aberrations over the chromosome complement, both intra- and inter-chromosomally, in order to relate, if possible, the location of the aberrations to chromosome structure.

Particular attention in all of the above aims was given to the possibilities of inter-individual differences. All results were analysed using the "Teddybear" statistical package (Wilson, 1979). This allowed for a powerful and methodical approach for the analysis of the results.

METHODS

Human peripheral blood lymphocytes were cultured using a standard microculture technique (e.g. Hungerford, 1965; see appendix I for details). Briefly, heparinized blood lymphocytes were stimulated with PHA-m (Difco) and cultured in Hams F10 medium supplemented with AB serum. Colchicine (Sigma) was added 1½ hours prior to harvest at a final concentration of 1 µg/ml. Hypotonic treatment was in 0.4% KCl solution for 15-30 minutes. The cells were fixed in chilled methanol-glacial acetic acid (3:1) mixture, centrifuged several times until the cell suspension was clear and dropped onto chilled, moist slides and flame dried.

Chromosome aberrations were scored in lymphocytes treated with Ara C or AM as follows.

Ara C: Ara C was added at final concentrations of 2.5, 5.0 or 10.0 µg/ml and treatment times before fixation were 2, 3 or 4 hours. Deionized water was added in the place of Ara C to the control cultures set up for each experiment. Five donors (3 females and 2 males) ranging in age from 19-26 years old were used. 200 cells were scored at each concentration and each control. Replicate cultures were not set up because of the problems involved in handling too many separate lymphocyte cultures at any one time. The above experiments required the handling of 60 separate cultures more-or-less together.

AM: Cells to be treated with AM were initially grown for 40 hours. At 40 hrs, AM was added at final concentrations of 0.01, 0.05, 0.10 or 0.15 µg/ml. Treatment time was for 6 hours. At 46 hours the cells were centrifuged out of the medium containing AM and washed 3 times in Hanks Balanced Saline solution and recultured in fresh medium for another 20 hours before fixation. Deionized water was added in place of AM to the control cultures set up in each experiment. 5 donors (3 females and 2 males) ranging in age from 19-26 years old were used. Three of these donors were the same as those used in the Ara C experiments. One replicate culture was set up in each case, making a total of 60 separate lymphocyte cultures to be handled at any one time. 200 cells were scored at each concentration.

Examination of the location of chromosome breaks was conducted on lymphocytes to which Ara C was added 3 hours before fixation at a final concentration of 5.0 µg/ml. In the case of AM, the 0.05 µg/ml concentration was used. These treatments gave the best results for studying chromosome breaks as there was a good percentage of cells with breaks, but the mitotic index was not too severly affected. For Ara C, 400 cells were studied from 3 of the donors. Only 250 cells could be studied from the other 2 donors because of the poor quality of the G banding on some of the slides. For AM, 400 cells were studied from each donor.

The effect of increasing concentrations of AM and Ara C on the SCE frequency was recorded in lymphocytes using the same treatment times as used in the G banding experiments above. For AM, the concentrations used were 0.01 and 0.05 µg/ml, and for Ara C, 2.5 and 10.0 µg/ml. For Ara C, four of the five donors from the dosage experiments were used for AM the same five from the AM dosage experiments were used. Deionized water was added in place of either Ara C or AM to the control cultures. One replicate culture was set up in each case and at least 25 cells were scored from each culture.

For G banding, flame dried preparations were incubated at 60^{o}C overnight and then treated with trypsin, prepared by adding deionized water to dehydrated trypsin (Difco) and made up to a 0.05% solution in 0.06M phosphate buffer at pH 8.0. Treatment with trypsin was for 1-2 minutes, after which the slides were thoroughly washed in phosphate buffer and stained in 10% Giemsa (BDH) for 6-8 minutes.

For differential staining of the chromatids (see Perry and Wolff, 1974) bromodeoxyuridine (BUdR) (Sigma) was added to the culture medium at a final concentration of 10 µg/ml All cultures were incubated in the dark. Flame dried slides were left for 5 days after preparation and then immersed in 1.0 µg/ml Hoechst 33258 for 12 minutes, rinsed in 2 x sodium saline citrate (SSC) and finally mounted with a coverslip in 2 x SSC. Slides were placed in a moist chamber and exposed to daylight for 24 hours, followed by removal of the coverslip and incubation in 2 x SCC at 60^{o}C for 2 hours, rinsing in phosphate buffer and staining in 10% Giemsa for 10-12 minutes.

Slides were coded by an assistant such that scoring was done "blind". Cells containing 46 chromosomes were selected under 125X magnification solely on the basis of adequacy of the chromosome spread (aberrations cannot be detected with any confidence at this magnification). All cells so selected were subsequently scored under 1250X magnification. G banded cells were selected under 1250X magnification solely on the basis of the quality of the G bands, and all cells so selected were either scored microscopically or photomicrographically.

The classification of Schoeller and Wolf (1970) was used to distinguish chromatid and chromosome breaks from gaps. Briefly, a break occurs when the "broken" chromatid or chromosome is completely displaced, or displaced at an angle greater than 90^{o} from either the other chromatid or the rest of the chromosome. However, when studying banded preparations one modification to this procedure was made. A break was scored regardless of whether or not the "broken" ends were displaced if the amount of missing chromatin was greater than the width of the chromatid under study. Aberrations other than those so defined above constitute gaps, and were not included in any of the analyses. For scoring of aberrations, standard criteria were used (e.g. Comings, 1974). In the case of Ara C only two types of breaks occurred, namely isochromatid and chromatid breaks (p. 9). Isochromatid breaks were scored as only one break rather than two. The classification of Vig (1971) was used for scoring the various types of breaks induced by AM. Isolated fragments (or breaks) were of the chromatid type if only one of the two chromatids was broken or deleted at any one point; where both chromatids were affected, the two sister fragments were scored as one chromosome fragment. Exchange and intra-chromosomal reunions were classed as chromosome or chromatid breaks, depending upon whether the origin could be traced to a single stranded or double stranded chromosome. Thus dicentrics and rings were considered as chromosome exchanges, whereas triradials were classed as chromatid exchanges.

The 1971 Paris Conference convention was used for assigning breakpoints to G bands, except that 6p11 and 6p12 could not be distinguished and were jointly labelled 6p11, as in the Paris Conference supplement of 1975. Relative sizes of chromosomes were calculated using the data of von Koskull and Aula (1973).

Data were analysed using the "Teddybear" computer programme of Wilson (1979). This programme can be used for analysis of variance, covariance, multivariate analysis of variance, discriminate analysis, and regression and correlation.

RESULTS

A. Effect of Dose of Ara C and Treatment Times on the Chromosome Aberration Frequency.

Ara C induced isochromatid and chromatid breaks (Fig. 1 exemplifies the breaks). An analysis of covariance showed that there was a significant ($p < 0.01$) linear relationship between chromatid (C) and isochromatid (I) breaks, specified by the equation

$$C = 22(5.0) + 1.6(0.2)I,$$

where the figures in brackets represent the standard errors (see appendix 5 for regression graph). The slope of the relationship does not change with concentration levels ($p = 0.82$), different treatment times ($p = 0.83$), nor treatment time by concentration ($p = 0.94$). Therefore the chromatid and isochromatid aberrations were pooled.

Because no replicate cultures were set up for the studies of the effects of different concentrations and treatment times of Ara C (p. 13) the differences in the number of breaks between donors were treated as the experimental error (Table 1). Table 1 shows that the variance attributable to donor variation (375.37) is very small compared to the variance attributed to either concentration (13915.27) or treatment time (14446.47). This suggests that the donor variation has very little influence on the amount of damage induced by Ara C for different concentrations and different treatment times. Therefore the data from the five donors was pooled.

Figs. 2 and 3 summarize the effects of Ara C at three concentrations and three periods of time before fixation. There was a significant difference in the breakage rate with both increasing concentration and increasing treatment time (Table 1). A Duncan's multiple range test showed that at all treatment times the effect of 5 µg/ml was significantly greater than the effect of 2.5 µg/ml ($p < 0.01$). The difference in breakage rate being more pronounced at 3 and 4 hour treatment times than at 2 hours (Fig. 2). At all treatment times there were still more breaks at 10 µg/ml, though the differences between 5 and 10 µg/ml were not

statistically significant with the present sample size ($p > 0.05$). A Duncan's multiple range test showed that there were significantly more breaks with longer treatment times at all three doses ($p < 0.01$), except for the low dose, short treatment time of 2.5 µg/ml for 2 hours (Fig. 3). Fig. 3 also shows the observed mean number of breaks per 1000 cells, obtained by summing and averaging the data of the three doses for each treatment time; and an expected mean, calculated in the same manner after assuming a doubling of aberrations with a doubling of treatment time (Fig. 4). The increasing divergence of these two means with increasing treatment time suggests that the number of Ara C induced aberrations does not increase as rapidly as it would if there were a linear relationship between treatment time and breakage rate.

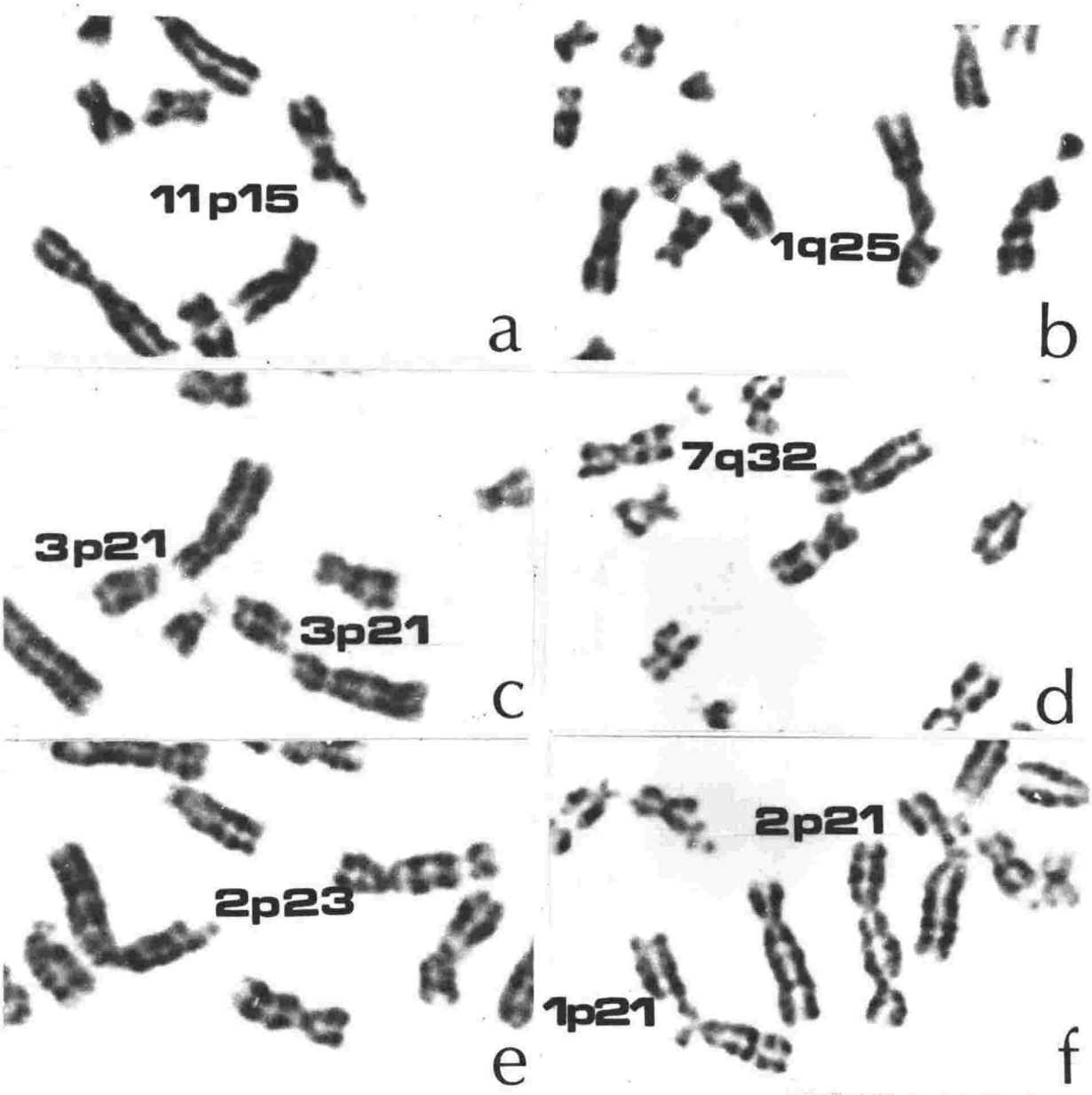
TABLE 1

Analysis of variance for the number of chromatid (including isochromatid) aberrations induced by Ara C at different concentrations and treatment times in cultured human lymphocytes.

SOURCE OF VARIATION	DF	MS	F	p
Concentration	2	13915.27	37.07	0.00
Treatment Time	2	14446.47	38.49	0.00
Concentration by Treatment Time	4	854.53	2.28	0.08
Error	36	375.37		
TOTAL	44	1673.97		

DF	Degrees of freedom
MS	Mean square
F	F-statistic
p	Probability

Fig.1. Aberrations induced by Ara C. Photographs a-f show chromatid aberrations. Photograph c also shows an isochromatid aberration.


11p15
a
1q25
b
3p21
3p21
c
7q32
d
2p23
e
2p21
1p21
f

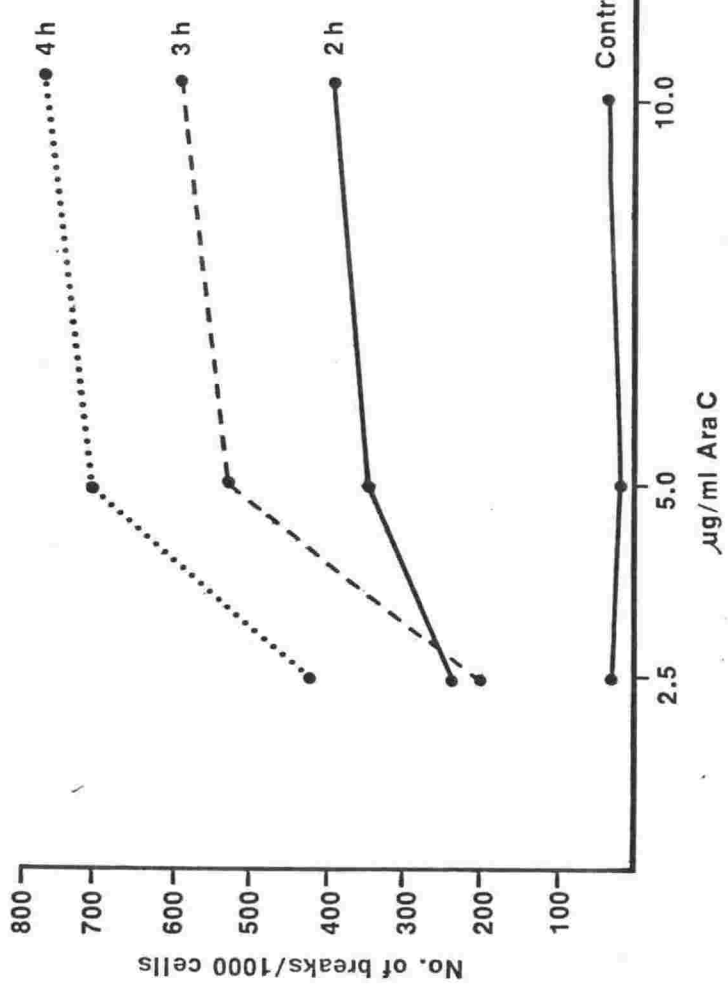


Fig. 2 Number of breaks caused by Ara C at 3 concentrations and 3 treatment times. Each point represents the data pooled from 5 donors

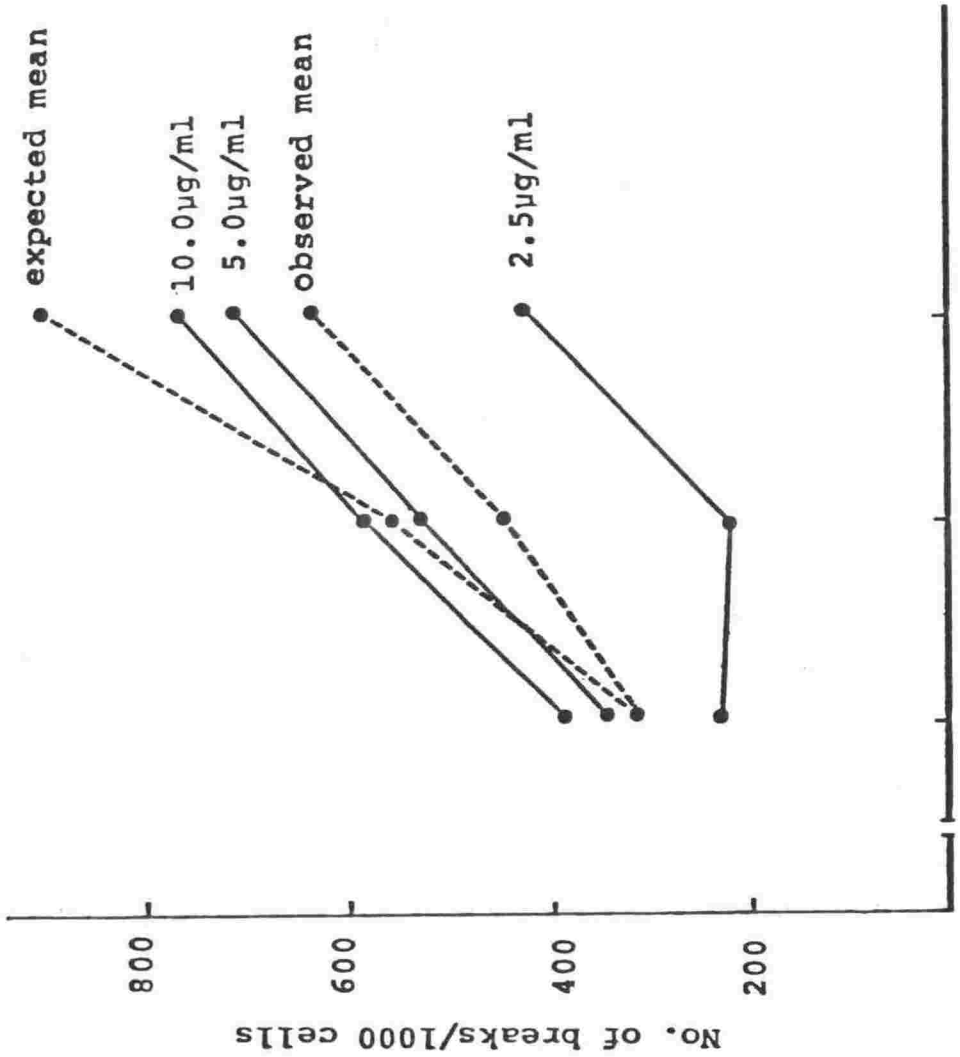


Fig.3 . Number of breaks caused by Ara C for 3 concentrations and 3 treatment times. Each point represents the pooled data from 5 donors. The observed mean was obtained by summing and averaging the data of the 3 doses for each treatment time. The expected mean was calculated by assuming a doubling of aberrations with a doubling of treatment time, calculated from the 2 hour treatment time.

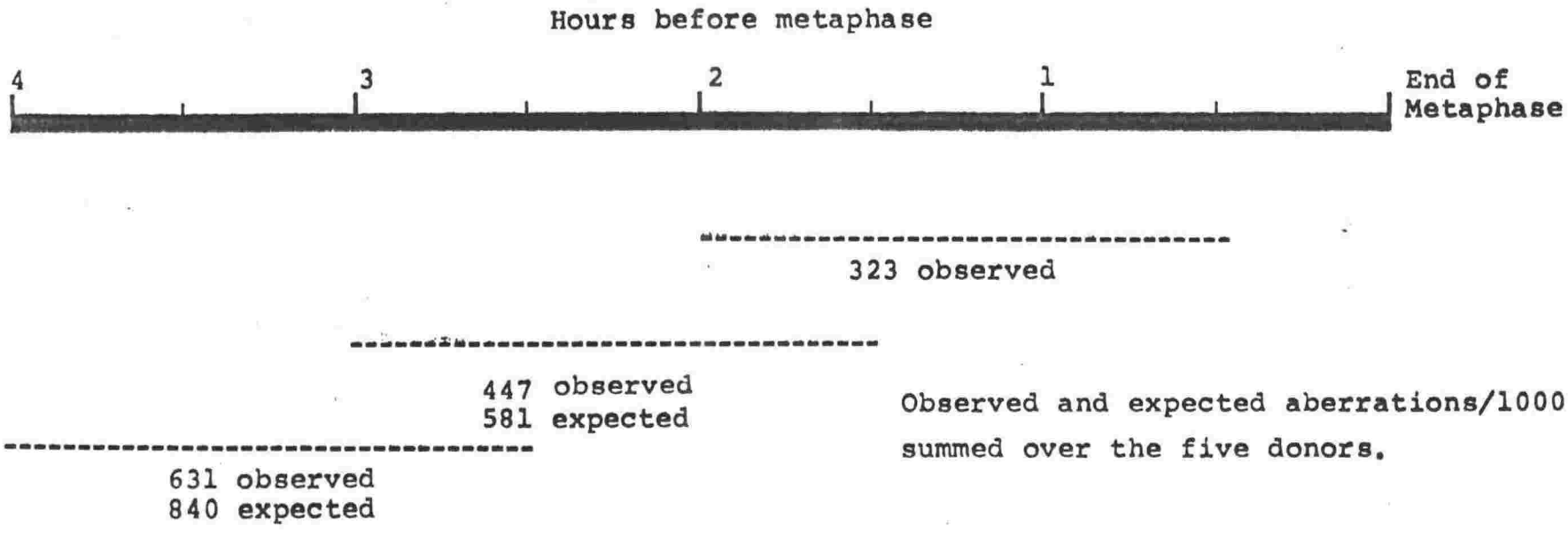


Fig. 4. The cell population scored at metaphase for each of the treatment times is represented by the dashed lines. Colchicine was added 1½ hours prior to fixation, regardless of the AraC treatment time.

Observed and expected frequencies of aberrations are given for each treatment time. Expected frequencies were calculated by assuming a doubling of aberrations with a doubling of treatment time, calculated from the 2 hour treatment time.

B. Dosage Effects of AM : Chromatid versus Chromosome Aberrations and Fragments versus Exchanges.

AM induced chromatid fragments and exchanges and also chromosome fragments and exchanges (Fig. 5 exemplifies the AM induced aberrations). A study was made of the frequency of the "total" aberrations with increasing concentrations of AM and then separate studies were made of the frequency of chromatid versus chromosome aberrations and the frequency of fragments versus exchanges with increasing concentrations of AM.

(i) Total Aberration Frequency

A Nested model of fixed effects (Table 2) shows that there is a significant difference ($p < 0.05$) between the five donors in the amount of total breakage induced by AM. A Duncan's multiple range test showed that the chromosomes of donors V(C) and X(A) had significantly fewer aberrations than donors U(H), W and Y (see also Fig. 6). There was also a significant difference in the number of aberrations ($p < 0.01$) with different concentration levels. These results caution against pooling data. However, the difference between donors was consistent at the four concentrations of AM ($p = 0.72$): i.e. donors who have a low amount of chromosome damage at low doses also have a low response at higher doses of AM. This does, in fact, allow the breaks from the 5 donors at each concentration level to be pooled. Fig. 6 summarizes the total number of breaks per 100 cells for each of the 5 individuals and gives the mean obtained from the pooled data. Overall the breakage rate increases sharply up to 0.05 µg/ml and then it begins to level off. The variation of total breakage over the four concentrations comprised a very highly significant ($p < 0.001$) linear component and a highly significant ($p < 0.01$) quadratic component (Table 2).

(ii) Chromatid versus Chromosome Aberration Frequency

Isochromatid breaks are morphologically indistinguishable from chromosome fragments and they have been scored as chromosome fragments in this study. The size of the error so introduced in this study of chromatid versus chromosome aberrations is unknown; it is inherrent in the scoring procedure.

Table 2 shows that the number of chromatid aberrations was significantly different ($p < 0.01$) from the number of chromosome aberrations: Fig. 7 shows that there were more chromatid aberrations than chromosome aberrations at all concentrations. However, the relationship between breakage and increasing dose (Fig. 7) was significantly different ($p < 0.05$) for the chromatid versus the chromosome aberrations (Table 2). This was mainly due to the relatively slow rate of increase of chromosome aberrations compared to that of chromatid aberrations at the 0.15 µg/ml concentration (Fig. 7).

Chromosome and chromatid aberrations were further classified into fragments and exchanges (Fig. 7). There were more chromatid exchanges than chromatid fragments at all concentrations. The chromosome fragments made up by far the greatest number of the chromosome aberrations: only a few chromosome exchanges were scored. It is interesting to note that the number of chromosome fragments actually decreased between the 0.10 and 0.15 µg/ml concentrations.

(iii) Fragment versus Exchange Aberration Frequency

The relationship between increasing concentrations of AM and fragment and exchange aberrations was also studied. Table 3 shows that there was a significant difference ($p < 0.01$) between the number of fragments and the number of exchanges induced by AM. Fig. 8 shows that there were more fragments than exchanges at all concentrations although this was largely because there were very few chromosome exchanges. The relationship between breakage and increasing dose was significantly different ($p < 0.05$) for the fragment versus exchange aberrations. Similar to the above, this was mainly due to the relatively slow rate of increase of exchanges compared to that of fragments between the 0.10 and 0.15 µg/ml concentrations.

Fragments were further classified as chromosome and chromatid fragments (Fig. 8). At all concentrations there were always more chromosome fragments than chromatid fragments. However, the frequency of chromosome fragments decreased above the 0.10 µg/ml concentration, whereas the frequency of chromatid fragments increased.

As already noted, the chromatid exchanges made up most of the exchange aberrations, as only a few chromosome exchanges were recorded (Fig. 8).

TABLE 2

A Nested model of fixed effects comparing chromatid and chromosome type aberrations induced by AM in cultured human lymphocytes for different donors and different concentrations of AM.

SOURCE OF VARIATION	DF	MS	F	P
Donors tested against R	4	601.49	3.44	0.03
Concentrations tested against R	3	5855.03	33.51	0.00
Concentrations (linear) tested against R	1	15694.27	89.83	0.00
Concentrations (quadratic) tested against R	1	1736.36	9.93	0.01
Concentrations (cubic) tested against R	1	134.47	0.77	0.38
Aberration Types	1	6372.45	104.04	0.00
Replicates	20	174.70	2.85	
Donors by Concentration tested against R	12	125.35	0.72	0.72
Donors by Aberration Types tested against TR	4	128.26	2.09	0.12
Concentrations by Aberration Types tested against TR	3	269.62	4.40	0.02
Donors by Concentrations by Aberration Types tested against TR	12	84.22	1.38	0.26
Error (TR)	20	61.25		
TOTAL	79	441.76		

Legend: R = Replicates
T = Aberration Types

TABLE 3

A Nested model of fixed effects comparing fragment and exchange aberration induced by AM in cultured human lymphocytes for different donors and different concentrations of AM.

SOURCE OF VARIATION	DF	MS	F	P
Donors tested against R	4	601.49	3.44	0.03
Concentrations tested against R	3	5855.03	33.51	0.00
Aberration types	1	5951.25	95.83	0.00
Replicates	20	174.70	2.81	
Donors by Concentration tested against R	12	125.35	0.72	0.72
Donors by Aberration Types tested against TR	4	101.69	1.64	0.20
Concentration by Aberration Types tested against TR	3	305.75	4.92	0.02
Donors by Concentrations by Aberration Types tested against TR	12	54.72	0.88	0.58
Error (TR)	20	62.12		
TOTAL	79	432.19		

Legend: R = Replicates
T = Aberration Types

Fig.5. Aberrations induced by AM. (a) chromatid fragment and chromosome exchange (dicentric); (b) chromosome fragments; (c) chromatid fragme (d) chromosome and chromatid fragment; (e) chromosome fragment and chromosome exchange; (f) chromatid exchange.

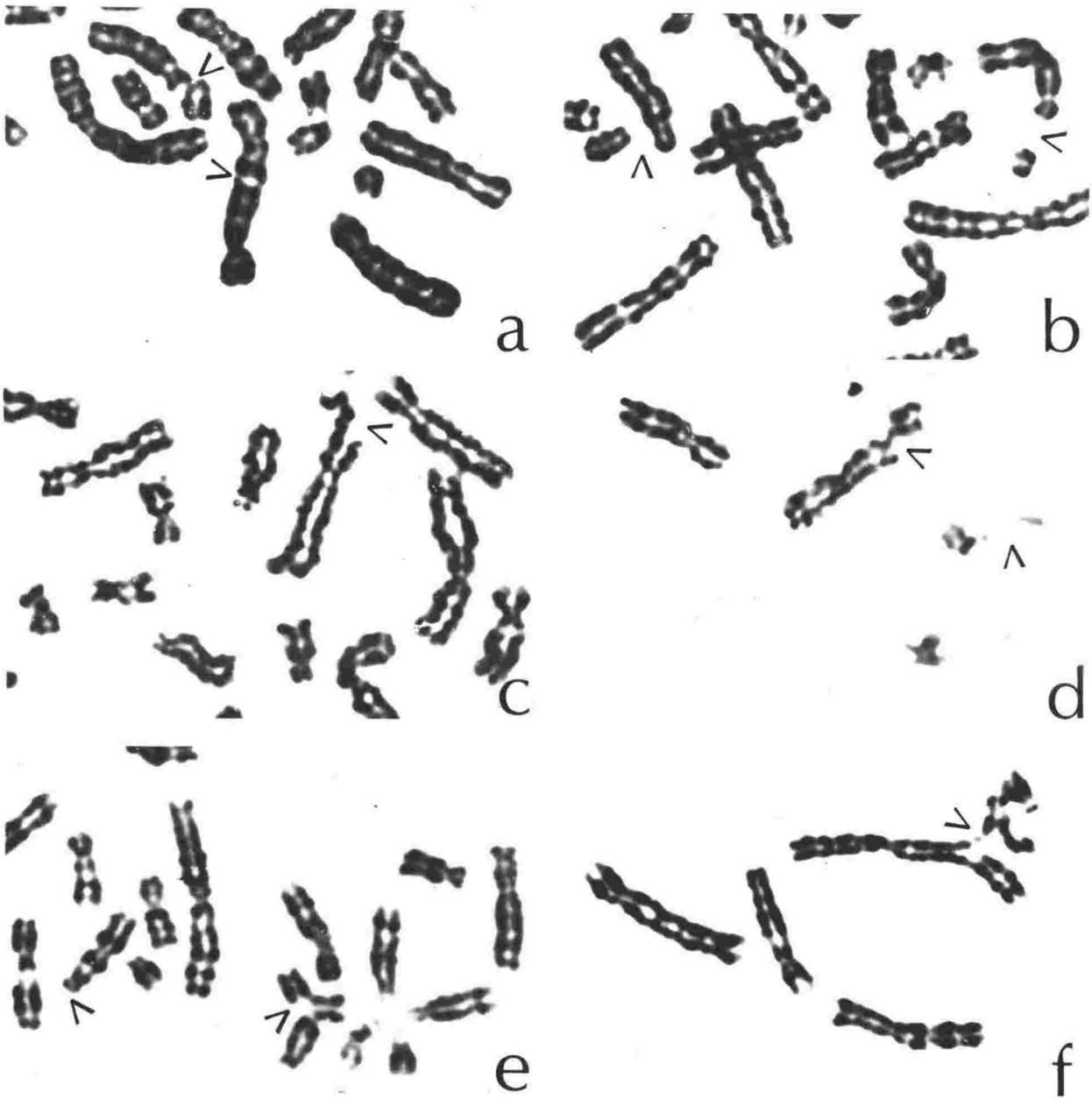

a
b
c
d
e
f

Fig. 6. Total number of aberrations induced by AM at 4 concentrations for donors U(H), V(C), W, X(A) and Y and the mean from the pooled data.

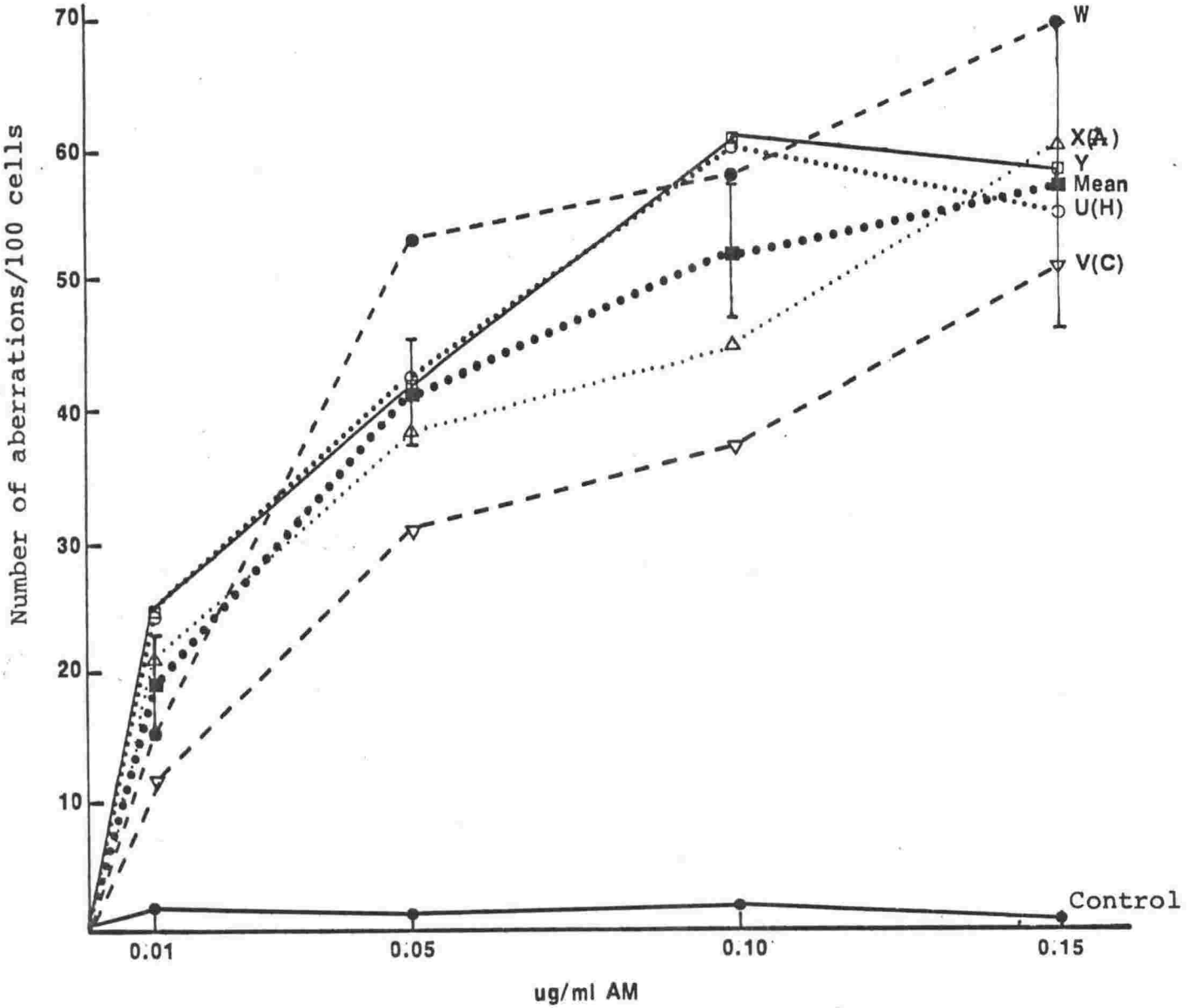

Number of aberrations/100 cells
70
60
50
40
30
20
10
W
X(A)
Y
Mean
U(H)
V(C)
Control
0.01
0.05
0.10
0.15
ug/ml AM

Fig. 7. Frequency of the chromatid and chromosome aberrations induced by AM at 4 concentrations. Each point represents the means from the pooled data of the 5 donors. The composition of the chromatid and chromosome aberrations relative to the fragment and exchange aberrations is also shown.

N.B. Vertical scale is different to fig. 6.

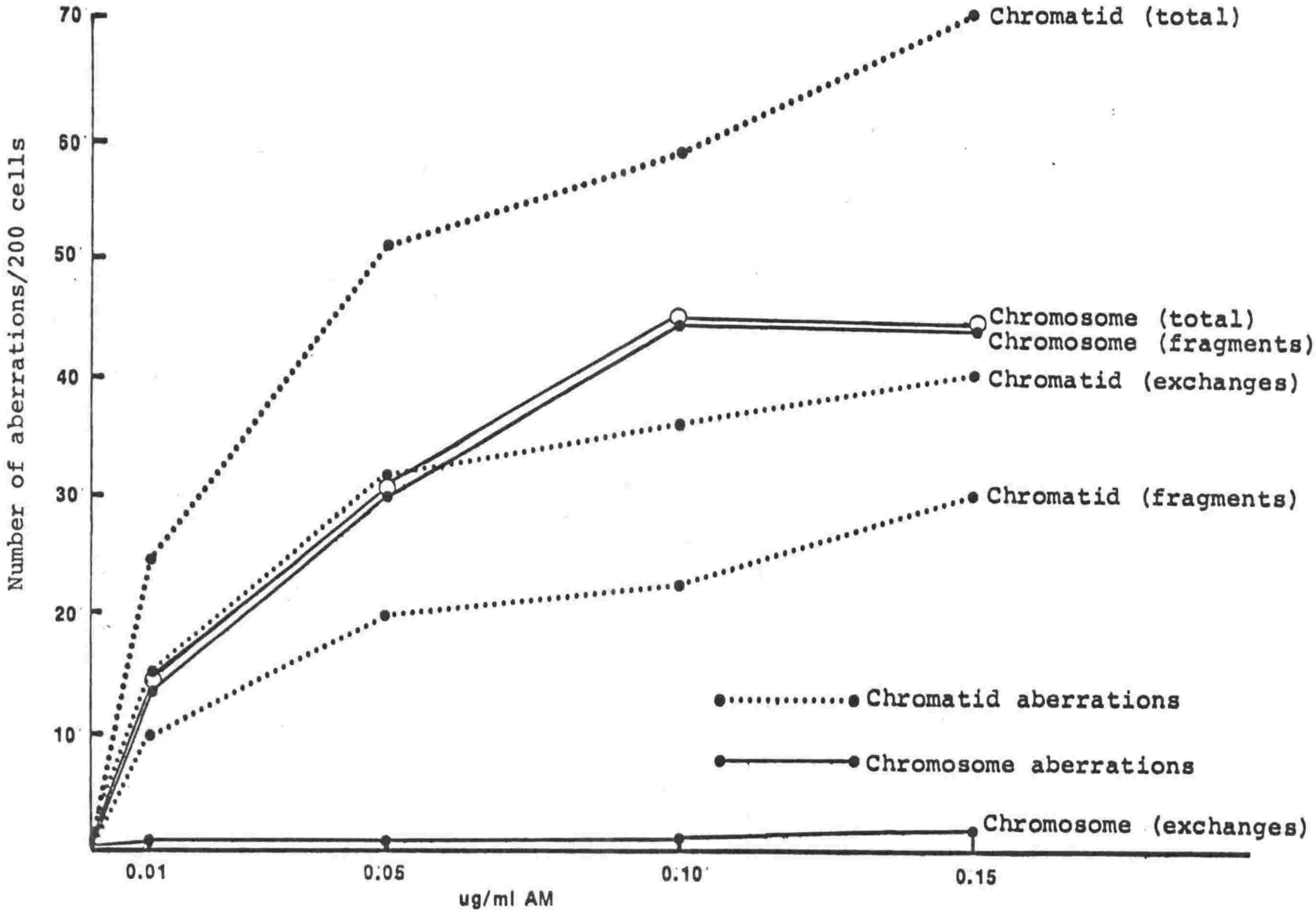
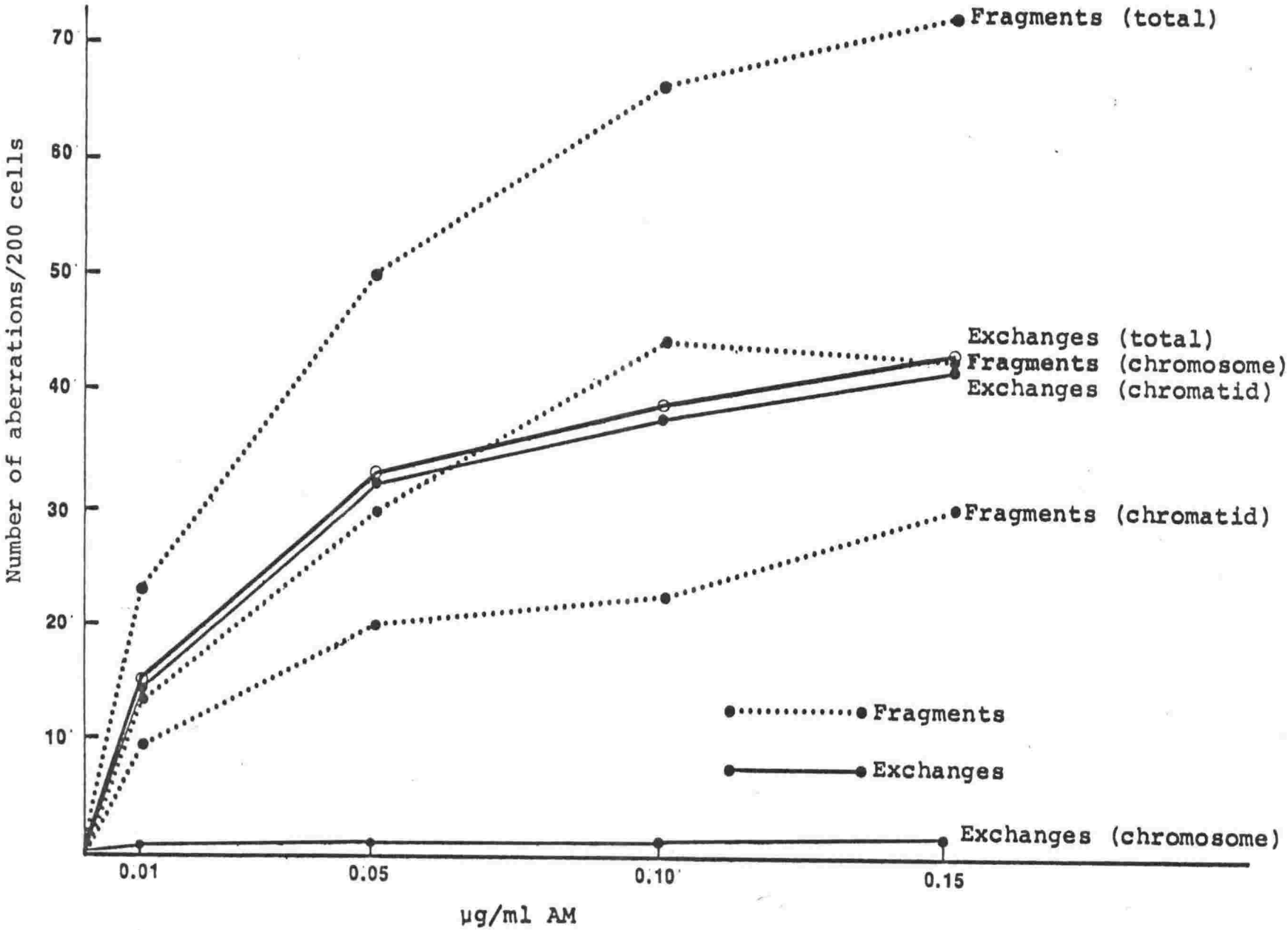

Number of aberrations/200 cells
70
60
50
40
30
20
10
0.01
0.05
0.10
0.15
ug/ml AM
Chromatid (total)
Chromosome (total)
Chromosome (fragments)
Chromatid (exchanges)
Chromatid (fragments)
Chromatid aberrations
Chromosome aberrations
Chromosome (exchanges)

Fig. 8. Frequency of the fragment and exchange aberrations induced by AM at 4 concentrations. Each point represents the means from the pooled data of the 5 donors. The composition of the fragment and exchange aberrations relative to the chromatid and chromosome aberrations is also shown.

N.B. Vertical scale is different to fig. 6.


Number of aberrations/200 cells
70
60
50
40
30
20
10
0.01
0.05
0.10
0.15
µg/ml AM
Fragments (total)
Exchanges (total)
Fragments (chromosome)
Exchanges (chromatid)
Fragments (chromatid)
Fragments
Exchanges
Exchanges (chromosome)

C. Summary of the Effects of Ara C and AM on the Aberration Frequency

(i) Both drugs caused an increase in chromosome damage with increasing concentration. For Ara C, increased treatment times caused an increase in the frequency of chromosome aberrations. (The effect of increasing treatment time with AM was not studied).

(ii) Ara C caused no significant inter-individual differences, neither for dosage nor for treatment time. However, for AM there were significant inter-individual differences.

(iii) AM caused more chromatid aberrations than chromosome aberrations; the relationship between breakage and increasing dose was significantly different for the chromatid versus the chromosome aberrations. Similarly, there were more fragments than exchanges produced by AM; and the relationship between breakage frequency and increasing dose was significantly different for the fragment versus the exchange aberrations.

D. Localization of Breaks in G Banded Chromosomes

For Ara C, a total of 1203 chromatid and isochromatid aberrations was studied, of which 909 (76%) were assigned to particular G bands. For AM, a total of 1281 aberrations was studied, of which 938 (74%) were assigned to particular G bands. In each case the remaining aberrations were not localized either because of poor banding or because in some cells too many aberrations made it difficult to accurately assign aberrations to a particular band.

Breaks induced by Ara C and AM were assigned to either a light band or a dark band. The percentage of breaks occurring in light staining regions was 66% for Ara C and 63% for AM. In some cases breaks were observed close to or at the interface of the light and dark bands. Such breaks occurred at a frequency of 20% (179/909) for Ara C and 17% (161/948) for AM. There is some doubt as to whether these breaks should be assigned to a separate "interface" class of breaks or assigned to the band that the breaks are visualized in. In this study breaks occurring close to an interface were always assigned to the band that they were visualized in. If the break appeared at the interface it was always assigned to the adjacent light band (see "Discussion" section p. 76 for the rationale for this).

A remarkable consistency was found (and verified by a chi-square test) in the frequency of breaks occurring in the light staining regions between the five individuals for both Ara C and AM (table 4). For AM, there is clearly no significant difference in the frequency of breaks occurring in the light bands between the four different types of aberrations (table 4 (b ii)): all aberration types showed approximately 63% occurrence in light G bands.

The frequency of breaks occurring in the light bands of Ara C and the light bands of AM is not significantly different ($p < 0.01$).

TABLE 4

The number of Ara C and AM induced aberrations occurring in the light and dark G bands in chromosomes of cultured human lymphocytes for different donors.

(a) Ara C

DONOR	H(U)	C(V)	E	A(X)	B	TOTAL
Light Bands	152	86	85	139	138	600
Dark Bands	81	42	41	73	72	309
% Light Bands	65	67	67	66	66	66

(b) AM

(i) Different Donors

DONOR	U(H)	V(C)	W	X(A)	Y	TOTAL
Light Bands	122	218	121	97	125	593
Dark Bands	74	67	79	69	66	355
% Light Bands	62	66	61	58	65	63

(ii) Different Aberration Types

ABERRATION TYPE	CHROMATID FRAGMENTS	CHROMATID EXCHANGES	CHROMOSOME FRAGMENTS	CHROMOSOME EXCHANGES
Light Bands	142	212	201	32
Dark Bands	79	124	137	21
% Light Bands	64	63	60	60

E. Distribution of Aberrations between Chromosomes and Chromosome Arms

The regression of breaks against relative chromosome length (see Appendix 2 for regression graphs) was significant ($p < 0.01$) and had an intercept consistent with being zero for both Ara C and AM. Hence, the ratio of breaks to relative length was considered an appropriate variate to compare chromosomes, chromosome arms and donors for each of the drugs.

To study the number of breaks in the p and q arms, two groups of chromosomes were considered.

(a) Chromosomes 1-12, 16-20, and the X chromosome, all of which have clearly defined p and q arms.

(b) Chromosomes 13-15, 21 and 22, all of which were considered to have no p arms.

The Y chromosome was not included since only one break was found in the male individuals studied.

(a) Chromosomes 1-12, 16-20 and X

I Ara C

No satisfactory statistical method could be devised to compare the distribution between chromosomes and chromosome arms of the chromatid versus the isochromatid aberrations. A cursory examination of the distribution of chromatid versus isochromatid aberrations showed no apparent difference. Quantitatively, the ratios of isochromatid to chromatid aberrations at regions of the chromosomes where high yields of aberrations allowed meaningful comparisons to be made were similar (see appendix 4). Therefore the two types of aberrations were pooled.

Table 5 shows that there was a significant difference ($p < 0.05$) between the chromosomes in the number of Ara C induced breaks per unit length of chromosome. However, a Duncan's multiple range test was not able to distinguish any one group of chromosomes as being different from others. Fig. 9 shows the chromosomes ranked according to the mean number of breaks per unit length, summed over the five donors. Chromosome 3 and the X chromosome had the most number of breaks per unit length while chromosomes 15 and 18 had the least. There was no significant difference in the mean number of breaks per unit length between the p and q arms.

Different donors showed a significant difference in the number of breaks per unit length. This significant difference was inconsequential because different numbers of cells were scored for different donors. However, the interaction of "donors by chromosomes" was consistent and this allowed the results from the five donors to be pooled and the breaks plotted to show their distribution relative to the chromosome bands (Fig. 10). Non-random distribution of breaks between various G bands is difficult to show statistically because methods of measuring the lengths of individual bands are not available. However, Fig. 10 shows a number of regions which appear noticeably "hot". These "hotspots", which for the purposes of this study are bands with 10 or more aberrations, occur at the following bands: 1q21, 1q32, 3p21, 3p14, 3p13, 4q31, 7q11, 7q32, 9q12, 12p12, 12q13, 16q24, 17q21 and Xp22.

II AM

The AM data were analysed firstly by studying the total aberrations induced by AM and secondly by studying the different types of aberrations that make up the total AM induced aberrations. In some instances heterogeneous data were pooled to enable comparisons to be made with the present Ara C study and other related studies. The justification for such pooling is biological: during cancer chemotherapy, for example, it is of little consequence to a cell whether cytotoxicity is induced by a chromatid exchange or a chromosome fragment.

(i) The Distribution of the Total Aberrations

Table 6 shows that there was a significant difference ($p < 0.01$) between the chromosomes in the mean number of aberrations per unit length. A Duncan's multiple range test was not able to distinguish one group of chromosomes from another. Fig. 9 shows the chromosomes ranked according to the number of aberrations per unit length summed over the five donors. The greatest number of aberrations per unit length were found in chromosomes 13 and 21, while the least were found in chromosomes 19 and 20. There was no significant difference in the number of breaks per unit length between the 5 donors, nor between the p and q arms. There was, however, a significant ($p < 0.01$) "chromosome by chromosome

arm" interaction; i.e. some chromosomes had more breaks per unit length in the p arm than the q arm, whereas for others the reverse was true.

Since there was no significant difference in the number of aberrations per unit length over the five donors, nor in the "donor by chromosome arm" interaction, the total number of aberrations for each donor was pooled. Fig. 11 shows that there was a non-random distribution of aberrations relative to the G bands, and "hotspots" occurred at 1p32, 1q11, 6p21, 6q21, 6q23, 13q12, 13q14, 14q24 and 22q13.

(ii) The Distribution of Chromosome and Chromatid Aberrations

Table 7 shows that there were significant differences ($p < 0.01$) in the mean number of chromosome aberrations per unit length between chromosomes, and between chromosome arms. A Duncan's multiple range test showed that there were significantly more aberrations per unit length in the q arm than the p arm. Table 7 shows that the difference was not consistent for different chromosomes: i.e. in some chromosomes there were more aberrations per unit length in the p arm, while in others there were more aberrations in the q arm. But overall there were more aberrations in the q arm. For chromatid aberrations there was a significant difference ($p < 0.01$) in the number of aberrations per unit length over the chromosomes (Table 8). However, there was no significant difference between the number of breaks per unit length in the p and q arms, although there was a significant ($p < 0.01$) "chromosome by chromosome arm" interaction.

Figs. 12 and 14 show that the distributions of chromosome and chromatid aberrations were different. The greatest number of chromosome aberrations per unit length were found in chromosomes 6 and 4 and the least in chromosomes 17 and 18, whereas the greatest number of chromatid aberrations per unit length were found in chromosomes 13 and 21 and the least in chromosomes 19 and 20.

The chromatid aberrations were further classified as chromatid fragments and chromatid exchanges. The chromatid fragments were randomly distributed over the chromosomes (Table 9): i.e. there was no significant difference in the mean number of breaks per unit length between chromosomes or between chromosome arms.

The distribution and frequency of the chromatid exchanges and "total exchanges" is similar (Fig. 8 and Tables 10 and 12) because relatively few chromosome exchanges were recorded. Therefore, chromatid exchanges and "total exchanges" are considered to be analogous, and both are referred to together as "total exchanges" in the next section. The chromosome aberrations were not further classified because mostly they were of the fragment type with only relatively few exchanges occurring.

(iii) The Distribution of Fragment and Exchange Aberrations

Table 11 shows significant differences ($p < 0.01$) in the mean number of fragments per unit length between chromosomes and between the p and q arms. For exchanges (i.e. "total exchanges") there were significant differences ($p < 0.01$) in the mean number of exchanges per unit length between donors, between chromosomes and between chromosome arms; and there was also a significant ($p < 0.01$) "chromosome by chromosome arm" interaction (Table 12). A Duncan's multiple range test showed that the chromosomes of Donor "W" were involved in significantly ($p < 0.01$) more exchanges than the other donors (see table of means appendix 3-12). There were significantly more exchanges per unit length in the p arm than the q arm, whereas for fragments the reverse was true. The distribution of fragments and exchanges was different over the chromosome complement (Figs. 13 and 15). The greatest numbers of fragments per unit length were found in chromosomes 6 and 4 and the least in chromosome 18. For exchanges the satellite chromosomes 21, 13 and 14 were involved in the most number of exchanges per unit length and chromosomes 19 and 20 were not involved in any exchanges. Fig. 16 shows the frequency of chromosomes involved in exchanges. For convenience exchanges involving three chromosomes are shown as exchanges involving only 2 chromosomes in their three combinations. The most common exchanges were those involving chromosomes 13 and 14 and chromosomes 13 and 15.

The fragments were further classified as chromatid fragments and chromosome fragments. As already discussed in the previous section, the chromatid fragments are distributed randomly between the chromosomes (Table 9). The distribution and frequency of the chromosome fragments and chromosome aberrations are similar (Tables 7 and 13, Fig. 7), because relatively few

chromosome exchanges were recorded. Therefore chromosome fragments and chromosome aberrations are considered to be analogous, and both are discussed together as chromosome aberrations in the previous section.

The exchanges were not further classified as most were chromatid exchanges and relatively few chromosome exchanges were recorded.

(b) Chromosomes 13-15, 21 and 22

The data in Tables 14 and 15 show no evidence for any difference in the mean number of breaks per unit length between the chromosomes or between the 5 donors.

TABLE 5

Analysis of variance for the total no. of breaks induced by Ara C in chromosomes of cultured human lymphocytes:- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	3.40	2.08	0.02
Donors	4	8.35	5.12	0.00*
Chromosome arms	1	2.17	1.33	0.25
Chromosomes by Donors	68	1.76	1.08	0.38
Chromosomes by chromosome arms	17	2.55	1.57	0.10
Donors by Chromosome arms	4	1.73	1.06	0.38
Error	68	1.63		
TOTAL	179	2.09		

* This significant difference is inconsequential because different numbers of cells were scored for different donors (see p. 20, second paragraph).

TABLE 6

Analysis of variance for the total no. of breaks induced by AM in chromosomes of cultured human lymphocytes:- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	3.62	6.42	0.00
Donors	4	1.02	1.81	0.14
Chromosome arms	1	0.53	0.95	0.33
Chromosomes by Donors	68	0.63	1.12	0.32
Chromosomes by chromosome arms	17	1.28	2.27	0.01
Donors by Chromosome arms	4	0.77	1.36	0.26
Error	68	0.56		
TOTAL	179	0.96		

TABLE 7

Analysis of variance for "chromosome" type aberrations induced by AM in chromosomes of cultured human lymphocytes: - chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.24	4.80	0.00
Donors	4	0.14	0.53	0.71
Chromosome arms	1	3.21	12.47	0.00
Chromosomes by Donors	68	0.18	0.71	0.92
Chromosomes by chromosome arms	17	0.65	2.52	0.00
Donors by chromosome arms	4	0.75	2.91	0.03
Error	68	0.26		
TOTAL	179	0.39		

TABLE 8

Analysis of variance for "chromatid" type aberrations induced by AM in chromosomes of cultured human lymphocytes:
- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.66	4.17	0.00
Donors	4	1.11	2.80	0.03
Chromosome arms	1	0.50	1.26	0.27
Chromosomes by Donors	68	0.41	1.04	0.44
Chromosomes by chromosome arms	17	1.02	2.57	0.00
Donors by chromosome arms	4	0.34	0.86	0.49
Error	68	0.40		
TOTAL	179	0.60		

TABLE 9

Analysis of variance for "chromatid fragments" aberrations induced by AM in chromosomes of cultured human lymphocytes:
- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	0.18	1.09	0.38
Donors	4	0.01	0.06	0.99
Chromosome arms	1	0.20	1.23	0.27
Chromosomes by Donors	68	0.15	0.96	0.56
Chromosomes by chromosome arms	17	0.12	0.74	0.76
Donors by chromosome arms	4	0.18	1.07	0.38
Error	68	0.16		
TOTAL	179	0.16		

TABLE 10

Analysis of variance for "chromatid exchanges" aberrations induced by AM in chromosomes of cultured human lymphocytes:
- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.11	5.92	0.00
Donors	4	1.30	6.89	0.00
Chromosome arms	1	2.51	13.34	0.00
Chromsomes by Donors	68	0.22	1.18	0.25
Chromosomes by chromosome arms	17	0.79	4.17	0.00
Donors by chromosome arms	4	0.28	1.51	0.21
Error	68	0.19		
TOTAL	179	0.39		

TABLE 11

Analysis of variance for "fragment" type aberrations induced by AM in chromosomes of cultured human lymphocytes: - chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.71	4.21	0.00
Donors	4	0.26	0.64	0.63
Chromosome arms	1	4.01	9.83	0.00
Chromosomes by Donors	68	0.34	0.84	0.76
Chromosomes by chromosome arms	17	0.58	1.44	0.15
Donors by chromosome arms	4	0.46	1.13	0.35
Error	68	0.41		
TOTAL	179	0.54		

TABLE 12

Analysis of variance for "exchange" type aberrations induced by AM in chromosomes of cultured human lymphocytes:
- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.35	5.87	0.00
Donors	4	1.16	5.06	0.00
Chromsome arms	1	2.12	9.25	0.00
Chromosomes by Donors	68	0.24	1.03	0.44
Chromosomes by chromosome arms	17	1.00	4.37	0.00
Donors by chromosome arms	4	0.48	2.07	0.09
Error	68	0.23		
TOTAL	179	0.45		

TABLE 13

Analysis of variance for "chromosome fragments" aberrations induced by AM in chromosomes of cultured human lymphocytes:
- chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	17	1.04	3.82	0.00
Donors	4	0.15	0.56	0.70
Chromosome arms	4	2.18	8.05	0.01
Chromosomes by Donors	68	0.15	0.54	0.99
Chromosomes by chromosome arms	17	0.58	2.15	0.02
Donors by chromosome arms	4	0.32	1.19	0.32
Error	68	0.27		
TOTAL	179	0.34		

TABLE 14

Analysis of variance for the total no. of breaks induced by Ara C in chromosomes of cultured human lymphocytes:- chromosomes 13, 14, 15, 21, 22.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	4	1.15	1.35	0.29
Donors	4	1.06	1.06	0.41
Error	16	0.85		
TOTAL	24	0.91		

TABLE 15

Analysis of variance for the total no. of breaks induced by AM in chromosomes of cultured human lymphoctyes:- chromosomes 13, 14, 15, 21, 22.

SOURCE OF VARIATION	DF	MS	F	P
Chromosomes	4	0.52	0.70	0.60
Donors	4	0.81	1.09	0.40
Error	16	0.74		
TOTAL	24	0.72		

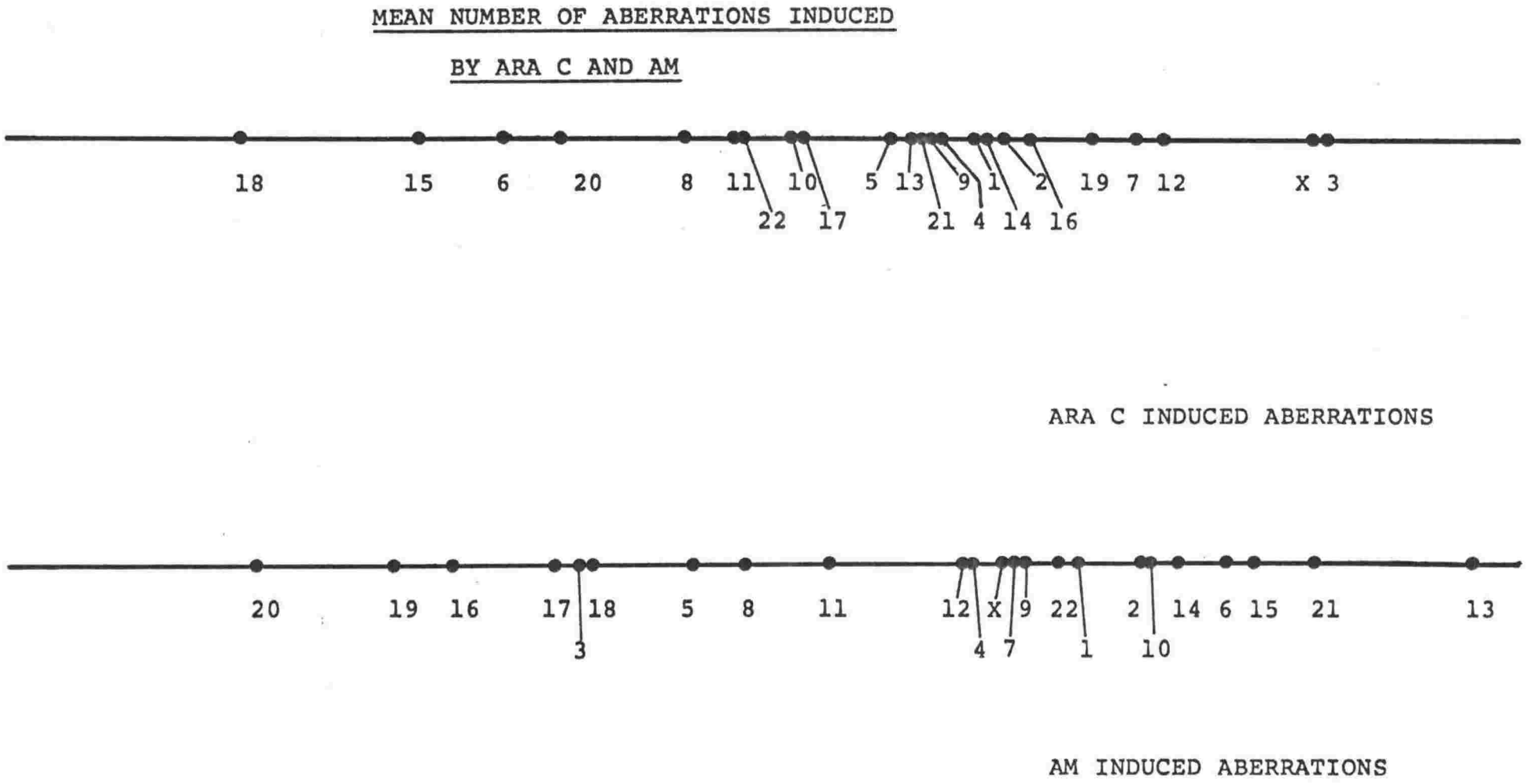


Fig. 9. Relative number of aberrations per unit length for each chromosome. Chromosomes on the right have more aberrations than those on the left

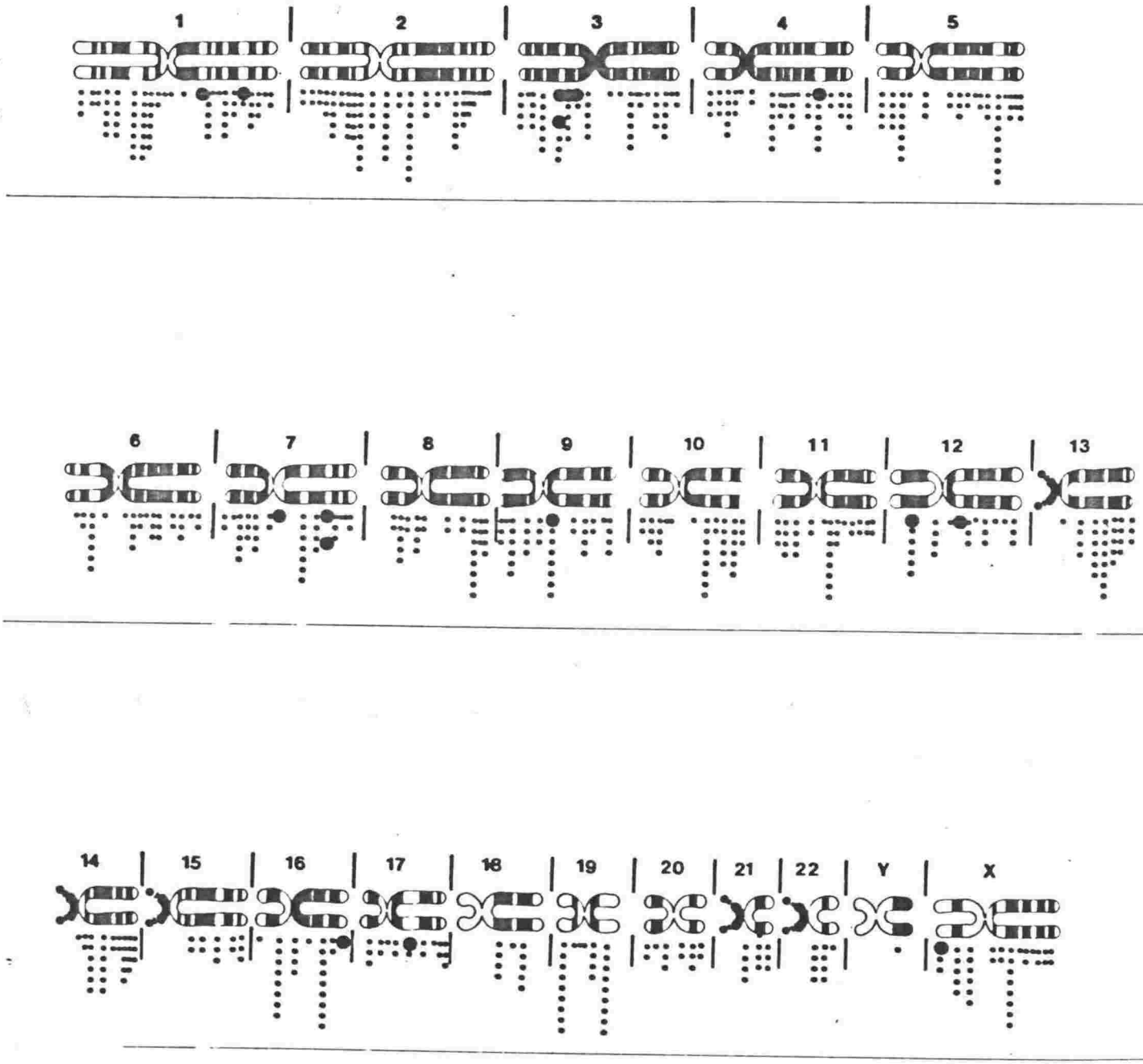


Fig. 10. Distribution of Ara C induced aberrations on the individual chromosomes. The banding pattern of the Paris Conference (1971) was used.
The large dots represent 10 aberrations (hotspot

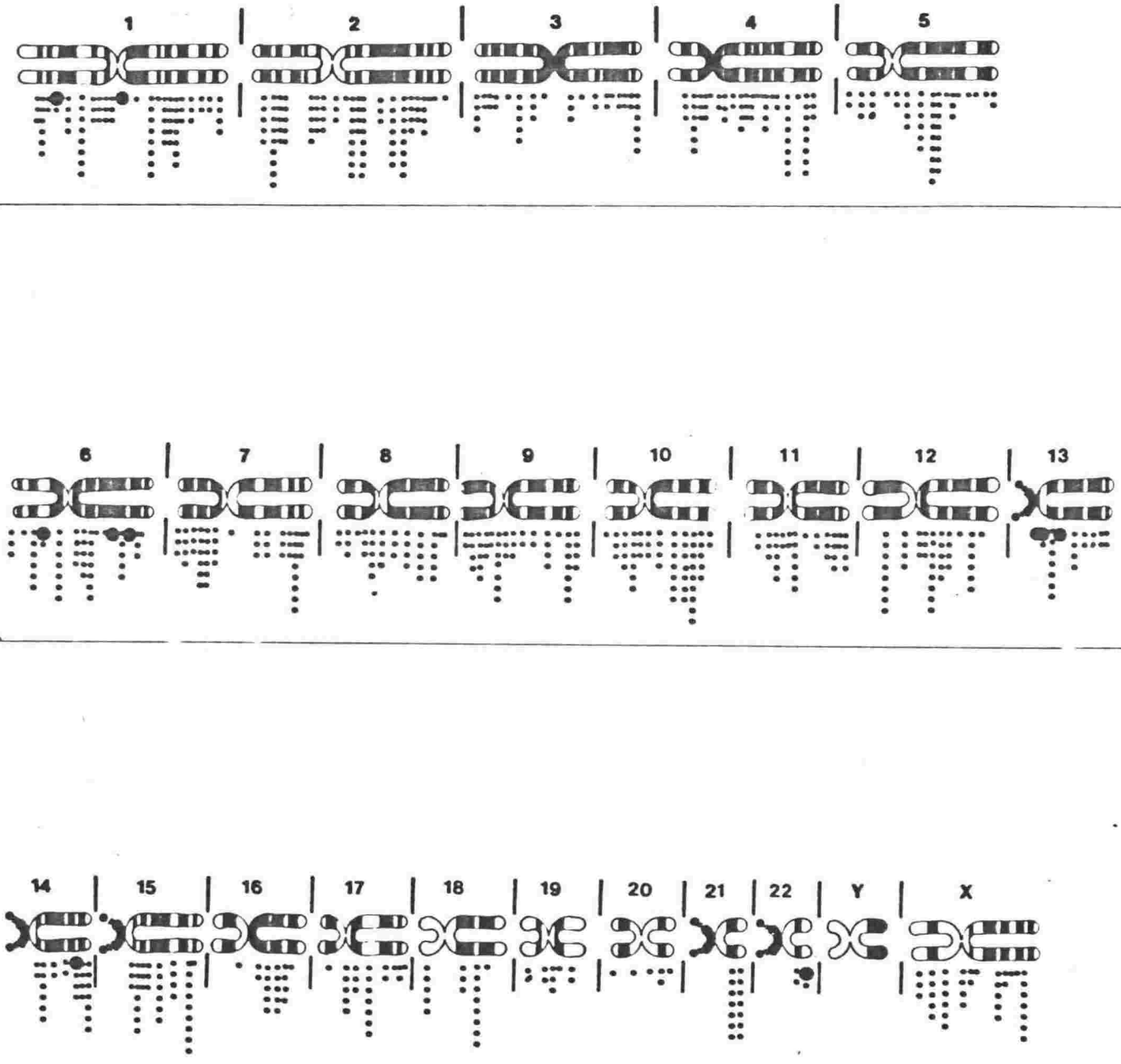


Fig. 11. Distribution of the total number of aberrations induced by AM on the individual chromosomes. The banding pattern of the Paris Conference (1971) was used.
The large dots represent 10 aberrations (hotspots).

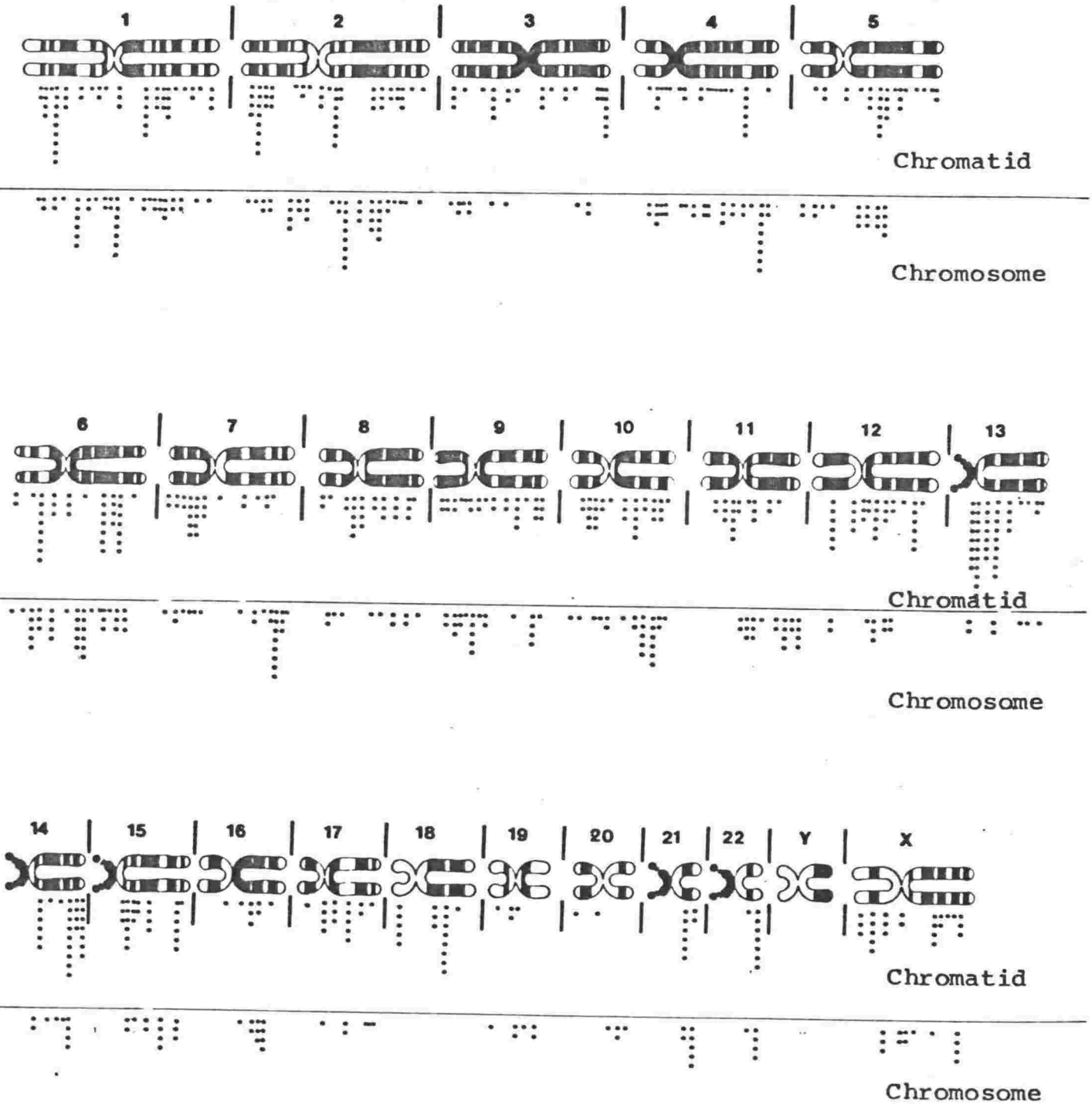


Fig. 12. Distribution of the chromatid and chromosome aberrations induced by AM on the individual chromosomes. The banding pattern of the Paris Conference (1971) was used.

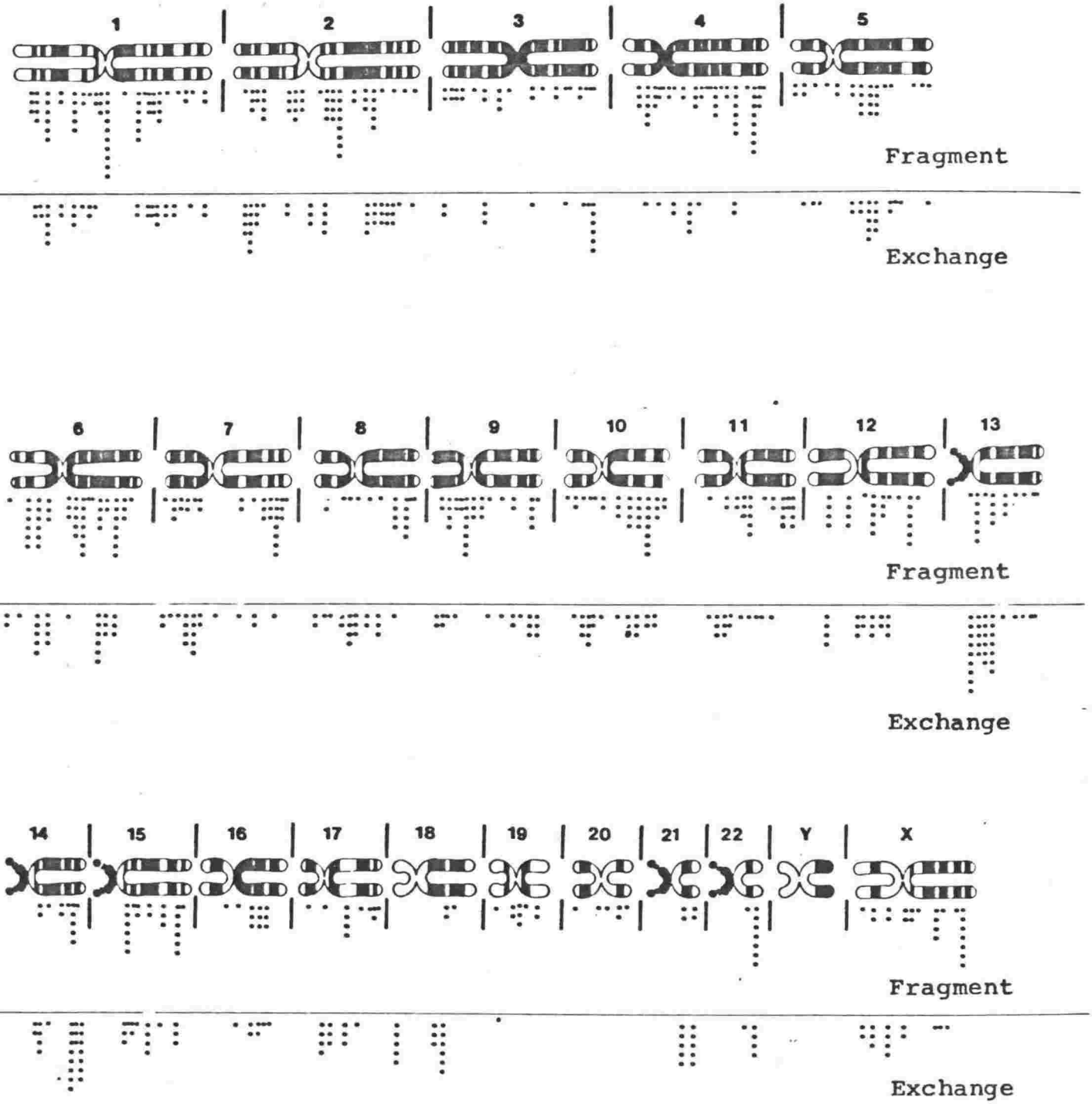
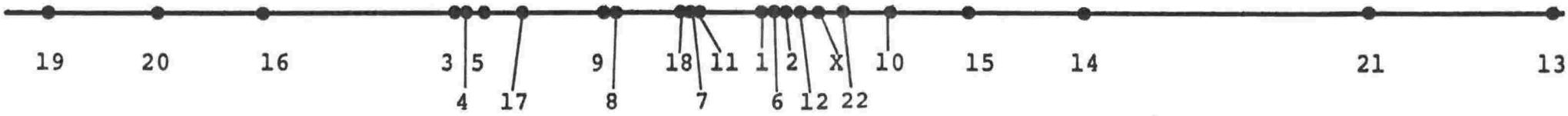


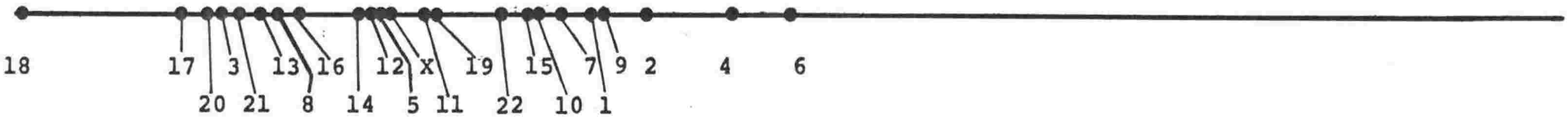
Fig. 13. Distribution of the fragment and exchange aberrations induced by AM on the individual chromosomes. The banding pattern of the Paris Conference (1971) was used.

MEAN NUMBER OF "CHROMATID" AND "CHROMOSOME" ABERRATIONS

INDUCED BY AM



"CHROMATID" TYPE ABERRATIONS

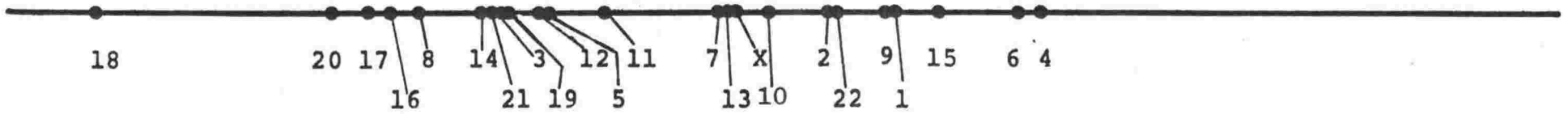


"CHROMOSOME" TYPE ABERRATIONS

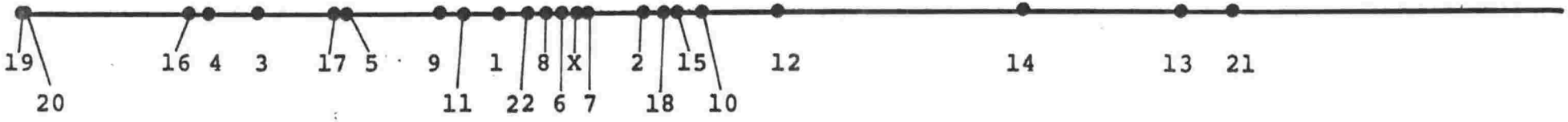
Fig. 14 Relative numbers of aberrations per unit length for all chromosomes. The chromatid and chromosome aberrations are drawn to the same scale. Chromosomes on the right have more aberrations than those on the left.

MEAN NUMBER OF "FRAGMENT" AND "EXCHANGE" ABERRATIONS

INDUCED BY AM



"FRAGMENT" TYPE ABERRATIONS



"EXCHANGE" TYPE ABERRATIONS

Fig. 15. Relative numbers of aberrations per unit length for all chromosomes. The fragment and exchange aberrations are drawn to the same scale. Chromosomes on the right have more aberrations than those on the left

	Chromosomes																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
1	1	6	-	1	1	2	1	1	3	3	-	-	2	5	1	-	4	1	-	-	1	-	1
2		1	1	6	-	1	1	1	2	2	3	5	3	2	1	-	-	1	-	-	1	1	2
3			-	1	-	1	-	1	1	2	1	1	1	-	-	-	1	1	-	-	-	-	-
4				1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
5					1	4	1	2	-	2	1	3	1	-	1	-	-	-	-	-	1	-	-
6						-	3	3	2	1	3	1	-	1	1	-	1	1	-	-	-	1	1
7							-	-	2	-	1	-	1	3	3	1	2	-	-	-	-	-	1
8								-	-	-	1	1	4	1	1	1	-	1	-	-	-	-	3
9									-	1	-	1	-	-	1	-	-	1	-	-	1	-	-
10										1	1	2	2	3	-	1	1	-	-	-	-	-	-
11											-	-	-	-	-	-	1	-	-	-	3	1	-
12												-	1	1	-	-	-	1	-	-	1	1	1
13													2	5	5	-	-	1	-	-	-	1	1
14														1	-	1	-	1	-	-	-	-	-
15															-	-	1	-	-	-	-	-	-
16																-	-	1	-	-	-	-	-
17																	-	-	-	-	-	-	-
18																		-	-	-	1	-	2
19																			-	-	-	-	-
20																				-	-	-	-
21																					2	-	-
22																						-	-
X																							1

Fig. 16. Diagram illustrating the frequencies with which different chromosomes are involved in AM induced exchanges.

F. Summary of the Distribution of Aberrations Induced by Ara C and AM

Analysis of variance on the mean number of aberrations per unit length for Ara C showed a significant difference in the number of breaks per unit length between chromosomes. No significant difference in the number of breaks per unit length were found for the remaining variates or interaction of variates.

The results obtained from analyses of variance of the distribution of total aberrations induced by AM and the various classes of AM induced aberrations are summarized in Table 16. A study of the distribution of the total number of aberrations induced by AM reveals a significant non-random distribution of aberrations between the chromosomes and a significant "chromosome by chromosome arm" interation. However, when the different classes of aberrations are studied, some of the other variates showed significant differences. All of the different classes of aberrations showed significant non-random distributions, except for the chromatid fragments. Significant differences in the mean number of aberrations per unit length of chromosome were shown by chromatid exchanges, total chromosome aberrations, total fragments, chromosome fragments and total exchanges. Significant variation in the number of aberrations between donors was shown by chromatid exchanges and total exchanges. Significant "chromosome by chromosome arm" interactions were shown by total chromatid aberrations, chromatid exchanges, total chromosome aberrations and total exchange aberrations.

Both Ara C and AM produced elevated frequencies of aberrations at certain positions along the chromosomes. The positions are referred to as "hotspots". The localization of the "hotspots" was different for each drug.

TABLE 16

Summary of the analysis of variance Tables (6-13) for the different types of aberrations induced by AM.

VARIATES	C	R	A	CR	CA	RA	Table No.
Total aberrations	*				*		6
Total chromatid (fragments and exchanges)	*				*		8
Chromatid fragments							9
Chromatid exchanges	*	*	*		*		10
Total chromosome	*		*		*		7
Total fragments (chromatid and chromosome)	*		*				11
Chromatid fragments							9
Chromosome fragments	*		*		*		13
Total exchanges	*	*	*		*		12

Legend: C = Chromosomes
R = Donors
A = Chromosome arms
* = $p<0.01$

G. Comparison between the Distribution of Ara C and AM Induced Aberrations

Three of the five individuals used in the Ara C studies were the same as three of the five individuals used in the AM studies (U(H), V(C), and X(A)). An analysis of variance was designed to compare the distribution of the aberrations over the chromosomes induced by Ara C and AM, using the three donors U(H), V(C) and X(A). Only fragment aberrations of the AM data were used because no exchanges were recorded for Ara C. There was a significant difference in the number of Ara C and AM induced aberrations (Table 17). However, no conclusions can be drawn from these differences since different concentrations and treatment times were used. The interesting feature of Table 17 is that although there was no significant difference in the mean number of aberrations per unit length between the p and q arms, there was a significant ($p < 0.05$) "drug by chromosome arm" interaction: AM caused more aberrations in the q arm than the p arm, whereas Ara C caused more aberrations in the p arm than the q arm (see table of means Appendix 3-17).

TABLE 17

Analysis of variance comparing the effects of Ara C and AM on the chromosomes of individuals U(H), V(C), and X(A) - chromosomes 1-12, 16-20, X.

SOURCE OF VARIATION	DF	MS	F	P
Drug	1	45.28	30.51	0.00
Chromosomes	17	2.40	1.62	0.11
Donors	2	2.64	1.78	0.18
Chromosome arms	1	0.35	0.23	0.63
Drugs by Chromosomes	17	2.26	1.53	0.14
Drugs by Donors	2	5.55	3.74	0.03
Drugs by Chromosome arms	1	8.02	5.40	0.03
Chromosomes by Donors	34	1.53	1.03	0.46
Chromosomes by Chromsome arms	17	2.13	1.43	0.18
Donors by Chromosome arms	2	0.04	0.03	0.97
Drugs by Chromosomes by Donors	34	1.69	1.14	0.35
Drugs by Chromosomes by Chromosome arms	17	1.76	1.19	0.32
Drugs by Donors by Chromosome arms	2	1.34	0.90	0.41
Chromosomes by Donors by Chromosome arms	34	1.44	0.97	0.53
Error	34	1.48		
TOTAL	215	1.99		

H. Sister Chromatid Exchange

Fig. 17 exemplifies differentially stained chromosomes showing SCE's.

(i) Ara C

Table 18 is an analysis of variance on the square root of counts and shows that there was no significant difference between the four donors, or between different concentrations, in the number of SCE's per cell.

(ii) AM

Table 19 is an analysis of variance on the square root of counts and shows that there was no significant difference between the five donors in the number of SCE's per cell. However, there was a significant difference ($p < 0.01$) between the concentrations in the number of SCE's per cell. A Duncan's multiple range test showed that the 0.01 µg/ml concentration caused significantly more SCE/cell than the controls and that the 0.05 µg/ml concentration caused significantly ($p < 0.05$) more SCE/cell than the 0.01 µg/ml concentration (Fig. 18).

TABLE 18

Analysis of variance on the square root of counts for SCE's induced by Ara C in chromosomes of cultured human lymphocytes.

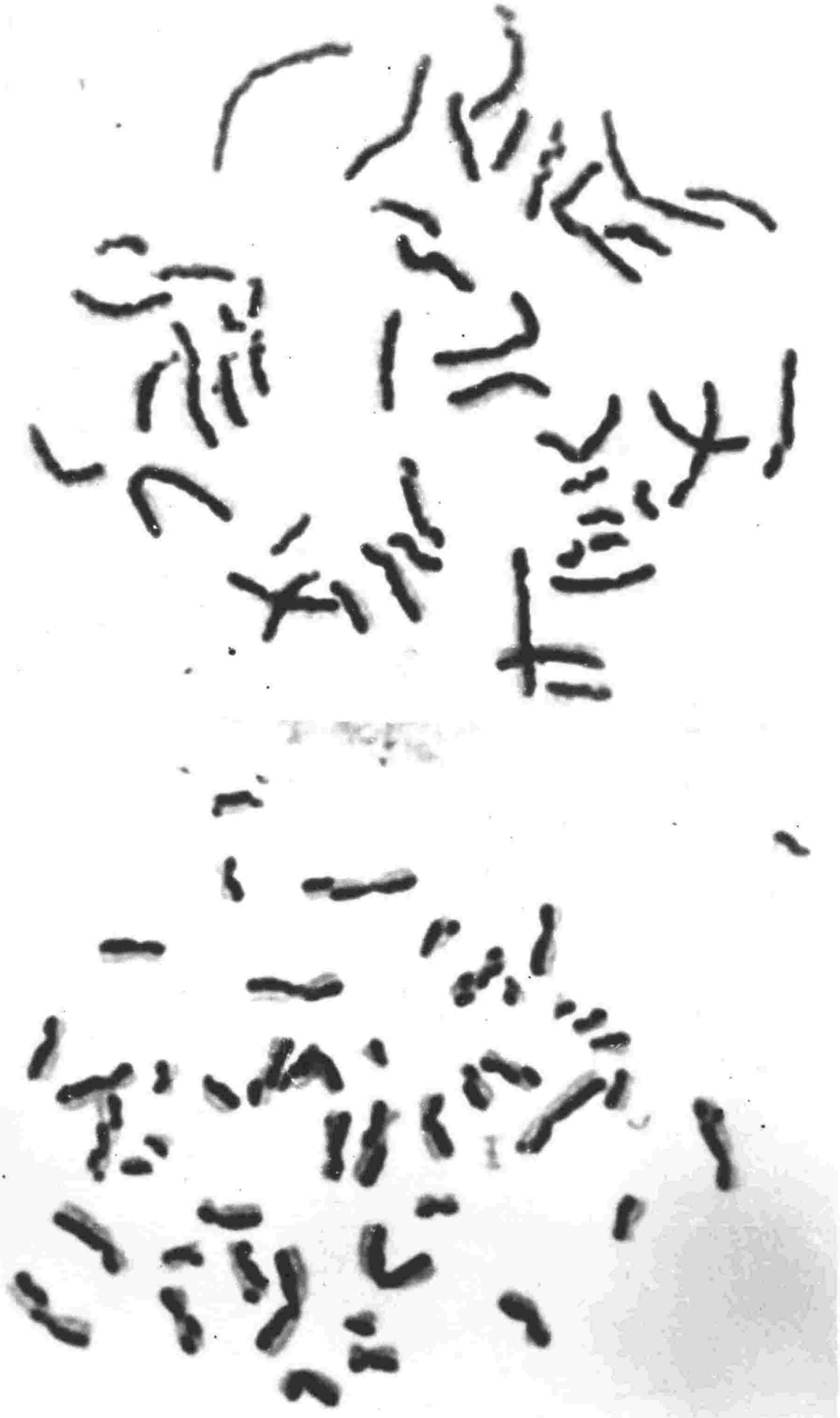
SOURCE OF VARIATION	DF	MS	F	P
Concentrations	2	0.13	0.41	0.66
Donors	3	0.47	1.49	0.22
Concentrations by Donors	6	0.27	0.87	0.52
Error	306	0.32		
TOTAL	317	0.32		

TABLE 19

Analysis of variance on the square root of counts for SCE's induced by AM in chromosomes of cultured human lymphocytes.

SOURCE OF VARIATION	DF	MS	F	P
Concentrations	2	83.49	177.60	0.00
Donors	4	0.30	0.65	0.63
Concentrations by Donors	8	0.26	0.55	0.82
Error	360	0.47		
TOTAL	374	0.91		

Fig.17. Two metaphase chromosome spreads of differentially stained chromosomes showing sister chromatid exchange.



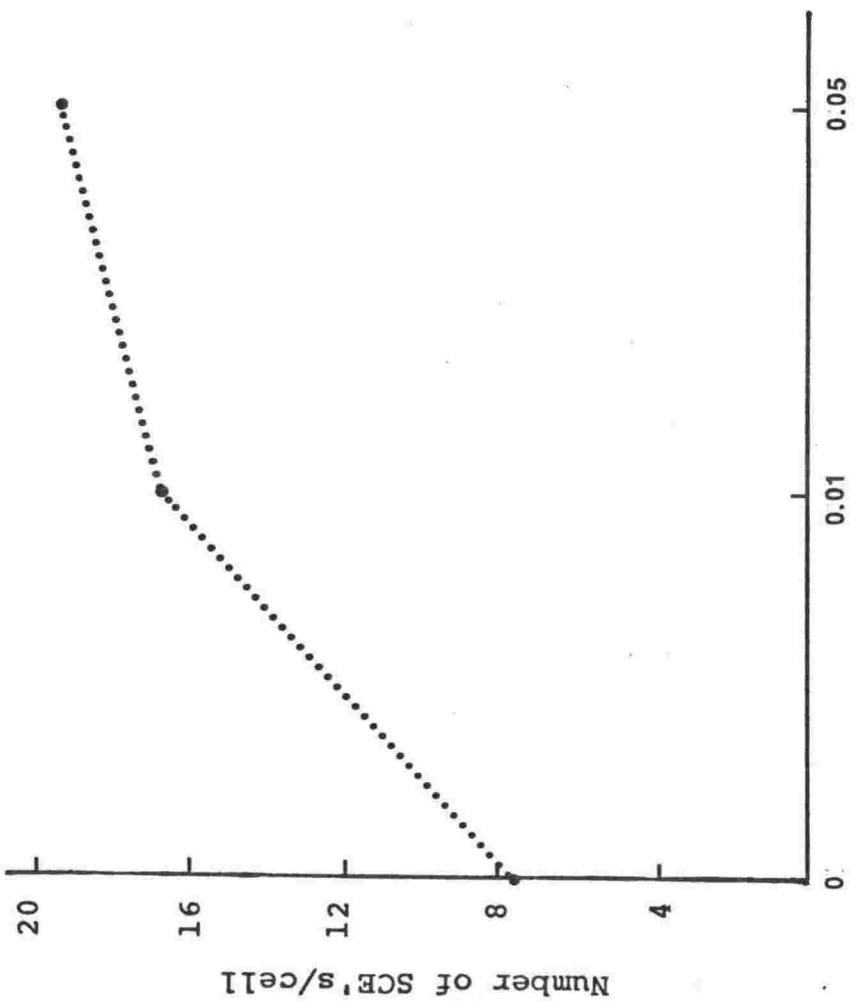


Fig.18. Number of SCE/cell at two concentrations of AM. Each point represents the data pooled from 5 donors

DISCUSSION

Ara C Induced Aberrations in relation to Dose and Treatment Times

For Ara C, the increase in the frequency of breaks with increasing dose, on the one hand, and increasing treatment time, on the other is not unexpected. This is because Ara C is known to induce chromosome breaks (Brewen, 1965; Brewen and Christie, 1967; and Kihlman et al., 1963) and similar relationships have been noted for other drugs. Indeed, Brewen (1965) used a concentration of Ara C of 11.6 µg/ml for 2, 3 and 4 hours and noted a relationship between breakage rate and time remarkably similar to that obtained in this study, as shown below:-

	Concentration	No. of Breaks/100 Cells at treatment times of		
		2 hours	3 hours	4 hours
Brewen (1965)	11.6 µg/ml	38	59	86
Present Study	10.0 µg/ml	39	59	76

Brewen, however, did not study the effect of different doses on the breakage rate.

In the present study the relationship between breakage rate and concentration was not linear, for all three treatment times. Thus, after a sharp increase in breakage rate between 2.5 and 5.0 µg/ml there was only a marginal further increase at 10.00 µg/ml (Fig. 2). Kihlman et al. (1963) studied the effect of Ara C for four hours at two concentrations (7.0 and 11.6 µg/ml, the latter being a little higher than the 10.0 µg/ml used in the present study). They found marginally fewer breaks at the higher concentration than the lower one. Thus there appears to be a saturation point above which any further increase in dose has a relatively small effect on the breakage rate. This levelling off with increasing dose between the 5 and 10.0 µg/ml concentrations superficially appears to be in conflict with the trend shown by the effect of increasing treatment times on the breakage rates (Figs. 2 and 3). Although the rate of increase in breakage decreased below that which was expected with increasing treatment time (Fig. 3), there was no indication of a levelling off with increasing treatment times (Fig. 2 and 3). Any explanation of the data must take into

account both the apparent "saturation" point, above which any further increase in dose had only a relatively small effect on the breakage rate, and the fact that increasing treatment times caused significant increases in the breakage rate.

One such explanation of these two features of the Ara C effect is that Ara C, rather than directly causing chromosome breaks, may induce points of "potential breakage" along chromosomes. These "potential breaks" may become chromosome breaks only after a given period of time; and a longer time converts more potential breaks into actual breaks. The sites of "potential breakage" may be confined to a limited number of sites along the chromosome such that the chromosome becomes saturated at a given concentration of Ara C. Other, perhaps simpler, explanations for the saturation of chromosome breaks with increasing dose are that the cell might retain Ara C only up to a particular concentration or that only a certain amount of Ara C may be phosphorylated into its active form (Ara CTP).

How Ara C could induce points of "potential breakage" mentioned above is not easily explained. The aberrations produced by Ara C in this study were induced in either late S or G_2 (Benedict et al., 1970; Brewen and Christie, 1967; Kihlman et al., 1963; see also in the introduction). If the breaks occurred in late S, they may have been caused by an inhibition of the last stages of DNA synthesis (Chou et al., 1975; Jones et al., 1976), either by inhibition of a specific DNA polymerase or the incorporation of Ara C (Ara CTP) into DNA as a fraudulent nucleoside. If the aberration occurred in G_2 then a precursor deficiency might result in the expression of a nuclease activity in the G_2 nucleus (Ahnstrom and Natarajan, 1966). Both the incorporation of Ara C (Ara CTP) into DNA as a fraudulent nucleoside and the action of a nuclease on specific points along the chromosome might be time dependent, thus accounting for the dependency of the production of chromosome breaks on time.

Although the frequency of Ara C induced aberrations increases with increasing period of treatment (Fig. 3), this frequency actually decreases relative to the "expected" aberration frequency (calculated by assuming a doubling of aberrations with a doubling of treatment time; see Figs. 3 and 4). The increasing divergence of the expected and observed may be explained by a decreasing sensitivity to Ara C in late S - early G_2 (i.e. the 4 hour treatment time) relative to late G_2 (i.e. the 2 hour treatment time). G_2 in the presence of Ara C is thought to be approximately 3 hours (Brewen, 1965). Previous studies with Ara C have described stages of the cell cycle which were more sentitive to Ara C than others, although none are in agreement with this study. Benedict et al. (1970), in their study with hamster fibroblast cells, reported that maximum sensitivity to Ara C was in the latter half of S and at the beginning of G_2. Similarly, Ayraud et al. (1976), using cultured human lymphocytes, found that Ara C was most effective during the late S phase as far as the production of chromatid breaks is concerned. Brewen and Christie (1967), however, presented data from human lymphocyte studies suggesting that Ara C produced chromatid breaks with equal efficiency throughout the G_2 phase.

Another explanation for the increasing divergence of the expected and observed aberration frequencies with increasing treatment time, is that aberrations induced in early G_2 or late S have more time to undergo repair than aberrations induced towards the end of G_2. This would cause a decrease in the number of aberrations in the late S or early G_2 stage of the cell cycle (4 hour treatment time). However, this explanation seems unlikely because Ara C is thought actually to inhibit repair of damage caused by a wide variety of agents (Hiss and Preston, 1977; Preston, 1980).

Mechanisms Responsible for Ara C Induced Aberrations

Interpretation of the dose kinetics of Ara C is difficult because of the levelling off of the breakage rate with increasing concentrations (Figs. 2 and 3). This prevented any study of the dose kinetics in relation to the "Breakage First" hypothesis and "Revell's Exchange" hypothesis.

According to the model proposed by Bender et al. (1974), Ara C induces single polynucleotide strand breaks either directly or through failure to complete polynucleotide chain assembly, or indirectly perhaps through stimulation of a nuclease activity.

The method by which Ara C causes chromosome damage is not fully understood. Benedict and Karon (1971) have shown that ultra-violet light can reduce the frequency of chromatid breaks induced by Ara C in both the S and G_2 phases of the cell cycle. This effect was correlated with a decrease in the uptake of tritiated thymidine following ultra-violet treatment and implies that the production of chromosome breaks by Ara C is closely related to replicative DNA synthesis. Subsequently, it was found that 5-azacytidine which, like Ara C, is incorporated into DNA, produced chromosomal breaks in the G_2 phase of the cell cycle. Other agents, however, such as hydroxyurea, which decrease DNA synthesis by inhibiting ribonucleotide reductase and are not incorporated into DNA, produced no chromatid breakage in the G_2 phase (Karon and Benedict, 1972). These results suggest that the mechanism by which DNA synthesis is decreased is important to the cell cycle differences found in the production of chromosome aberrations caused by the various inhibitors of DNA synthesis. There is no conclusive evidence, however, that incorporation into DNA is the basis for chromatid aberration production by Ara C.

Natarajan and Obe (1978) described a possible mechanism for the production of chromatid breaks in chromosomes of Chinese hamster cells. They showed that the treatment of G_2 X-irradiated Chinese hamster cells with Neurospora endonuclease increased the frequency of all types of chromatid aberrations. Neurospora endonuclease is specific for cleaving single stranded DNA. Therefore, Natarajan and Obe interpreted their results as being the consequence of the enzyme converting some of the X-ray induced single strand breaks into double strand breaks, which could interact to provide aberrations. It is possible that such a mechanism could operate in cells exposed to Ara C, converting directly induced single strand breaks or single strand breaks arising during the repair of base damage, into double strand breaks.

There have been numerous reports that the majority of chromatid aberrations induced by Ara C are of the incomplete type; i.e. terminal deletions, non union isochromatid deletions, and a striking lack of chromatid interchanges (Brewen, 1965; Kihlman et al., 1963; Nichols and Heneen, 1964). The results of the present study agree with these reports and may be explained by the observation that Ara C has been shown to inhibit the repair of damage induced by ultra-violet irradiation, mitomycin C and 8-hydroxyquinoline in Chinese hamster cells and human fibroblasts (Hiss and Preston, 1977) and X-irradiation induced damage of human lymphocytes (Preston, 1980). "Complete" aberrations (e.g. exchanges) imply repair of breakage of chromosomes. The proposed mode of action of Ara C inhibition of repair (Preston, 1980) is via the incorporation of several Ara C molecules into the repaired replicating DNA, distorting the DNA helix and preventing the polymerase from functioning.

The frequency of isochromatid breaks (29%, see appendix 5) was higher than expected considering the period of the cell cycle during which the cells were exposed to Ara C (i.e. late S and G_2). Isochromatid breaks can be produced "directly" or "indirectly". However, nearly all chromosome breaking agents, except for ionizing radiations,appear incapable of producing "directly" induced isochromatid aberrations (Bender et al., 1974). Therefore isochromatid aberrations produced by Ara C are probably produced "indirectly". It is unlikely that the isochromatid breaks are due to the chance occurrence of two independent chromatid breaks at the equivalent loci, because of the high frequency of isochromatid breaks. However, if a locus on a chromatid is more susceptible to damage than others, then the corresponding locus on the sister chromatid would presumeably also be more susceptible to damage. Thus, some isochromatid aberrations may be due to the occurrence of two "independent" breaks at the same site of sister chromatids. However, the frequency of these breaks was not able to be quantified in the present study.

It is unlikely that isochromatid aberrations are "chromosome type" aberrations (i.e. lesions produced before DNA replication and then replicated into both chromatids) since the regression of isochromatid aberrations against chromatid aberrations does not change significantly with increasing time

of treatment ("Results" section, p. 17). Thus the ratio of isochromatid breaks to chromatid breaks produced in G_2 (2 hours treatment) is no different from the ratio produced in late S early G_2 (4 hours treatment).

Isochromatid aberrations may be produced by a method similar to that proposed in Revell's "Exchange Hypothesis" (see Introduction, p. 3). In fact, the Exchange Hypothesis predicts a ratio of 1:2.5 of isochromatid breaks to chromatid breaks, which is very close to that found in this study (1:2.45). However, when consideration is given to the period of the cell cycle during which the cells were exposed to Ara C, the ratio of 1:2.45 is still high. "Recombination repair" may be important in the development of isochromatid aberrations. Indeed, the similarity between exchanges giving rise to chiasmata during meiosis and the mutagen-induced exchanges in somatic cells has been long recognized, and it has been proposed that normal recombination and induced exchanges make use of common pathways in the cell (Evans, 1967).

AM induced Aberrations in Relation to Dose and the Cell Cycle

The frequency of AM induced aberrations rose sharply with increasing concentrations and then began to level off. Indeed, after 0.10 µg/ml the frequency of the total chromosome aberrations decreased (Fig. 7). This may be explained by the action of AM on the cell cycle. Hittleman and Rao (1975) presented data which suggest that cells exposed to AM suffer a dose dependent mitotic delay, primarily due to the prolongation of G_2. AM did not affect the progression of G_1 cells into S. The G_2 delay was least in cells treated during G_2; and cells treated during S exhibited a greater delay in their entry into mitosis than did those treated in G_2. The delay of cells in G_2 would have the effect of increasing the frequency of chromatid aberrations relative to chromosome aberrations, because cells in G_2 produce only chromatid aberrations. This difference in the relative frequencies of chromatid and chromosome aberrations would increase with dose, as exemplified in this study (Fig. 7), because cells would be held in G_2 for longer periods of time. The decrease in chromosome aberrations after 0.10 µg/ml may be explained by cells in G_1 or early S (a stage of the cell cycle when most chromosome aberrations are produced), being held for longer periods of time in G_2 and not progressing through to mitosis.

Another possible explanation for the levelling off of aberrations with increasing concentrations of AM is one similar to that given for Ara C. If there are only a limited number of sites along the chromosome where AM is able to intercalate (AM is known to intercalate into the DNA e.g. Di Marco, 1975), a "saturation point" would be reached, above which any further intercalation of AM could not be achieved.

It is of course possible that the cause of the levelling off of AM induced aberrations with increasing concentration is a combination of both explanations given here.

Although cells in this study were not exposed to AM for the final 20 hours before fixation, the effect of AM will still presumeably be present. This is because AM is retained within the cells due to its intercalation into DNA and is thus not easily removed by washing (Hittleman and Rao, 1975).

Mechanisms Responsible For AM Induced Aberrations

As was the case for Ara C, any interpretation of the dose kinetics of AM was limited because of the levelling off of the breakage rate with increasing dose (Figs. 6 and 7). The statistical analysis (Table 2) shows that the increase in breakage rate with increasing concentration of AM fits both linear and quadratic dose kinetics. The effects of a greater range of concentrations of AM on chromosome breakage need to be studied before any definite trend can be shown statistically.

As Vig (1971, 1973) found, there were significantly more chromatid aberrations than chromosome aberrations in the present study (Fig. 7). If isochromatid breaks were incorrectly classified as chromosome fragments, then this difference in the frequency of chromatid and chromosome aberrations would be magnified. The frequency of chromatid fragments was always lower than the frequency of chromosome fragments, but was parallel to the frequency of chromatid exchanges (Fig. 7). The chromosome fragment class was unlikely to contain a high proportion of isochromatid fragments because the frequency of chromosome fragments is considerably higher than the frequency of chromatid fragements (Fig. 7). The lack of chromosome exchanges may indicate that AM is capable of causing breaks in G_1 chromosomes at a time when reunions are not easily accomplished, and that exchanges do not reflect the

expected breakage reunion type of sequence suggested by the "Breakage First" hypothesis. On the other hand, if the chromosome fragments were of the isochromatid type, then their frequency should not exceed that of the chromatid fragments on the basis of "Revell's Exchange" hypothesis. Therefore, neither of the two main chromosome breakage hypotheses fit the data, as was the case for Ara C induced aberrations. The two hypotheses, however, were derived from cells exposed to ionizing radiation and are thus not necessarily applicable to chromosome damage induced by chemical agents.

The model proposed by Bender et al. (1974) provides possible mechanismsfor the production of aberrations by AM (see categories 2 and 3, pages 5-7). However, the model does not take into account the interaction of the various effects that AM has on the cell. AM has been shown to intercalate into DNA (Di Marco, 1975), inhibit RNA and DNA synthesis (Meriwether et al., 1972; Di Marco et al., 1971; Di Marco, 1975) and differentially injure cells with regard to the cell cycle (Barranco, et al., 1973). However, it is not known how these effects are interrelated to produce chromosome aberrations.

Sister Chromatid Exchange

(i) Ara C

As expected (see "Introduction" p. 9), no significant difference was found in the frequency of SCE's with increasing doses of Ara C, nor was there any evidence of a significant difference in the number of SCE's between donors. Raposa (1978) studied the effect of 2 µg/ml of Ara C on cultured human lymphocytes and found two populations of cells. One population had approximately a two fold increase over the control level of SCE, whereas in the other population the SCE frequency was approximately the same as the controls. Although Raposa offers no explanation for these data, it is likely that the two cell populations represent the difference between cells that underwent DNA synthesis in the presence of Ara C and those that were in G_2 in the presence of Ara C. It is possible that, at a concentration as low as 2.0 µg/ml, some cells may be able to pass through the S phase into G_2.

(ii) AM

The frequency of SCE increased with increasing dose of AM. One major difference between the effect of AM on the chromosome aberration frequency and the SCE frequency was that there was no significant difference in the frequency of SCE's between donors, whereas the chromosome aberration frequency was significantly different for some donors. This difference between chromosome aberrations and SCE's may reflect the different mechanisms that cause them. Nevstad (1978) has shown that the frequency of SCE's is a more sensitive indicator of the absence or presence of AM, particularly at lower concentrations. It may be that the increase in frequency of SCE's could be used to monitor the in vivo concentration for patients undergoing chemotherapy.

Intra-chromosome Distribution of Aberrations

All of the aberrations induced by Ara C and AM were assigned to either a dark or light band. However, 20% of the Ara C induced aberrations and 17% of the AM induced aberrations were difficult to assign with confidence because the breaks were close to or at an interface between light and dark bands. Such aberrations were always assigned to the band that they were visualized in. If the break appeared at the interface it was in fact assigned to the light band adjacent to the light/dark interface. In the present study, and in agreement with von Koskull and Aula (1977) who studied breaks induced by measles and Fanconi's anemia, a large portion of the breaks occurred close to one end of a light band. However, some breaks occurring close to or at an interface are possibly always visualized as occurring in the lightly stained area because a small deleted segment of a dark band can remain microscopically undetectable. Breaks occurring close to or at an interface and in the distal (relative to the centromere) part of the dark band will tend to be assigned to the light band. This is because confident assignment of such breaks to the dark band requires conviction that there has indeed been a loss of a portion of the dark band. This error leads, therefore, to an over representation of breaks in light regions. Corresponding breaks in the distal portion of a light band will, on the other hand, be correctly assigned, since the absence of a dark piece distal to the light band is easily determined.

The problem does not arise for breaks in the proximal part of light or dark bands. Buckton (1976) studied the effects of X-irradiation on cultured human lymphocytes and found approximately 30% of all breaks occurred at the light/dark interface. She suggested two possible causes for increased breakage at the interface. Visually there is a difference in the chromosome morphology at the interface; this boundary region may be more fragile. Alternatively, if a break occurs in a band, that break might be more readily repaired because the chromosome structure on both sides of the break is similar. If either of Buckton's suggestions are correct, aberrations occurring close to or at an interface should be regarded as a separate class. However, in the present study breaks were scored either in the light band they were visualized in or, in the case of breaks occurring at the interface, the breaks were scored in the light band adjacent to the interface. This seemed just as appropriate as scoring the "interface" breaks as a separate class, for three reasons. Firstly, it is not known whether the structure of the chromosome is different at the interface to either the light and/or the dark band and therefore no justification can be given to scoring "interface" breaks as a separate class on this count. Secondly, any error in assigning breaks to a light or dark band would normally only occur in those breaks close to or at an interface and in the distal part of a dark band; this error is probably not significant in the present study. Thirdly, the scoring of aberrations solely in either a light or dark band allows one to compare the results of the present study with those of previous ones.

Because the light staining regions of G banded (or Q banded) chromosomes are estimated to be about 50% of the chromosome length (Holmberg and Jonasson, 1973) one would expect half of the breakpoints to occur in light staining regions, if the breakpoints were located randomly. This was clearly not the case for both Ara C and AM (table 4). Similar localizations of breaks in the light G bands (or its equivalent pale Q bands) have been reported in earlier studies, and with a variety of agents. It is unclear whether this is partly or completely an artifact of the banding and scoring procedures, or an indication of a real structural difference in chromosomes. Seabright (1973) and Savage et al. (1973), using G banding and X-irradiation, found that all chromosome breaks occurred in the light staining regions. Holmberg and Jonasson (1973) used Q

banding with X-irradiation and found 60% of the breaks occurred in the pale fluorescing regions. Similarly, Aula and von Koskull (1976) found all spontaneously occurring breaks arose in light staining regions of G bands; and Aymé et al. (1976) using R banding, found between 50% and 70% of breaks arose in the light staining regions, i.e. in the dark staining regions of G banded chromosomes. In the present study, 67% of breaks induced by Ara C were found in the light staining G bands, whereas Ayraud et al. (1976) found nearly all Ara C induced breaks in the light staining regions of R banded preparations. Therefore, any conclusions regarding the occurrence of breaks in the light or dark bands should be treated carefully until a satisfactory explanation can be given regarding the discrepancies between different studies that use similar chromosome breaking agents.

Inter-chromosome Distribution of Aberrations

(i) Ara C

The distribution of Ara C induced breaks between chromosomes was non-random (Figs. 9 and 10). Although it was not possible to evaluate statistically the chromatid breaks relative to individual G bands, particular regions of some chromosomes were broken more often than others (Fig. 10). Four of these "hotspots" (3p14, 3p13, 7q32 and 9q12) either coincided with or were adjacent to those reported by Ayraud et al. (1976), who also studied the distribution of Ara C induced aberrations in cultured human lymphocytes. Ayraud et al. used R banding to localize their chromatid aberrations; this may explain the one band discrepancy between some of the "hotspots" they found and those found in this study (see the previous section).

Four of the "hotspots" (3p1, 3p2, 9q1 and 16q2) were found to be "hotspots" of spontaneous breakages by Aula and von Koskull (1976), and four (3p1, 7q3, 9q1, and 16q2) were found to be "hotspots" of spontaneous breakage by Aymé et al. (1976). 3p1, 9q1 and 16q2, therefore, are "hotspots" found in all three studies. These "hotspots" of spontaneous breakage are probably susceptible to damage and this susceptibility may be enhanced in the presence of a mutagenic agent. The band 9q1 is in the region of a secondary

constriction; and secondary constrictions are, in general, susceptible to damage by a wide variety of agents (e.g. Morad et al., 1973). Chromosomes 3 and 16 also had an excess of breaks in studies on unbanded chromosomes by Lubs and Samuelson (1967) and Obe and Luers (1972), and in banded preparations by San Roman and Bobrow (1973).

In one individual of the present study (donor H(U)), 38 breaks out of 333 were localized in the 3p1 - 3p2 region, and 16 others in the 7p3 region. However, this trend was not found in the other donors, so that the localization of these "hotspots" may be a characteristic of the individual concerned.

The method by which Ara C causes chromosome damage is not fully understood and consequently it is difficult to give an adequate explanation of the existence of hotspots found in this study. Ayraud et al. (1976) found that nearly all of their breaks were located in the light regions of R banded chromosomes. As these are regions of late replicating DNA these authors suggested that Ara C produced chromosome damage most effectively during the late S phase of the cell cycle. However, the present study showed that 67% of the Ara C induced breaks occurred in the light staining G bands and, therefore, no conclusion can be drawn regarding the timing of Ara C induced aberrations.

(ii) AM

The distribution of aberrations between chromosomes was non-random for AM induced lesions (Figs. 9 and 11). There was a significant difference in the mean number of breaks per unit length between the p and q arms for different chromosomes (Table 6); i.e. although overall there was no significant difference between the mean number of breaks per unit length of the p and q arms, when the chromosomes were considered separately, some had more breaks per unit length in the p arm than the q arm, whereas for others the reverse was true. This inter-arm interaction was a reflection of the overall non-random distribution of aberrations within the chromosome complement.

A number of "hotspots" induced by AM were located within the chromosome complement (Fig. 11). None of them coincided with those found for Ara C. However, some of the "hotspots" coincided with those from other studies, as shown in the Table below:

BAND	AGENT	AUTHORS
14q2	Spntaneous breakage	von Koskull and Aula, 1976
1q1, 1q3, 6p2, 6q2	Fanconi's anemia	von Koskull and Aula, 1976
14q2	Spontaneous breakage	Mattei et al. 1979
6p21	Busulphan	Honeycombe, 1978
1q11	Mitomycin C	Morad et al., 1973
1q32	X-irradiation	Holmberg and Jonasson, 1973
1p32	Chlorambucil	Reeves and Margoles, 1974

It is noteworthy that 4 of the 12 "hotspots" found by von Koskull and Aula coincided with 4 of the "hotspots" induced by AM in the present study. Fanconi's anemia is thought to be associated with a defective DNA repair system (Sasaki, 1975) or possibly a deficiency in an endonuclease necessary for removing an abnormal DNA strand in the repair process. However, the important cellular activity of AM is attributed to its inhibitory action on RNA and DNA synthesis. The basic mechanism of AM action is thought to be via formation of a specific complex with DNA, which is then stabilized by a variety of factors (see Di Marco, 1975). As with many other chromosome breaking agents, it is difficult to relate the biochemical activities of AM to the cytogenetic effects (as, for example, the clustering of aberrations on chromosome 6 and 13).

In an endeavour to understand the action of AM more clearly the various types of aberrations induced by AM were studied separately.

(a) Chromosome and Chromatid Aberrations

The different distributions of chromosome and chromatid aberrations (Fig. 12) may reflect differences in the mechanisms that produce chromosome and chromatid aberrations. Other

studies have shown that the mechanisms that produce chromosome and chromatid aberrations are different. For example, Bender et al. (1974) concluded that the chromosome aberrations produced by Ara C in G_1 were of different origin to the chromatid and isochromatid aberrations produce in S and G_2.

If the chromatid aberrations are further divided into chromatid exchanges and chromatid fragments, the reason for the non-random distribution of chromatid aberrations is found to be attributable to the non-random distribution of the chromatid exchanges (Table 10), since the chromatid fragments were distributed randomly (Table 9). Chromatid exchanges make up nearly all the exchanges and are discussed in the next section on "fragment and exchange aberrations".

The chromosome aberrations are nearly all of the fragment type. Chromosome aberrations are distributed non-randomly (Table 7) and there were significantly more breaks per unit length in the q arm than the p arm. This inter-arm interaction is discussed in the following section.

(b) Fragments and Exchanges

Fragments and exchanges were distributed differently along the chromosomes (Fig. 13). This contrasts with the findings based on groups of chromosomes by Vig (1971) who concluded that there was a similarity between the distribution of fragments and exchanges. In the present study both types of aberrations also had significantly different numbers of aberrations per unit length between the p and q arms.

Fragments had significantly more breaks in the q arm than the p arm, as did the chromosome aberrations (see the previous section), and exchanges had more breaks in the p arms than the q arms. A difference in the interaction between arms has been reported to my knowledge in only one other study. Savage et al. (1973) using X-irradiation reported that the distribution of exchange points between chromosome arms or chromosomes was not in proportion to chromosome length, although distribution between the arms of a given chromosome is in agreement with chromosome arm length. They concluded that the discrepancies arise from between chromosomes and not within them. This contrasts with the results of the present study, where there

were significant differences in the number of aberrations per unit length of chromosome between the p and q arms, i.e., there was also a difference between arms within chromosomes. There is no clear explanation for this phenomenon, since p and q arms are labelled by convention, with the shorter arm labelled p. However, one difference between p and q arms is that points along the p arms are closer to the centromeres and telomeres than points along the q arms.

Comings (1968) suggested that both the centromeres and telomeres are attached to the nuclear membrane, as well as being the sites for the initiation of DNA synthesis. Although Buckton (1976) points out that there is no clear evidence for the attachment of the telomeres and the centromeres to the nuclear membrane in human lymphocytes, a membrane association might well be present and might account for the increased rate of exchange at or near the centromeres and telomeres, due either to the close proximity of these regions to each other or to a greater vulnerability at the periphery of the nucleus. The closer proximity of points along the p arm to the centromere and the telomere compared to points along the q arm, and the greater vulnerability of centromeres and telomeres to damage, would lead to a greater number of breaks per unit length of chromosome in the p arm compared to the q arm. In the present study there were more exchanges per unit length of chromosome in the p arm than the q arm although, at least visually (Fig. 13), there appears to be no concentration of aberrations at the telomeres or centromeres. Also, the above explanation conflicts with the occurrence of more fragments per unit length of chromosome in the q arm than the p arm, and gives no explanation for there being a significant "drug by chromosome arm" interaction (Table 17) between Ara C induced aberrations, on the one hand, and AM induced aberrations on the other.

When the total number of fragments is further subdivided into chromatid fragments and chromosome fragments, it becomes evident that the non-random distribution of fragment aberrations and the inter-arm interaction is due to the distribution of chromosome fragments, because the chromatid fragments have no inter-arm effect and are distributed randomly. This difference in distribution of chromosome and chromatid aberrations may again be due to the difference in origin of the two types of aberrations. A better comparison between the fragments and

exchanges, in view of the differences between the distribution of chromosome and chromatid aberrations (Fig. 12), is one between chromatid fragments and "total exchanges" (exchanges are made up mostly of chromatid aberrations, Fig. 8). Chromatid fragments show a random distribution (Table 9). The spatial arrangement of some chromosomes during interphase may allow for increased opportunities for exchange. The satellite chromosomes (13, 14, 15, 21, 22) showed a higher susceptibility towards being involved in exchanges than other chromosomes (Figs. 13 and 15).

Vig (1971), who also studied the distribution of AM induced aberrations, found that chromosomes 21 and 22 had the highest number of exchanges per unit length. In the present study, chromosomes 13 and 14, and 13 and 15 were the ones most likely to be involved in exchanges. This may be a result of the intranuclear distribution of the chromosomes rather than an intrinsic peculiarity in the chromosome. Acrocentric chromosomes are thought to be associated with the nucleolar organising region of these chromosomes when they combine to form a common nucleolus (Schmid, 1969). The close proximity of the satellite chromosomes may allow for increased opportunities for exchanges between members of this group of chromosomes.

One of the most striking features of the AM data was the differences in the distributions of the different types of aberrations over the chromosome complement. Such differences have been reported in previous studies. Morad et al. (1973) reported a striking difference in the distribution of mitomycin C induced chromatid exchanges and chromatid fragments. Cook et al. (1975) studied the distribution of X-ray induced aberrations and also found a marked difference in the distribution of various types of chromosome aberrations. They concluded that although different regions of the chromosome may vary in their ability to undergo any particular form of structural rearrangement, many of the observed deviations could be explained in terms of the differential accuracy with which some aberrations can be detected. In the present study the aberrations were divided into four main classes, and although there was a small bias towards the exclusion of exchanges rather than fragments, because of the

difficulty in assigning the aberration to a band, this bias would not be sufficient to explain the marked difference in the distribution of the fragment and exchange aberrations.

The exchange aberrations showed a significant difference in the number of aberrations between donors. This was largely due to chromosomes from donor W having significantly more exchanges than those of the other donors. In the dosage experiments it was interesting to note that, overall, Donor W had the greatest number of AM induced aberrations (Fig. 6), although this was mainly due to the large number of fragments rather than exchanges (see table of means Appendix 3-3). The fact that AM induces significantly more exchanges in Donor W along with an indication from the dosage experiments that Donor W is the most susceptible to damage, suggests that individuals respond differentially to AM. This is supported by the observation that donors V(C) and X(A) had significantly fewer aberrations than the others in the dosage experiments.

Relevance of the Present Study to Cancer Chemotherapy

In vitro cell cultures are one of the most frequently used cytogenetic test systems. The concentrations of AM and Ara C used in the present study are similar to those in the tissues or plasma of patients during chemotherapy (Bachur et al., 1974; Benjamin et al., 1977; Ho and Freireich, 1975). However, *in vitro* experiments can only be regarded as a complementary tool to *in vivo* experiments and not an alternative. *In vivo* conditions cannot be imitated. Schoeller and Wolf (1970) summarized some of the differences between *in vivo* and *in vitro* conditions. In living organisms, metabolic effects may alter the mode of action of a given compound. Certain agents need activation within the organism to become effective, while *in vitro* the original form is maintained. Some compounds produce organ-specific effects while appearing ineffective in *in vitro* experiments.

In achieving the optimum concentration of a chemotherapeutic agent at the target cells of an individual undergoing chemotherapy, there are two important considerations to be made. Firstly, the characteristics of the individuals undergoing chemotherapy must be known. The capacity of a drug to have an antineoplastic effect is influenced by age, sex, race and previous therapy of the individual. Secondly, the anti-

neoplastic activity of a chemotherapeutic agent is profoundly affected by pharmacological parameters such as dosage, route and schedule of administration, the half-life of the drug, and the amount of the drug excreted in the urine.

The main goal of chemotherapy is to select doses of a drug that will kill cancer cells faster than they are replaced, without overdosing the patient (Skipper, 1971). In the present study, chromosome damage, rather than cell death, was studied. However, a good correlation has been demonstrated between chromosome damage and cell death for AM (Hittleman and Rao, 1975) and for Ara C (Jones et al., 1976).

Overdosing the patient has important considerations in cancer chemotherapy. One of the major drawbacks of chemotherapeutic agents is their relative inability to select between neoplastic and non-neoplastic cells, thus inducing the toxic and virtually ubiquitous side effects associated with most chemotherapeutic agents. Therefore the 'levelling off effect' in the present study (Figs 2 and 6) may have important implications in cancer chemotherapy if a similar levelling off can be demonstrated in vivo. Any dose above the onset of the levelling off of the chromosome breakage rate would be superfluous and would only increase the chance of overdosing the patient.

The Ara C studies suggest that 'treatment time' is an important factor when considering cell toxicity. Levelling off of the breakage rate was demonstrated with increasing concentration of Ara C. However, no such levelling off was observed with increasing treatment times (Fig. 2). This may also have important practical applications in cancer chemotherapy, because it implies that optimum cytotoxicity can be induced by using Ara C at low doses for long periods of time. Ideally the dose at the target cell should be analogous to that which initiated the levelling off of the breakage rate in the present in vitro study.

Inter-individual differences are important in cancer chemotherapy. A large number of studies have been carried out on individual variation regarding drug metabolism. From such studies it has been established that drug metabolizing enzymes are under genetic control and the genetic component is rather substantial, although they are often affected by other drugs, age, sex and even physical stress (e.g. Vessell et al., 1971;

Whitaker and Price Evans, 1970). The present *in vitro* studies reflect the individual variation regarding drug metabolism. There was a significant difference between donors in the amount of AM induced chromosome aberrations, although for Ara C there was no such inter-individual variation.

The existence of 'hotspots' revealed by Ara C and AM treatment gave no basis upon which any valid correlations between the mechanism of activity of Ara C and AM, on the one hand, and the pattern of distribution of aberrations, on the other, could be formulated. One possibility is that drugs which are able to produce 'hotspots' at any of the sites along the chromosome that are important, directly or indirectly, in cell division, may be useful as chemotherapeutic agents. Thus 'hotspots' induced by Ara C and AM may represent points along the chromosome important for cell division.

SUMMARY (Compare the "Aims of this study" p. 11)

A study of chromosome aberrations induced by 1-β-D-arabinofuranosylcytosine (Ara C) and Adriamycin (AM) in the chromosomes of cultured human lymphocytes was made and the following results were obtained and discussed.

(i) There were significant increases in the frequency of aberrations with increasing concentrations for both Ara C (2.5, 5.0 and 10.0 µg/ml) and AM (0.01, 0.05, 0.10 and 0.15 µg/ml). A "levelling off" was observed after 5.0 µg/ml for Ara C and after 0.05 µg/ml for AM. It was suggested that the levelling off may have been a result of there being only a limited number of sites along the chromosome where either AM or Ara C could interact to produce aberrations: a levelling off would be reached when these sites are "occupied". Other possibilities are that cells may retain the drugs only up to a particular concentration or that only certain amounts of the drug may be altered into forms that are able to interact with the chromosome to produce aberrations.

(ii) There was no indication of a levelling off in the frequency of aberrations with increasing treatment time. The levelling off in the frequency of aberrations with increasing concentrations of Ara C superficially appears to be in conflict with the trend shown by the effect of increasing treatment times on the breakage rate. It was suggested that Ara C, rather than directly causing breaks, may induce points of "potential breakage" along the chromosomes. These potential breaks became breaks only after a given period of time; longer treatment time converts more potential breaks into actual breaks.

The frequency of Ara C induced aberrations increased

case for cells exposed to AM. One major difference between the effect of AM on the chromosome aberration frequency and the SCE frequency was that SCE's showed no significant differences between donors, whereas the chromosome aberration frequency was different for different donors.

(iv) There was a linear relationship between the frequency of chromatid and isochromatid aberrations with increasing treatment time and increasing concentrations of Ara C.

AM caused more chromatid aberrations than chromosome aberrations. The relationship between the frequency of aberrations and increasing dose was significantly different for the chromatid versus the chromosome aberrations. This was explained in terms of AM and its effect on the cell cycle. There were more fragments than exchanges induced by AM; and the relationship between increasing dose was significantly different for the fragment versus the exchange aberrations.

(v) More aberrations were located in the light G bands than the dark G bands.

Both drugs showed a distinct clustering of aberrations in some regions of the chromosomes (hotspots), although the location of AM induced hotspots was different from the location of those induced by Ara C. The distribution of AM induced chromatid aberrations was different from the distribution of the chromosome aberrations as were the distributions of the fragment and exchange aberrations. The different types of aberrations also differed in the number of AM induced aberrations per unit length between the p and q arms. There were more aberrations per unit length in the p arm than in the q arm for exchanges, whereas for fragments and chromosome aberrations the reverse was true. For chromatid aberrations, there was no significant difference in the number of aberrations per unit length between the p and q arms.

The distribution of the aberrations was discussed in relation to previous studies. However, no conclusions could be drawn relating the distribution of aberrations to chromosome structure.

(vi) The relevance of the present <u>in vitro</u> studies to cancer chemotherapy, was discussed.

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APPENDIX 1

Method For Culturing Human Peripheral Blood Lymphocytes

Reagents

Hams F10: (Dry Powder, Microbiological Associates)
- made up and stored at 4°C for up to 14 days.
- contains various amino acids, vitamins and buffered salts.

AB Serum: can be stored at -20°C indefinitely.
- supplies serum proteins necessary to support the metabolism of cultures.

Phytohaemagglutinin-m: (Dry Powder, Difco).
- reconstituted PHA can be stored at -20°C for up to 6 months.
- activates in vitro lymphocytes to start dividing.

Gentamicin Sulphate: (Roussel)
- antibiotic

Colchicine: (Sigma)
- added to culture medium at a final concentration of 0.01 mg/ml. A stock solution(0.01 mg /ml) was normally kept at 4°C for up to 6 months.
- colchicine allows cells to accumulate at metaphase.

Hypotonic Solution: 0.4% KCl made up fresh before use.
- hypotonic swells the cells and promotes dispersion of metaphase chromosomes.

Fixative: 3 parts of analar methanol: 1 part of analar glacial acetic acid.
- made up fresh before use and stored in a refrigerator.

Media:	Hams F10	100mls	adjusted to pH 7.0 using
	PHA-m	2mls	either CO_2 or $NaHCO_3$
	Gentamicin	0.1ml	

Dispense:	6mls	media	per 20 ml sterile universal container
	3mls	AB Serum	

To set up Culture

Venous blood was drawn off into a vacuotainer containing Lithium heparin. 0.4mls of whole blood was added to each culture vessel and incubated at 30°C for 66 hours.

To harvest Culture

1. 0.1µg/ml of colchicine was added to each culture vessel 1½ hours prior to harvest.

2. The contents of the culture vessels were transferred to 12 ml graduated conical centrifuge tubes and spun for 5 minutes at 800 rpm.

3. The supernatant was discarded to 0.5 mls and the cells were resuspended in the remaining supernatant. About 3 mls of prewarmed (37°C) hypotonic was added for 15 minutes at 37°.

4. The cells were resuspended if necessary, then 5mls of chilled fixative was added by squirting it vigorously into the centrifuge tube. This first fixative addition resulted in hemolysis of the red blood cells and conversion of the haemoglobin to a dark brown acid hematin.

5. The fixed cells were spun at 800 rpm for 5 minutes, then the supernatant was discarded to about 1 ml and fresh fixative added. This step was repeated until the cells were white and the supernatant was clear (normally 2-3 times).

6. As much supernatant as possible, without disturbing the cell button, was removed and the cells resuspended in a few drops of fresh fixative to produce a cloudy suspension.

7. Using a pasteur pipette held 15-20cm above a slide, the cells were dropped onto a clean chilled slide and passed through a small flame to ignite the alcohol in the fixative.

8. The slide was examined under a microscope (phase contrast) and the quality of the metaphase was observed. If the cells were too scarce, the "cloudy suspension" in the centrifuge tube was concentrated; if the cells were too dense the suspension was diluted.

9. If the slides were to be banded, the procedure in the method section was followed. Otherwise, the slides were left in an incubator overnight at 60°C and stained in 10% Giemsa (B.H.D.) the next morning.

10. Slides were mounted in Duco Industrial clear lacquer (Number, 35-928).

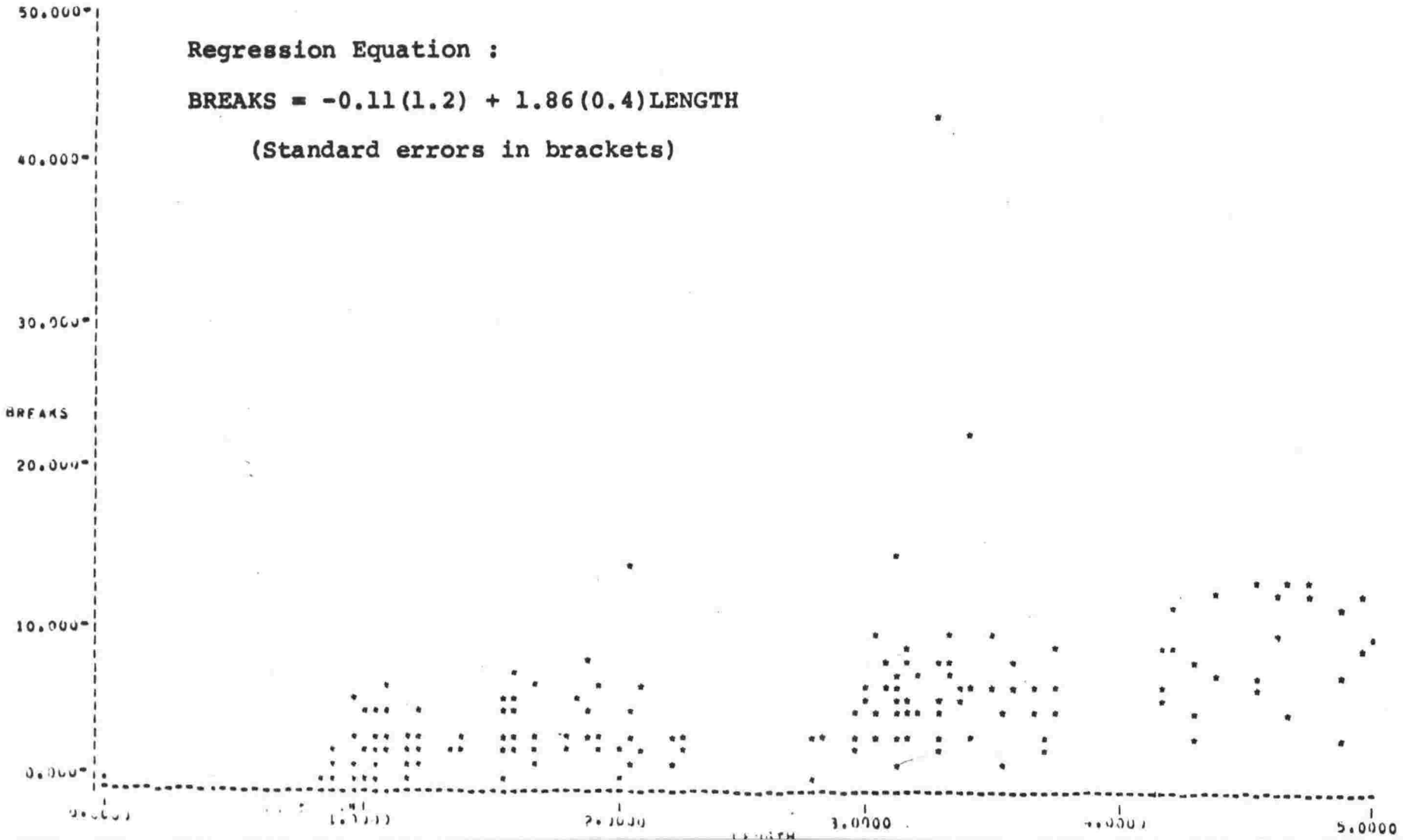
Appendix 2

Regression of Ara C induced aberrations against relative length

Regression Equation :

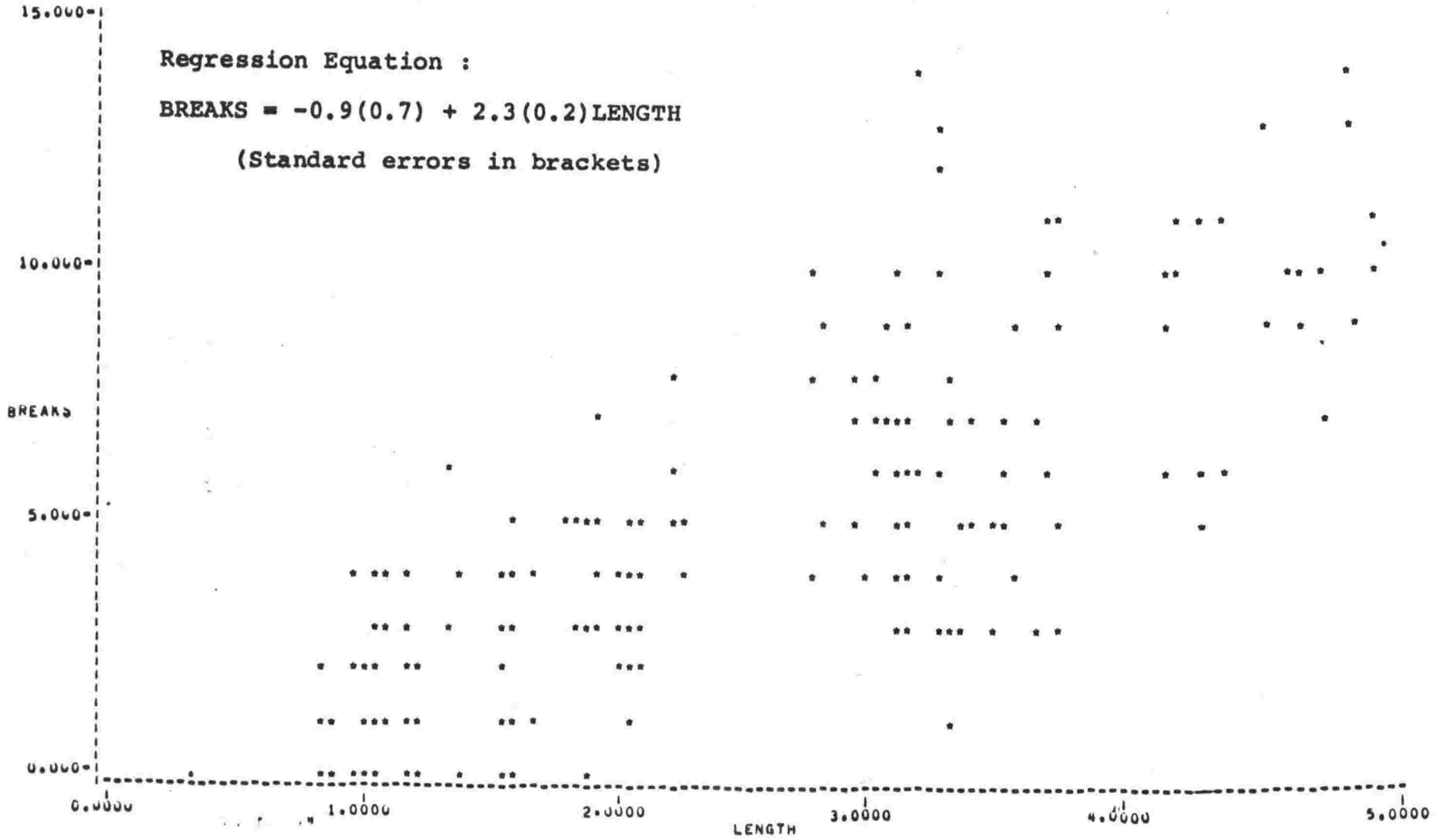
BREAKS = -0.11(1.2) + 1.86(0.4)LENGTH

(Standard errors in brackets)



Appendix 2

Regression of AM induced aberrations against relative length



Appendix 3

Analysis of variance tables and their tables of means for tables 1-3, 5-15 and 17-19 from the "result" section

Legend

3-1	=	The "1" refers to the table number from the "result" section.
A	=	Chromosome arms
C	=	Chromosomes
c	=	Cubic
D	=	Replicate (tables 2 and 3) or drug (table 17)
H	=	Time
L	=	Concentration
l	=	Linear
q	=	Quadratic
R	=	Donors
T	=	Types of aberration

Appendix 3-1

ANALYSIS OF VARIANCE FOR CONCENTRATIONS AND TREATMENT TIMES FOR Ara C

SOURCE OF VARIATION	SS	DF	MS	F	P
L	27830.53	2	13915.27	37.07	0.00
H	28892.93	2	14446.47	38.49	0.00
L H	3418.13	4	854.53	2.28	0.08
ERROR	13513.20	36	375.37		
TOTAL	73654.80	44	1673.97		

Table of means

Factor L

CONCENTRATION	MEAN
2.5µg/ml	58.73
5.0µg/ml	105.60
10.0µg/ml	115.87

Factor H

TIME	MEAN
2 hours	64.60
3 hours	89.33
4 hours	126.27

Appendix 3-1

Table of means (cont.)

L H MEANS

TIME(hours)	2	3	4
2.5µg/ml	47.20	44.40	84.60
5.0µg/ml	69.40	105.80	141.60
10.0µg/ml	77.20	117.80	152.60

Appendices 3-2 and 3-3

Appendices 3-2 and 3-3 are "Nested" models of fixed effects; "D" (the replicates are within "R" (the donors), within "L" (the concentrations) and within "T" (the aberration types). Any interactions with "T" are tested against the "T" by "D" interaction which is assumed to be the random error.

Appendix 3-2

ANALYSIS OF VARIANCE COMPARING CHROMATID AND CHROMOSOME TYPE ABERRATIONS FOR DIFFERENT DONORS AND DIFFERENT CONCENTRATIONS OF AM

SOURCE OF VARIATION	SS	DF	MS	F	P
R	2405.95	4	601.49	3.44	0.03
	tested against D				
L	17565.10	3	5855.03	33.51	0.00
	tested against D				
Ll	15694.27	1	15694.27	89.83	0.00
	tested against D				
Lq	1736.36	1	1736.36	9.93	0.01
	tested against D				
Lc	134.47	1	134.47	0.77	0.38
	tested against D				
T	6372.45	1	6372.45	104.04	0.00
D	3494.00	20	174.70	2.85	0.02
R L	1504.15	12	125.35	0.72	0.72
	tested against D				
R Ll	152.41	4	38.10	0.22	0.93
	tested against D				
R Lq	653.02	4	163.25	0.93	0.46
	tested against D				
R Lc	698.72	4	174.68	0.99	0.43
	tested against D				
R T	513.05	4	128.26	2.09	0.12
	tested against T D				
L T	808.85	3	269.62	4.40	0.02
	tested against T D				
Ll T	380.88	1	380.88	6.22	0.02
	tested against T D				
Lq T	18.04	1	18.04	0.29	0.59
	tested against T D				
Lc T	409.93	1	409.93	6.69	0.02
	tested against T D				
P L T	1010.65	12	84.22	1.38	0.26
	tested against T D				
P Ll T	292.98	4	73.25	1.20	0.34
	tested against T D				
P Lq T	441.87	4	110.47	1.80	0.17
	tested against T D				
P Lc T	275.80	4	68.95	1.13	0.37
	tested against T D				
ERROR	1225.00	20	61.25		
TOTAL	34899.20	79	441.76		

Appendix 3-2

Tables of means for chromatid and chromosome type aberrations

Factor R

DONOR	MEAN
U(H)	45.69
V(C)	32.75
W	47.69
X(A)	40.56
Y	46.31

Factor L

CONCENTRATION	MEAN
0.01µg/ml	19.15
0.05µg/ml	41.15
0.10µg/ml	52.20
0.15µg/ml	57.90

Factor T

TYPE	MEAN
Chromatid	51.53
Chromosome	33.68

R L MEANS

DONOR	0.01µg/ml	0.05µg/ml	0.10µg/ml	0.15µg/ml
U(H)	24.25	42.75	60.25	55.50
V(C)	12.00	30.50	36.75	51.75
W	15.00	53.75	57.75	64.25
X(A)	20.00	38.50	44.50	59.25
Y	24.50	40.25	61.75	58.75

Appendix 3-2

Table of means (cont.)

R T MEANS

DONOR	Chromatid	Chromosome
U(H)	57.25	34.13
V(C)	42.38	23.13
W	54.88	40.50
X(A)	45.50	35.63
Y	57.63	35.00

L T MEANS

CONCENTRATION	Chromatid	Chromosome
0.01µg/ml	24.50	13.80
0.05µg/ml	51.70	30.60
0.10µg/ml	58.70	45.70
0.15µg/ml	71.20	44.60

Appendix 3-3

ANALYSIS OF VARIANCE COMPARING FRAGMENT AND EXCHANGE TYPE ABERRATIONS FOR DIFFERENT DONORS AND DIFFERENT CONCENTRATIONS OF AM

SOURCE OF VARIATION	SS	DF	MS	F	P
R	2405.95	4	601.49	3.44	0.03
	tested against D				
L	17565.10	3	5855.03	33.51	0.00
	tested against D				
Ll	15694.27	1	15694.27	89.84	0.00
	tested against D				
Lq	1736.36	1	1736.36	9.94	0.01
	tested against D				
Lc	134.47	1	134.47	0.77	0.39
	tested against D				
T	5951.25	1	5951.25	95.83	0.00
D	3494.00	20	174.70	2.81	0.02
	tested against D				
R L	1504.15	12	125.35	0.71	0.72
	tested against D				
R Ll	152.41	4	38.10	0.22	0.92
	tested against D				
R Lq	653.02	4	163.25	0.93	0.46
	tested against D				
R Lc	698.72	4	174.68	1.00	0.43
	tested against D				
R T	406.75	4	101.69	1.64	0.20
	tested against T D				
L T	917.25	3	305.75	4.92	0.02
	tested against T D				
Ll T	835.65	1	13.46	13.46	0.00
	tested against T D				
Lq T	40.60	1	40.60	0.65	0.43
	tested against T D				
Lc T	41.00	1	41.00	0.66	0.43
	tested against T D				
R L T	656.75	12	54.72	0.88	0.58
	tested against T D				
R Ll T	215.60	4	53.90	0.87	0.50
	tested against T D				
R Lq T	382.98	4	94.75	1.54	0.23
	tested against T D				
R Lc T	58.16	4	14.54	0.23	0.93
	tested against T D				
ERROR	1242.00	20	62.10		
TOTAL	34143.20	79	432.19		

Appendix 3-3

Tables of means for fragment and exchange aberrations

Factor R

DONOR	MEAN
U(H)	45.69
V(C)	32.75
W	47.69
X(A)	40.56
Y	46.31

Factor L

CONCENTRATION	MEAN
0.01µg/ml	19.15
0.05µg/ml	41.15
0.10µg/ml	52.20
0.15µg/ml	57.90

Factor T

TYPE	MEAN
Fragment	51.22
Exchange	33.98

R L MEANS

DONOR	0.01µg/ml	0.05µg/ml	0.10µg/ml	0.15µg/ml
U(H)	24.25	42.75	60.25	55.50
V(C)	12.00	30.50	36.75	51.75
W	15.00	53.75	57.75	64.25
X(A)	20.00	38.50	44.50	59.25
Y	24.50	40.25	61.75	58.75

Appendix 3-3

Table of means (cont.)

R T MEANS

DONOR	Fragment	Exchange
U(H)	54.63	36.75
V(C)	37.63	27.88
W	58.63	36.75
X(A)	51.38	29.75
Y	53.88	38.75

L T MEANS

CONCENTRATION	Fragment	Exchange
0.01µg/ml	23.30	15.00
0.05µg/ml	47.70	34.60
0.10µg/ml	63.90	40.50
0.15µg/ml	70.00	45.80

Appendix 3-5

ANALYSIS OF VARIANCE FOR TOTAL No. OF BREAKS INDUCED BY ARA C

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	57.73	17	3.40	2.08	0.02
R	33.39	4	8.35	5.12	0.00
A	2.17	1	2.17	1.33	0.25
C R	119.77	68	1.76	1.08	0.38
C A	43.42	17	2.55	1.57	0.10
R A	6.91	4	1.73	1.06	0.38
ERROR	110.84	68	1.63		
TOTAL	374.25	179	2.09		

Tables of means

Factor R

DONOR	MEAN
H(U)	2.26
C(V)	1.41
E	1.22
A(X)	2.16
Y	2.13

Appendix 3-5

Table of means (cont.)

Factor A

ARM	MEAN
P	1.95
Q	1.73

Factor C

CHROMOSOME	MEAN
1	2.01
2	2.07
3	2.73
4	1.92
5	1.82
6	1.04
7	2.35
8	1.41
9	1.89
10	1.60
11	1.49
12	2.40
16	2.12
17	1.62
18	0.49
19	2.24
20	1.16
X	2.71

Appendix 3-5

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	1.92	2.10
2	2.43	1.71
3	4.15	1.30
4	1.88	1.95
5	2.06	1.57
6	0.90	1.18
7	2.07	2.62
8	1.55	1.26
9	1.87	1.92
10	1.61	1.59
11	1.64	1.34
12	3.49	1.30
16	1.73	2.50
17	1.11	2.13
18	0.00	0.98
19	2.16	2.31
20	1.15	1.17
X	3.29	2.13

R A MEANS

ARMS	H(U)	C(V)	E	A(X)	B
P	2.64	1.57	1.30	2.30	1.91
Q	1.87	1.23	1.16	2.03	2.35

Appendix 3-5

Table of means (cont.)

C R MEANS

CHROMOSOME	H(U)	C(V)	E	A(X)	B
1	2.52	1.50	1.26	2.61	2.15
2	3.54	0.79	1.69	2.64	1.70
3	7.29	1.28	1.16	1.59	2.31
4	1.69	1.06	2.34	2.30	2.20
5	1.58	1.00	1.44	2.09	2.99
6	0.95	0.50	0.63	1.47	1.65
7	4.92	0.96	2.00	1.63	2.21
8	2.56	0.76	0.60	1.63	1.49
9	0.99	1.36	1.68	2.64	2.79
10	1.86	1.38	1.22	2.19	1.35
11	1.31	1.29	0.51	2.41	1.94
12	2.92	2.42	1.49	2.93	2.20
16	3.87	1.70	1.21	1.43	2.38
17	0.73	2.10	0.73	2.00	2.55
18	0.00	0.50	0.50	0.73	0.73
19	0.41	3.65	1.82	2.69	2.61
20	0.84	1.39	0.49	1.78	1.30
X	2.61	1.70	1.28	4.19	3.77

Appendix 3-6

ANALYSIS OF VARIANCE FOR TOTAL No. OF BREAKS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	61.46	17	3.62	6.42	0.00
R	4.07	4	1.02	1.81	0.14
A	0.53	1	0.53	0.95	0.33
C R	42.86	68	0.63	1.12	0.32
C A	21.78	17	1.28	2.27	0.01
R A	3.06	4	0.77	1.36	0.26
ERROR	38.30	68	0.56		
TOTAL	172.08	179	0.96		

Tables of means

Factor R

DONOR	MEAN
U(H)	1.65
V(C)	1.65
W	1.91
X(A)	1.43
Y	1.70

Appendix 3-6

Table of means (cont.)

Factor A

ARM	MEAN
P	1.62
Q	1.72

Factor C

CHROMOSOME	MEAN
1	2.22
2	2.35
3	1.18
4	1.98
5	1.42
6	2.53
7	2.07
8	1.53
9	2.10
10	2.35
11	1.71
12	1.98
16	0.91
17	1.13
18	1.20
19	0.79
20	0.52
X	2.07

Appendix 3-6

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	2.21	2.23
2	2.36	2.33
3	1.22	1.14
4	2.00	1.96
5	1.04	1.81
6	2.52	2.54
7	2.69	1.45
8	1.67	1.39
9	2.54	1.67
10	2.36	2.34
11	1.52	1.89
12	2.21	1.75
16	0.19	1.64
17	0.22	2.04
18	1.22	1.18
19	0.59	0.99
20	0.38	0.67
X	2.18	2.01

R A MEANS

ARMS	U(H)	V(C)	W	X(A)	Y
P	1.48	1.71	2.05	1.25	1.59
Q	1.82	1.59	1.76	1.62	1.82

Appendix 3-6

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
1	2.08	2.64	1.72	2.27	2.39
2	2.08	2.95	2.05	2.54	2.12
3	1.88	1.02	1.33	0.55	1.12
4	2.01	2.54	1.39	2.30	1.67
5	1.03	1.88	2.26	0.69	1.26
6	3.17	2.85	1.95	2.36	2.31
7	1.51	2.32	2.55	2.23	1.75
8	0.75	2.11	2.11	1.04	1.63
9	2.36	1.88	2.52	2.00	1.77
10	2.23	1.91	3.02	0.94	3.65
11	1.82	1.99	2.12	0.67	1.92
12	2.24	1.17	2.85	1.92	1.72
16	0.97	1.21	1.21	0.71	0.48
17	1.22	0.73	1.53	1.20	0.96
18	1.96	1.10	1.60	0.49	0.85
19	0.83	0.50	1.41	0.41	0.82
20	0.00	0.42	0.42	0.88	0.89
X	1.60	0.48	2.29	2.61	3.36

Appendix 3-7

ANALYSIS OF VARIANCE FOR "CHROMOSOME" TYPE ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	21.06	17	1.24	4.80	0.00
R	0.55	4	0.14	0.53	0.71
A	3.21	1	3.21	12.47	0.00
C R	12.48	68	0.18	0.71	0.92
C A	11.05	17	0.65	2.52	0.00
R A	3.00	4	0.75	2.91	0.03
ERROR	17.55	68	0.26		
TOTAL	68.93	179	0.39		

Tables of means

Factor R

DONOR	MEAN
U(H)	0.75
V(C)	0.78
W	0.64
X(A)	0.64
Y	0.69

Appendix 3-7

Table of means (cont.)

Factor A

ARM	MEAN
P	0.57
Q	0.84

Factor C

CHROMOSOME	MEAN
1	0.98
2	1.07
3	0.35
4	1.22
5	0.63
6	1.32
7	0.94
8	0.45
9	1.01
10	0.90
11	0.71
12	0.61
16	0.48
17	0.29
18	0.00
19	0.71
20	0.33
X	0.63

Appendix 3-7

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	0.91	1.05
2	0.96	1.18
3	0.49	0.22
4	1.25	1.19
5	0.65	0.60
6	1.35	1.30
7	0.83	1.05
8	0.48	0.42
9	1.44	0.58
10	0.59	1.20
11	0.00	1.42
12	0.37	0.85
16	0.00	0.96
17	0.00	0.58
18	0.00	0.00
19	0.59	0.83
20	0.00	0.67
X	0.32	0.94

R A MEANS

ARMS	U(H)	V(C)	W	X(A)	Y
P	0.51	0.80	0.67	0.44	0.43
Q	0.99	0.76	0.62	0.85	0.96

Appendix 3-7

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
1	0.79	1.02	0.81	1.14	1.14
2	1.20	1.52	0.53	1.02	1.08
3	0.58	0.31	0.61	0.00	0.27
4	1.80	1.80	0.64	0.94	0.94
5	0.56	1.00	0.88	0.23	0.46
6	1.72	1.63	0.59	1.33	1.33
7	0.70	1.37	0.85	0.80	0.98
8	0.16	0.60	0.45	0.30	0.74
9	0.99	0.83	1.04	1.41	0.75
10	0.80	1.22	0.53	0.47	1.46
11	1.02	1.02	0.51	0.50	0.50
12	0.57	0.14	0.89	0.73	0.70
16	0.72	0.24	0.72	0.48	0.24
17	0.49	0.24	0.24	0.48	0.00
18	0.00	0.00	0.00	0.00	0.00
19	0.83	0.50	0.99	0.41	0.82
20	0.00	0.42	0.42	0.41	0.41
X	0.58	0.16	0.85	0.94	0.63

Appendix 3-8

ANALYSIS OF VARIANCE FOR "CHROMATID" TYPE ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	28.17	17	1.66	4.17	0.00
R	4.44	4	1.11	2.80	0.03
A	0.50	1	0.50	1.26	0.27
C R	27.96	68	0.41	1.04	0.44
C A	17.35	17	1.02	2.57	0.00
R A	1.37	4	0.34	0.86	0.49
ERROR	27.01	68	0.40		
TOTAL	106.80	179	0.60		

Tables of means

Factor R

DONOR	MEAN
U(H)	0.90
V(C)	1.00
W	1.26
X(A)	0.79
Y	1.01

Appendix 3-8

Table of means (cont.)

Factor A

ARM	MEAN
P	1.05
Q	0.94

Factor C

CHROMOSOME	MEAN
1	1.28
2	1.36
3	0.77
4	0.78
5	0.82
6	1.32
7	1.16
8	1.05
9	1.03
10	1.52
11	1.17
12	1.37
16	0.43
17	0.89
18	1.15
19	0.08
20	0.27
X	1.43

Appendix 3-8

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	1.30	1.27
2	1.40	1.31
3	0.73	0.81
4	0.75	0.81
5	0.39	1.25
6	1.17	1.46
7	1.86	0.47
8	1.19	0.91
9	1.11	0.96
10	1.77	1.27
11	1.52	0.81
12	1.84	0.90
16	0.19	0.67
17	0.22	1.55
18	1.22	1.08
19	0.00	0.17
20	0.38	0.17
X	1.80	1.07

R A MEANS

ARMS	U(H)	V(C)	W	X(A)	Y
P	0.98	0.90	1.38	0.81	1.16
Q	0.83	1.10	1.14	0.77	0.86

Appendix 3-8

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
1	1.29	1.84	0.90	1.14	1.25
2	0.88	1.83	1.52	1.51	1.04
3	1.30	0.44	0.72	0.55	0.86
4	0.21	0.85	0.74	1.35	0.73
5	0.47	1.00	1.38	0.46	0.80
6	1.45	1.77	1.36	1.02	0.98
7	0.81	1.11	1.71	1.43	0.77
8	0.60	1.35	1.66	0.74	0.89
9	1.36	0.72	1.48	0.59	1.02
10	1.43	1.01	2.49	0.47	2.19
11	0.81	1.82	1.61	1.67	1.42
12	1.67	1.03	1.96	1.19	1.01
16	0.24	0.97	0.48	0.24	0.24
17	0.73	0.73	1.29	0.72	0.96
18	1.96	0.86	1.60	0.49	0.85
19	0.00	0.00	0.42	0.00	0.00
20	0.00	0.42	0.00	0.48	0.48
X	1.01	0.32	1.44	1.68	2.73

Appendix 3-9

ANALYSIS OF VARIANCE FOR "CHROMATID FRAGMENTS"

ABERRATIONS INDUCED BY AM?

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	3.07	17	0.18	1.09	0.38
R	0.04	4	0.01	0.06	0.99
A	0.20	1	0.20	1.23	0.27
C R	10.82	68	0.15	0.96	0.56
C A	2.06	17	0.12	0.74	0.76
R A	0.71	4	0.18	1.07	0.38
ERROR	11.25	68	0.16		
TOTAL	28.16	179	0.16		

Tables of means

Factor R

DONORS	MEAN
U(H)	0.38
V(C)	0.41
W	0.38
X(A)	0.37
Y	0.38

Appendix 3-9

Table of means (cont.)

Factor R

Arm	Mean
p	0.35
q	0.42

Factor C

Chromosome	Mean
1	0.62
2	0.45
3	0.49
4	0.51
5	0.30
6	0.48
7	0.40
8	0.30
9	0.48
10	0.43
11	0.33
12	0.35
16	0.29
17	0.40
18	0.15
19	0.18
20	0.19
X	0.58

Appendix 3-9

Table of means (cont.)

C A Means

Chromosome	p	q
1	0.62	0.61
2	0.57	0.32
3	0.49	0.49
4	0.50	0.51
5	0.13	0.46
6	0.36	0.60
7	0.51	0.29
8	0.12	0.48
9	0.44	0.51
10	0.30	0.57
11	0.38	0.27
12	0.36	0.34
16	0.19	0.39
17	0.22	0.58
18	0.00	0.30
19	0.19	0.17
20	0.38	0.00
X	0.53	0.63

R A Means

Arms	U(H)	V(C)	W	X(A)	Y
$\bar{p}$	0.24	0.37	0.33	0.40	0.41
q	0.52	0.46	0.43	0.34	0.35

Appendix 3-9

Table of means (cont.)

C R Means

Chromosome	U(H)	V(C)	W	X(A)	Y
1	0.81	0.92	0.45	0.57	0.35
2	0.31	0.52	0.53	0.57	0.32
3	0.72	0.58	0.29	0.29	0.57
4	0.21	0.64	0.64	0.83	0.21
5	0.12	0.44	0.35	0.23	0.34
6	1.00	0.63	0.14	0.27	0.36
7	0.29	0.44	0.26	0.77	0.26
8	0.15	0.76	0.15	0.30	0.15
9	0.76	0.60	0.72	0.16	0.16
10	0.37	0.16	0.69	0.47	0.47
11	0.00	0.49	0.66	0.17	0.31
12	0.28	0.14	0.00	1.04	0.28
16	0.24	0.73	0.24	0.00	0.24
17	0.49	0.00	0.80	0.24	0.48
18	0.50	0.25	0.00	0.00	0.00
19	0.00	0.00	0.41	0.00	0.49
20	0.00	0.00	0.00	0.48	0.48
X	0.58	0.16	0.48	0.31	1.37

Appendix 3-10

ANALYSIS OF VARIANCE FOR "CHROMATID EXCHANGES"

ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	18.98	17	1.11	5.92	0.00
R	5.20	4	1.30	6.89	0.00
A	2.51	1	2.51	13.34	0.00
C R	15.07	68	0.22	1.18	0.25
C A	13.35	17	0.79	4.17	0.00
R A	1.13	4	0.28	1.51	0.21
ERROR	12.82	68	0.19		
TOTAL	69.07	179	0.39		

Table of means

Factor R

DONORS	MEAN
U(H)	0.52
V(C)	0.46
W	0.89
X(A)	0.42
Y	0.66

Appendix 3-10

Table of means (cont.)

Factor A

Arm	Mean
p	0.71
q	0.47

Factor C

Chromosome	Mean
1	0.62
2	0.82
3	0.34
4	0.25
5	0.50
6	0.73
7	0.73
8	0.78
9	0.62
10	1.02
11	0.67
12	1.02
16	0.14
17	0.44
18	1.05
19	0.00
20	0.00
X	0.86

Appendix 3-10

Table of means (cont.)

C A Means

Chromosome	p	q
1	0.67	0.57
2	0.83	0.82
3	0.24	0.43
4	0.25	0.25
5	0.26	0.74
6	0.81	0.64
7	1.34	0.12
8	1.07	0.48
9	0.66	0.58
10	1.47	0.57
11	1.41	0.20
12	1.48	0.57
16	0.00	0.28
17	0.00	0.87
18	1.21	0.89
19	0.00	0.00
20	0.00	0.00
X	1.27	0.44

R A Means

Arms	U(H)	V(C)	W	X(A)	Y
p	0.74	0.53	1.05	0.41	0.81
q	0.30	0.38	0.73	0.43	0.51

Appendix 3-10

Table of means (cont.)

C R Means

Chromosome	U(H)	V(C)	W	X(A)	Y
1	0.47	0.69	0.45	0.57	0.91
2	0.57	0.89	0.98	0.94	0.72
3	0.58	0.14	0.43	0.27	0.29
4	0.00	0.11	0.11	0.52	0.52
5	0.35	0.44	1.03	0.23	0.46
6	0.45	0.59	1.22	0.76	0.62
7	0.52	0.52	1.44	0.66	0.52
8	0.45	0.75	1.51	0.44	0.74
9	0.60	0.44	0.76	0.43	0.86
10	1.06	0.53	1.80	0.00	1.71
11	0.81	0.49	0.96	0.00	1.11
12	1.39	0.89	1.96	0.14	0.73
16	0.00	0.24	0.24	0.24	0.00
17	0.24	0.49	0.49	0.49	0.49
18	1.47	0.85	1.59	0.49	0.85
19	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00
X	0.43	0.16	0.96	1.36	1.37

Appendix 3-11

ANALYSIS OF VARIANCE FOR "FRAGMENT" TYPE ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	29.15	17	1.71	4.21	0.00
R	1.05	4	0.26	0.64	0.63
A	4.01	1	4.01	9.83	0.00
C R	23.37	68	0.34	0.84	0.76
C A	9.94	17	0.58	1.44	0.15
R A	1.83	4	0.46	1.13	0.35
ERROR	27.71	68	0.41		
TOTAL	97.06	179	0.54		

Tables of means

Factor R

DONOR	MEAN
U(H)	1.02
V(C)	1.13
W	0.96
X(A)	0.93
Y	0.93

Appendix 3-11

Table of means (cont.)

Factor A

ARM	MEAN
P	0.84
Q	1.14

Factor C

CHROMOSOME	MEAN
1	1.44
2	1.32
3	0.78
4	1.67
5	0.88
6	1.64
7	1.15
8	0.66
9	1.42
10	1.23
11	0.97
12	0.87
16	0.63
17	0.60
18	0.15
19	0.79
20	0.52
X	1.16

Appendix 3-11

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	1.34	1.53
2	1.34	1.31
3	0.92	0.65
4	1.75	1.58
5	0.78	0.97
6	1.44	1.84
7	1.13	1.16
8	0.48	0.85
9	1.88	0.96
10	0.89	1.58
11	0.38	1.55
12	0.73	1.02
16	0.19	1.06
17	0.22	0.97
18	0.00	0.30
19	0.59	0.99
20	0.38	0.67
X	0.74	1.57

R A MEANS

ARMS	U(H)	V(C)	W	X(A)	Y
P	0.70	1.09	0.89	0.83	0.71
Q	1.33	1.18	1.03	1.03	1.15

Appendix 3-11

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
1	1.61	1.83	1.14	1.36	1.25
2	1.25	1.79	0.89	1.50	1.20
3	1.16	0.89	0.75	0.29	0.84
4	1.80	2.32	1.28	1.78	1.15
5	0.56	1.32	1.23	0.46	0.80
6	2.49	2.04	0.72	1.60	1.34
7	0.70	1.54	0.85	1.57	1.09
8	0.30	1.36	0.60	0.45	0.59
9	1.76	1.43	1.76	1.41	0.75
10	1.01	1.38	1.22	0.79	1.78
11	0.84	1.50	1.17	0.67	0.65
12	0.85	0.28	0.75	1.64	0.84
16	0.72	0.97	0.97	0.24	0.24
17	0.73	0.24	1.05	0.48	0.48
18	0.50	0.25	0.00	0.00	0.00
19	0.83	0.50	1.41	0.41	0.82
20	0.00	0.42	0.42	0.89	0.89
X	1.17	0.32	1.06	1.25	2.00

Appendix 3-12

ANALYSIS OF VARIANCE FOR "EXCHANGE" TYPE ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	22.91	17	1.35	5.87	0.00
R	4.64	4	1.16	5.06	0.00
A	2.12	1	2.12	9.25	0.00
C R	16.18	68	0.24	1.03	0.44
C A	17.05	17	1.00	4.37	0.00
R A	1.90	4	0.48	2.07	0.09
ERROR	15.61	68	0.23		
TOTAL	80.43	179	0.45		

Tables of means

Factor R

DONOR	MEAN
U(H)	0.64
V(C)	0.55
W	0.95
X(A)	0.50
Y	0.78

Appendix 3-12

Table of means (cont.)

Factor A

ARM	MEAN
P	0.79
Q	0.57

Factor C

CHROMOSOME	MEAN
1	0.78
2	1.02
3	0.40
4	0.32
5	0.54
6	0.89
7	0.92
8	0.87
9	0.68
10	1.11
11	0.74
12	1.23
16	0.29
17	0.53
18	1.05
19	0.00
20	0.00
X	0.91

Appendix 3-12

Table of means (cont.)

C A MEANS

CHROMOSOME	P	Q
1	0.86	0.70
2	1.02	1.02
3	0.31	0.49
4	0.25	0.38
5	0.26	0.81
6	1.08	0.70
7	1.56	0.29
8	1.19	0.54
9	0.66	0.70
10	1.47	0.76
11	1.14	0.34
12	1.85	0.62
16	0.00	0.58
17	0.00	1.07
18	1.22	0.89
19	0.00	0.00
20	0.00	0.00
X	1.34	0.44

R A MEANS

ARMS	U(H)	V(C)	W	X(A)	Y
P	0.78	0.72	1.15	0.42	0.88
Q	0.49	0.38	0.73	0.59	0.67

Appendix 3-12

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
1	0.47	0.81	0.57	0.91	1.13
2	0.84	1.16	1.16	1.04	0.92
3	0.72	0.14	0.58	0.27	0.29
4	0.21	0.21	0.11	0.52	0.52
5	0.47	0.55	0.99	0.23	0.46
6	0.68	0.81	1.22	0.76	0.98
7	0.81	0.78	1.71	0.66	0.66
8	0.45	0.75	1.51	0.59	1.04
9	0.60	0.44	0.76	0.59	1.02
10	1.22	0.53	1.80	0.16	1.87
11	0.98	0.49	0.96	0.00	1.29
12	1.38	1.53	2.10	0.28	0.87
16	0.24	0.24	0.24	0.48	0.24
17	0.49	0.49	0.49	0.72	0.49
18	0.47	0.86	1.60	0.49	0.85
19	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00
X	0.43	0.16	1.23	1.37	1.37

Appendix 3-13

ANALYSIS OF VARIANCE FOR "CHROMOSOME FRAGMENTS" ABERRATIONS INDUCED BY AM

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
C	17.60	17	1.04	3.82	0.00
R	0.60	4	0.15	0.56	0.70
A	2.18	4	2.18	8.05	0.01
C R	10.03	68	0.15	0.54	0.99
C A	9.91	17	0.58	2.15	0.02
R A	1.28	4	0.32	1.19	0.32
ERROR	18.44	68	0.27		
TOTAL	60.05	179	0.34		

Tables of means

Factor R

DONORS	MEAN
U(H)	0.64
V(C)	0.72
W	0.58
X(A)	0.56
Y	0.58

Appendix 3-13

Table of means (cont.)

Factor A

Arm	Mean
p	0.50
q	0.72

Factor C

Chromosome	Mean
1	0.82
2	0.87
3	0.30
4	1.16
5	0.58
6	1.16
7	0.75
8	0.36
9	0.94
10	0.80
11	0.64
12	0.52
16	0.34
17	0.19
18	0.00
19	0.71
20	0.33
X	0.58

Appendix 3-13

Table of means (cont.)

C A Means

Chromosome	p	q
1	0.72	0.92
2	0.77	0.98
3	0.43	0.16
4	1.25	1.06
5	0.65	0.51
6	1.08	1.24
7	0.62	0.87
8	0.36	0.36
9	1.44	0.45
10	0.59	1.01
11	0.00	1.28
12	0.37	0.68
16	0.00	0.67
17	0.00	0.39
18	0.00	0.00
19	0.59	0.83
20	0.00	0.67
X	0.21	0.94

R A Means

Arms	U(H)	V(C)	W	X(A)	Y
p	0.46	0.71	0.56	0.43	0.36
q	0.81	0.72	0.60	0.69	0.80

Appendix 3-13

Table of means (cont.)

C R Means

Chromosome	U(H)	V(C)	W	X(A)	Y
1	0.79	0.90	0.69	0.80	0.91
2	0.94	1.26	0.37	0.92	0.88
3	0.44	0.31	0.46	0.00	0.26
4	1.59	1.69	0.64	0.94	0.94
5	0.44	0.88	0.88	0.23	0.46
6	1.49	1.40	0.59	1.34	0.98
7	0.41	1.11	0.58	0.80	0.83
8	0.15	0.60	0.45	0.15	0.45
9	0.99	0.83	1.03	1.25	0.59
10	0.64	1.22	0.53	0.31	1.31
11	0.85	1.01	0.51	0.50	0.33
12	0.57	0.14	0.75	0.59	0.56
16	0.48	0.24	0.72	0.23	0.00
17	0.24	0.24	0.24	0.24	0.00
18	0.00	0.00	0.00	0.00	0.00
19	0.83	0.50	0.99	0.41	0.82
20	0.00	0.42	0.42	0.41	0.41
X	0.58	0.15	0.58	0.94	0.63

Appendix 3-14

ANALYSIS OF VARIANCE FOR TOTAL No. OF BREAKS INDUCED BY ARA C

Chromosomes 13,14,15,21,22

SOURCE OF VARIATION	SS	DF	MS	F	P
C	4.59	4	1.15	1.35	0.29
R	3.61	4	0.90	1.06	0.41
ERROR	13.56	16	0.85		
TOTAL	21.76	24	0.91		

Tables of means

Factor C

CHROMOSOME	MEAN
13	1.88
14	2.03
15	0.86
21	1.92
22	1.50

Factor R

DONOR	MEAN
H(U)	1.35
C(V)	1.69
E	1.08
A(X)	2.11
B	1.95

Appendix 3-14

Table of means (cont.)

C R MEANS

CHROMOSOME	H(U)	C(V)	E	A(X)	B
13	0.92	2.45	1.53	2.40	2.10
14	3.30	1.32	0.99	2.60	1.95
15	0.00	1.08	1.08	1.06	1.06
21	0.00	1.94	0.97	2.86	3.81
22	2.52	1.68	0.84	1.65	0.83

Appendix 3-15

ANALYSIS OF VARIANCE FOR TOTAL No. OF BREAKS INDUCED BY AM

Chromosomes 13,14,15,21,22

SOURCE OF VARIATION	SS	DF	MS	F	P
C	2.08	4	0.52	0.70	0.60
R	3.24	4	0.81	1.09	0.40
ERROR	11.92	16	0.74		
TOTAL	17.24	24	0.72		

Table of means

Factor C

CHROMOSOME	MEAN
13	3.04
14	2.42
15	2.57
21	2.70
22	2.17

Factor R

DONOR	MEAN
U(H)	2.94
V(C)	3.10
W	2.33
X(A)	2.32
Y	2.22

Appendix 3-15

Table of means (cont.)

C R MEANS

CHROMOSOME	U(H)	V(C)	W	X(A)	Y
13	3.67	3.06	3.98	2.10	2.40
14	1.98	2.31	2.64	2.27	2.92
15	3.60	2.88	1.44	1.77	3.19
21	2.91	3.88	1.94	3.81	0.95
22	2.52	3.36	1.68	1.85	1.65

Appendix 3-17

ANALYSIS OF VARIANCE COMPARING THE EFFECTS OF ARA C AND AM ON INDIVIDUALS U(H),V(C), AND X(A)

Chromosomes 1-12,16-20,X

SOURCE OF VARIATION	SS	DF	MS	F	P
D	45.27	1	45.28	30.51	0.00
C	40.84	17	2.40	1.62	0.11
R	5.27	2	2.64	1.78	0.18
A	0.35	1	0.35	0.23	0.63
D C	38.49	17	2.26	1.53	0.14
D R	11.10	2	5.55	3.74	0.03
D A	8.02	1	8.02	5.40	0.03
C R	52.04	34	1.53	1.03	0.46
C A	36.26	17	2.13	1.43	0.18
R A	0.08	2	0.04	0.03	0.97
D C R	57.57	34	1.69	1.14	0.35
D C A	29.96	17	1.76	1.19	0.32
D R A	2.68	2	1.34	0.90	0.41
C R A	49.96	34	1.44	0.97	0.53
ERROR	50.46	34	1.48		
TOTAL	427.36	215	1.99		

Tables of means

Factor D

DRUG	MEAN
AM	1.03
ARA C	1.94

Appendix 3-17

Table of means (cont.)

Factor C	MEAN
1	1.90
2	1.92
3	2.08
4	1.82
5	1.16
6	1.51
7	1.89
8	1.18
9	1.60
10	1.43
11	1.34
12	1.84
16	1.49
17	1.05
18	0.33
19	1.41
20	0.89
X	1.18

Factor R	
DONOR	MEAN
U(H)	1.64
V(C)	1.27
X(A)	1.55

Appendix 3-17

Table of means (cont.)

Factor A

ARM	MEAN
P	1.52
Q	1.44

D C MEANS

CHROMOSOME	AM	ARA C
1	1.60	2.21
2	1.51	2.32
3	0.78	3.34
4	1.97	1.68
5	0.78	1.56
6	2.04	0.97
7	1.27	2.51
8	0.70	1.65
9	1.53	1.66
10	1.06	1.81
11	1.01	1.67
12	0.93	2.76
16	0.64	2.33
17	0.49	1.61
18	0.25	0.41
19	0.58	2.25
20	0.44	1.34
X	0.91	2.84

Appendix 3-17

Table of means (cont.)

D R MEANS

DRUG	U(H)	V(C)	X(A)
AM	1.02	1.13	0.93
ARA C	2.26	1.41	2.16

C R MEANS

CHROMOSOME	U(H)	V(C)	X(A)
1	2.06	1.66	1.99
2	2.40	1.29	2.07
3	4.22	1.08	0.94
4	1.74	1.69	2.04
5	1.07	1.16	1.27
6	1.72	1.27	1.53
7	2.81	1.25	1.60
8	1.43	1.06	1.04
9	1.38	1.40	2.02
10	1.43	1.38	1.49
11	1.08	1.40	1.54
12	1.89	1.35	2.29
16	2.30	1.33	0.83
17	0.73	1.17	1.24
18	0.25	0.37	0.37
19	0.63	2.07	1.55
20	0.42	0.91	1.33
X	1.89	1.01	2.72

Appendix 3-17

Table of means (cont.)

D A MEANS

DRUG	P	Q
AM	0.87	1.18
ARA C	2.18	1.71

C A MEANS

CHROMOSOME	P	Q
1	1.76	2.05
2	2.13	1.70
3	3.21	0.95
4	2.09	1.56
5	1.29	1.04
6	1.35	1.67
7	1.64	2.14
8	1.19	1.16
9	2.02	1.17
10	1.23	1.64
11	1.16	1.52
12	2.60	1.08
16	0.96	2.01
17	0.56	1.54
18	0.00	0.66
19	1.31	1.52
20	0.80	0.97
X	2.12	1.62

Appendix 3-17

Table of means (cont.)

R A MEANS

DONOR	P	Q
U(H)	1.67	1.60
V(C)	1.33	1.21
X(A)	1.57	1.53

Appendix 3-18

ANALYSIS OF VARIANCE ON THE SQUARE ROOT OF COUNTS FOR SCE's INDUCED BY ARA C

SOURCE OF VARIATION	SS	DF	MS	F	P
L	0.26	2	0.13	0.41	0.66
R	1.41	3	0.47	1.49	0.22
L R	1.64	6	0.27	0.87	0.52
ERROR	96.97	306	0.32		
TOTAL	100.48	317	0.32		

Tables of means

Factor L

CONCENTRATION	MEAN
CONTROL	2.61
2.5µg/ml	2.55
10.0µg/ml	2.55

Factor R

DONOR	MEAN
C(V)	2.63
E	2.52
A(X)	2.49
B	2.65

Appendix 3-18

Table of means (cont.)

L R MEANS

CONCENTRATION	C(V)	E	A(X)	B
CONTROL	2.83	2.54	2.49	2.60
2.5µg/ml	2.55	2.55	2.43	2.55
10.0µg/ml	2.51	2.48	2.55	2.67

Appendix 3-19

ANALYSIS OF VARIANCE ON THE SQUARE ROOT OF COUNTS FOR SCE's INDUCED BY AM

SOURCE OF VARIATION	SS	DF	MS	F	P
L	166.98	2	83.49	177.60	0.00
R	1.22	4	0.30	0.65	0.63
L R	2.07	8	0.26	0.55	0.82
ERROR	169.23	360	0.47		
TOTAL	339.50	374	0.91		

Tables of means

Factor L

CONCENTRATION	MEAN
CONTROL	2.70
0.01µg/ml	4.00
0.05µg/ml	4.20

Factor R

DONOR	
U(H)	3.56
V(C)	3.63
W	3.69
X(A)	3.57
Y	3.69

Appendix 3-19

Table of means (cont.)

L R MEANS

CONCENTRATION	U(H)	V(C)	W	X(A)	Y
CONTROL	2.67	2.73	2.78	2.68	2.61
0.01µg/ml	3.98	3.97	3.95	3.89	4.20
0.05µg/ml	4.04	4.22	4.36	4.14	4.27

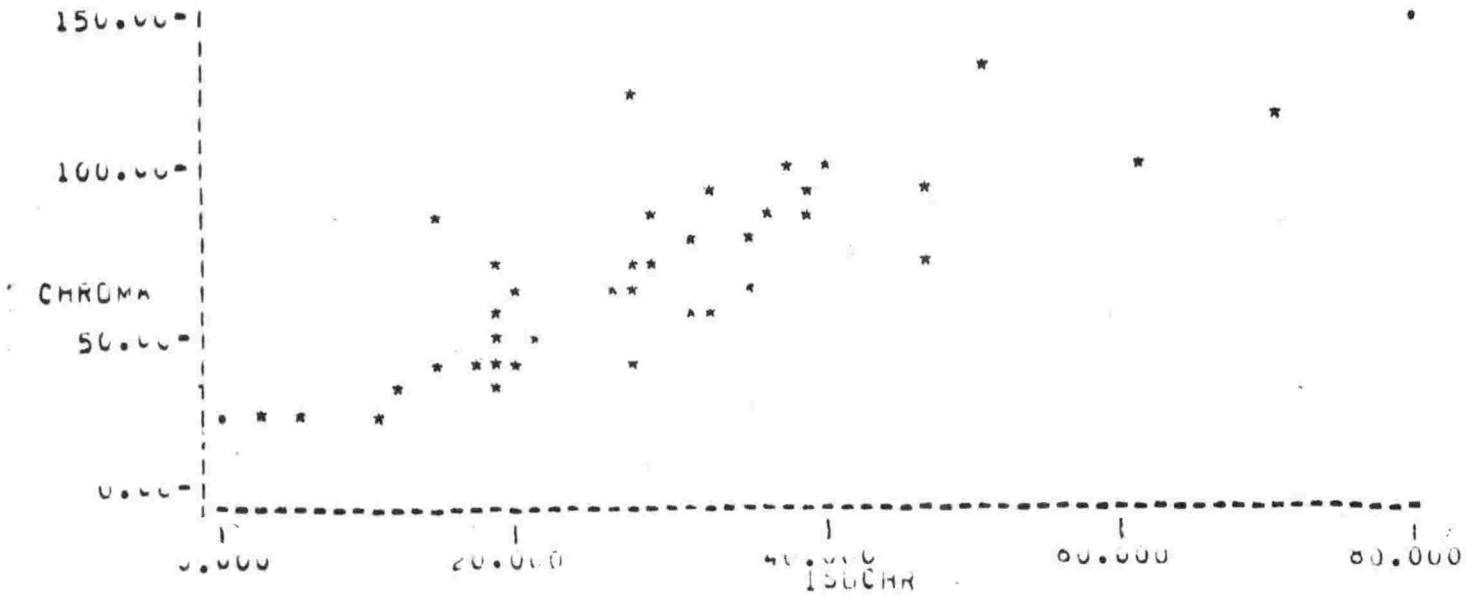
Appendix 4

The Number of Chromatid and Isochromatid Aberrations at "Hotspots" Induced by Ara C.

Band No.	Chromatid	Isochromatid	Total
1q21	8	2	10
1q32	7	4	11
3p21	16	7	23
3p14	10	4	14
3p13	7	4	11
4q31	14	1	15
7q11	7	3	10
7q32	15	5	20
9q12	9	8	17
12p12	10	5	15
12q13	7	3	10
16q24	7	3	10
17q21	8	3	11
Xp22	9	3	12
Totals	134	55	189

Appendix 5

Regression of Ara C induced isochromatid breaks against chromatid breaks



Regression Equation :

CHROMATID = 22(5) + 1.6(0.2)ISOCHROMATID

(Standard errors are in brackets)

THE NUMBER OF ABERRATIONS /200 CELLS INDUCED BY ARA C AT THREE CONCENTRATIONS AND THREE TREATMENT TIMES

DONORS	H(U)			C(V)			E			A(X)			B		
TREATMENT TIMES(hrs.)	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
2.5µg/ml															
isochromatid	17	15	31	12	18	33	27	5	20	12	14	19	3	11	19
chromatid	37	39	79	29	31	55	43	22	61	31	43	44	25	27	69
TOTAL	54	54	110	41	49	88	70	27	57	43	57	63	28	38	88
5.0µg/ml															
isochromatid	31	47	51	27	29	38	18	15	28	12	26	35	21	31	39
chromatid	58	74	135	43	74	99	53	88	123	34	66	62	50	79	98
TOTAL	89	121	186	70	103	137	71	103	151	46	92	87	71	110	137
10.0µg/ml															
isochromatid	20	37	71	18	33	29	27	35	62	19	39	41	18	27	47
chromatid	41	90	122	73	98	89	69	80	101	42	88	105	59	62	96
TOTAL	61	127	193	91	131	118	96	115	163	61	127	146	77	89	143

THE NUMBER OF ABERRATIONS /200 CELLS INDUCED BY ARA C AT THREE CONCENTRATIONS AND THREE TREATMENT TIMES

TREATMENT TIME (hrs.)	TOTALS			
	2	3	4	
2.5µg/ml				TOTAL
isochromatid	71	63	122	256
chromatid	165	162	308	635
TOTAL	236	225	430	891
5.0µg/ml				
isochromatid	109	148	191	448
chromatid	238	381	517	1136
TOTAL	347	529	708	1584
10.0µg/ml				
isochromatid	102	171	250	523
chromatid	284	418	513	1215
TOTAL	386	589	763	1738

Total no. of isochromatid breaks = 1227

Total no. of chromatid breaks = 2986

% of isochromatid breaks = 29

FREQUENCIES OF ABERRATIONS INDUCED BY FOUR CONCENTRATIONS OF AM

DONOR U(H)

concentration µg/ml	aberrations /200 cells	CHROMATID			CHROMOSOME			TOTAL
		fragments	exchanges	rings	fragments	exchanges	rings	
control	3	2	0	0	0	1	0	3
	2	1	0	0	0	1	0	2
0.01	13	11	21	0	20	0	0	52
	19	11	17	0	16	1	0	45
0.05	27	23	31	1	38	0	1	94
	22	18	37	0	21	1	0	77
0.10	29	29	53	0	41	1	1	125
	31	21	40	1	51	3	0	116
0.15	15	18	21	0	25	0	0	64
	51	43	61	1	51	1	1	158

FREQUENCIES OF ABERRATIONS INDUCED BY FOUR CONCENTRATIONS OF AM

DONOR V(C)

concentration µg/ml	aberrations /200 cells	CHROMATID			CHROMOSOME			TOTAL
		fragments	exchanges	rings	fragments	exchanges	rings	
control	2	2	0	0	0	0	0	2
	2	2	0	0	0	0	0	2
0.01	17	11	10	0	8	1	0	30
	12	7	8	0	3	0	0	18
0.05	18	12	21	0	19	1	0	53
	23	19	27	1	22	0	0	69
0.10	44	21	38	0	31	1	1	92
	29	11	19	1	23	1	0	55
0.15	39	23	40	1	27	3	2	96
	57	29	37	3	35	5	2	111

FREQUENCIES OF ABERRATIONS INDUCED BY FOUR CONCENTRATIONS OF AM

DONOR W

concentration µg/ml	aberrations /200 cells	CHROMATID			CHROMOSOME			TOTAL
		fragments	exchanges	rings	fragments	exchanges	rings	
control	1	1	0	0	0	0	0	1
	1	1	0	0	0	0	0	1
0.01	19	5	8	0	9	0	0	22
	15	9	11	0	18	0	0	38
0.05	46	23	47	1	46	0	1	118
	41	19	37	0	40	1	0	97
0.10	47	17	38	0	61	0	1	117
	48	20	37	0	56	1	0	114
0.15	56	28	57	0	39	1	0	125
	54	29	53	0	50	0	0	132

FREQUENCIES OF ABERRATIONS INDUCED BY FOUR CONCENTRATIONS OF AM

DONOR X(A)

concentration µg/ml	aberrations /200 cells	CHROMATID			CHROMOSOME			TOTAL
		fragments	exchanges	rings	fragments	exchanges	rings	
control	2	2	0	0	0	0	0	2
	3	3	0	0	0	0	0	3
0.01	18	10	19	0	16	0	0	45
	16	9	13	0	13	0	0	35
0.05	29	16	31	1	30	1	0	79
	27	15	27	0	33	0	0	75
0.10	34	13	32	0	46	0	0	91
	39	17	26	0	42	2	0	87
0.15	45	28	43	0	58	2	0	131
	40	24	39	1	41	1	0	106

FREQUENCIES OF ABERRATIONS INDUCED BY FOUR CONCENTRATIONS OF AM

DONOR Y

concentration µg/ml	aberrations /200 cells	CHROMATID			CHROMOSOME			TOTAL
		fragments	exchanges	rings	fragments	exchanges	rings	
control	2	2	0	0	0	0	0	2
	1	1	0	0	0	0	0	1
0.01	18	9	27	0	12	0	0	48
	21	16	13	0	20	1	0	50
0.05	29	21	33	1	19	1	0	75
	33	14	41	0	29	2	0	86
0.10	47	26	47	0	49	1	0	123
	43	23	57	0	41	3	0	124
0.15	51	31	21	0	47	2	0	101
	53	22	59	0	52	1	0	134

Appendix 7

Summary of the distribution over the chromosomes of the different types of aberrations induced by AM

Legend

p	=	p arm of the chromosome
q	=	q arm of the chromosome
T/F	=	Chromatid fragment
T/E	=	Chromatid exchange
S/F	=	Chromosome fragment
S/E	=	Chromosome exchange

SUMMARY OF ABERRATIONS INDUCED BY AM

chromosome	U(H) p T/F	U(H) p T/E	U(H) p S/F	U(H) p S/E	U(H) q T/F	U(H) q T/E	U(H) q S/F	U(H) q S/E	V(C) p T/F	V(C) p T/E	V(C) p S/F	V(C) p S/E	V(C) q T/F	V(C) q T/E	V(C) q S/F	V(C) q S/E
1	4	3	2	0	3	1	5	0	4	3	2	1	4	3	6	0
2	0	1	2	1	3	4	6	1	2	3	4	1	2	4	6	1
3	2	2	2	0	3	2	1	1	2	0	2	0	2	1	0	0
4	0	0	3	0	2	0	6	2	1	0	4	0	3	1	4	1
5	0	0	1	0	1	3	1	1	1	1	2	0	1	1	2	1
6	2	2	3	1	4	0	6	0	1	2	2	1	3	1	7	0
7	0	2	1	0	2	0	1	2	0	2	2	1	3	0	4	0
8	0	1	0	0	1	1	1	0	1	2	1	0	3	1	2	0
9	1	1	3	0	3	2	1	0	1	1	3	0	2	1	0	0
10	1	2	0	0	0	2	4	1	0	1	2	0	1	1	3	0
11	0	2	0	0	0	1	5	1	1	1	0	0	1	1	6	0
12	0	3	0	0	2	0	4	0	0	1	0	0	1	3	1	0
13	-	-	-	-	1	9	2	0	-	-	-	-	3	5	2	0
14	-	-	-	-	0	4	1	1	-	-	-	-	1	3	2	1
15	-	-	-	-	3	4	3	0	-	-	-	-	3	3	2	0
16	0	0	0	0	1	0	2	1	1	0	0	0	1	1	1	0
17	0	0	0	0	2	1	1	1	0	0	0	0	0	2	1	0
18	0	2	0	0	2	1	0	0	0	1	0	0	1	1	0	0
19	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
21	-	-	-	-	1	2	0	0	-	-	-	-	0	3	1	0
22	-	-	-	-	1	2	0	0	-	-	-	-	1	1	2	0
X	1	1	1	0	2	1	2	0	0	0	0	0	1	1	1	0

SUMMARY OF ABERRATIONS INDUCED BY AM (cont.)

chromosome	W p T/F	W p T/E	W p S/F	W p S/E	W q T/F	W q T/E	W q S/F	W q S/E	X(A) p T/F	X(A) p T/E	X(A) p S/F	X(A) p S/E	X(A) q T/F	X(A) q T/E	X(A) q S/F	X(A) q S/E
1	1	1	3	1	3	3	3	0	2	3	4	1	3	2	3	2
2	2	3	1	1	2	5	2	0	3	4	2	0	1	3	6	1
3	1	1	3	1	1	2	0	0	1	0	0	0	1	2	0	0
4	1	0	0	0	3	1	6	0	2	1	0	0	2	2	6	0
5	0	1	2	0	3	6	2	0	0	0	0	0	2	2	2	0
6	0	3	2	0	1	4	1	0	0	1	3	0	2	4	5	0
7	1	5	0	1	0	1	4	0	3	2	2	0	0	1	2	0
8	0	3	1	0	1	4	1	0	0	1	0	0	2	1	1	1
9	2	1	2	0	1	3	3	0	0	1	4	0	1	1	1	1
10	1	4	1	0	2	2	1	0	0	0	0	0	3	0	2	1
11	1	3	0	0	2	0	3	0	0	0	0	0	1	0	3	0
12	0	3	1	0	0	4	2	1	2	0	1	0	1	1	1	1
13	-	-	-	-	5	6	1	1	-	-	-	-	2	4	1	1
14	-	-	-	-	1	6	1	0	-	-	-	-	2	4	1	0
15	-	-	-	-	1	0	2	1	-	-	-	-	0	4	1	0
16	0	0	0	0	1	1	3	0	0	0	0	0	0	1	1	1
17	1	0	0	0	1	2	1	0	0	0	0	0	1	2	1	1
18	0	1	0	0	0	4	0	0	0	0	0	0	0	2	0	0
19	0	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0
20	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0
21	-	-	-	-	1	1	0	0	-	-	-	-	0	3	1	0
22	-	-	-	-	0	1	1	0	-	-	-	-	1	0	1	0
X	0	3	1	1	3	1	2	0	0	2	0	0	1	1	3	0

SUMMARY OF ABERRATIONS INDUCED BY AM (cont.)

Y

chromosome	p				q			
	T/F	T/E	S/F	S/E	T/F	T/E	S/F	S/E
1	2	4	4	1	1	4	4	1
2	2	2	3	0	0	4	4	2
3	2	1	0	0	2	1	2	0
4	0	1	2	0	2	2	3	0
5	0	0	0	0	3	4	4	0
6	1	1	2	1	1	3	4	1
7	1	2	1	0	0	0	4	1
8	0	2	1	1	1	1	1	0
9	0	2	1	0	1	2	2	1
10	0	3	1	0	3	4	6	1
11	1	3	0	0	0	1	2	1
12	0	1	0	0	2	2	4	1
13	-	-	-	-	2	6	0	0
14	-	-	-	-	1	6	2	0
15	-	-	-	-	2	3	4	0
16	0	0	0	0	1	0	0	1
17	0	0	0	0	2	2	0	0
18	0	1	0	0	0	1	0	0
19	0	0	0	0	0	0	2	0
20	1	0	0	0	0	0	1	0
21	-	-	-	-	0	1	0	0
22	-	-	-	-	0	1	1	0
X	2	2	0	0	1	1	2	0

TOTALS

chromosome	p				q			
	T/F	T/E	S/F	S/E	T/F	T/E	S/F	S/E
1	13	14	15	4	14	13	21	3
2	9	13	12	3	8	20	24	5
3	8	4	7	1	9	8	3	1
4	4	2	10	0	12	6	25	3
5	1	2	5	0	10	16	11	2
6	4	9	12	3	11	12	23	1
7	5	13	6	2	5	2	15	3
8	1	9	3	1	8	8	6	1
9	4	6	13	0	8	9	7	2
10	2	10	4	0	9	9	16	3
11	3	9	9	0	4	3	19	2
12	2	8	2	0	6	10	12	3
13	-	-	-	-	13	30	6	1
14	-	-	-	-	5	23	7	2
15	-	-	-	-	9	14	12	1
16	1	0	0	0	4	3	7	3
17	1	0	0	0	6	9	4	2
18	0	5	0	0	3	9	0	0
19	0	0	3	0	1	0	5	0
20	2	0	0	0	0	0	4	0
21	-	-	-	-	2	10	2	0
22	-	-	-	-	3	5	5	0
X	3	8	2	1	8	5	10	0