Canad.J.Genet.Cytol. 11, 1-24 (1969). LSD 1877

Canadian Journal of Genetics and Cytology

Volume XI March 1969 Number 1

THE INTERACTION OF VARIOUS DRUGS WITH HUMAN CHROMOSOMES[1]

MAIMON M. COHEN, PH.D.

Division of Human Genetics, Department of Pediatrics, School of Medicine, State University of New York at Buffalo, and Buffalo Children's Hospital, Buffalo, New York

The technical advances of the last decade, particularly in the fields of tissue culture and cytogenetics, have extended the study of induced mutagenesis to the chromosomal level. Four general classes of exogenous agents exist which are capable of inducing chromosomal damage, and thereby, also possibly gene mutations; these include radiation, some viruses, physical stimuli, and a variety of chemicals. This last group of compounds can be subdivided into those which affect DNA, RNA and protein synthesis, the DNA precursors, antibiotics, alkylating agents, nitroso-compounds and the heterogenous miscellaneous group (1 to 6). Additionally, recent investigations have focused on several types of common environmental agents including tranquilizers (7, 8) oral contraceptives (9), fungicides (10), pesticides (11) and air pollutants (12, 13). To categorize all the chemical agents which have been shown to cause chromosomal damage is beyond the scope of this presentation, but the feasibility and flexibility of the use of cytogenetic investigation as an assay for screening exogenous agents will be demonstrated in this paper. To this end, experiments with three drugs will be presented: streptonigrin, a streptomyces derived antibiotic; mitomycin C, also a streptomyces derived antibiotic and, lysergic acid diethylamide (LSD-25), a powerful hallucinogen.

The most frequently used human cytogenetic test system for the investigation of exogenous agents is the peripheral lymphocyte. The obvious advantages of this cell type derive from its ease of access and relatively simple techniques of culture yielding large numbers of dividing cells suitable for study. The usual method of cell harvest and slide preparation is that of Moorehead *et al.* (14) with slight modifications. Most modifications have resulted in the use of micromethods, necessitating only a few drops of peripheral blood. In addition to peripheral lymphocytes, fibroblasts derived from primary cultures of skin and other organs, as well as bone marrow cells, have also been used.

Two basic approaches to the screening of any agent can be utilized in such systems, i.e., in vitro and in vivo experimentation. In vitro studies necessitate the use of peripheral lymphocytes from normal individuals who have not been exposed to the agent in question (nor any other known chromosome breaking agent), radiation or recent viral infections. Such 'normal' cells are are cultured and the test agent is added to the culture medium at various times and concentrations during the normal 72-hour culture period. In this way, a dose-response relationship can be established between chromosomal damage and both concentration of the drug and length of exposure time. Additionally, drug effects on the rate of mitosis are readily apparent. Several slides from each culture are prepared and coded. Usually 25 metaphases per slide are obtained

[1]Substance of the Annual Invitation Lecture given to the Genetics Society of Canada, at the University of Manitoba, Winnipeg, Manitoba, May 16, 1968.

Manuscript received Dec. 16, 1968.

by individuals who do not participate in the actual microscopic scoring of the cells, and a minimum of 100 cells per treatment is studied. Well-spread mitoses are selected under low magnification (250 ×), and chromosomes are scored for abnormalities under oil immersion phase contrast microscopy (approximately 1560 ×). Once a cell is selected under low power, it is included in the study.

Abnormalities of the chromosomes (Fig. 1) are scored as breaks only if a clear discontinuity and chromatid nonalignment are visible. Breaks are classified as 'chromatid' if only one chromatid is affected, and 'isochromatid' if both sister chromatids are broken at the same location. Both these abnormalities are scored as single breaks. Single fragments are included with chromatid breaks while 'double fragments' are scored as isochromatid breaks. Dicentric chromosomes and 'quadriradial' or exchange configurations are considered as containing two breaks. Attenuated, pale-staining chromosomal regions, other than normal secondary constrictions without distal nonalignment, are scored separately as 'gaps' but are not included in the calculation of breakage rates. Whenever possible, each break is assigned to a given identifiable chromosome or chromosome group according to the Denver classification (15). However, in some cases, unidentified fragments are observed which are not classifiable.

Streptonigrin (SN)

SN, a derivative of *Streptomyces flocculus*, exhibits both antitumor and antibiotic activities but has rarely been used clinically due to its extreme cytotoxicity. Previous investigations indicate that SN has profound effects on macromolecular synthesis in microorganisms. The drug induces bacteriophage production in lysogenic bacteria while inhibiting the net synthesis of bacterial DNA (16). In the presence of SN, a marked increase in genetic recombination is observed during mixed bacteriophage infections (17). Furthermore, SN initiates rapid in vivo degradation of *E. coli* DNA (18). Because of these striking effects on macromolecular synthesis, tests of the activity of SN on human chromosomes seemed warranted.

Figure 2 illustrates the effect of SN on mitosis of peripheral lymphocytes derived from normal individuals. The cells were exposed to three concentrations of the drug (0.10, 0.01 and 0.001 μg/ml for 6, 12, 18, 24, 30 and 36 hours. The lowest concentration of SN showed no statistically significant deviation from the mitotic rate in the control cultures. However, at both the 0.01 and the 0.10 μg/ml level, a statistically significant suppression of mitosis occurred. This depression was linearally correlated with the length of exposure time.

A total of 395 SN induced chromosome breaks and rearrangements of various types were observed in 701 treated cells (0.56 breaks/cell) as opposed to four chromatid breaks in 150 control cells (0.03 breaks/cell). This latter figure compares favorably with the range of control breakage rates of 0.0-6.0% encountered in most cytogenetic laboratories. Both the mean number of breaks per cell and the percentage of cells with breaks increased with concentration. The mean number of breaks per cell was 0.28, 0.70 and 1.00 for concentrations of 0.001, 0.01 and 0.1 μg/ml of SN respectively, whereas 19.7, 35.1 and 42.2% of the cells exhibited chromosomal breaks at these same concentrations. An increased number of abnormalities was also observed upon lengthened exposure time (19).

The assignment of SN induced chromosomal aberrations among the seven groups of chromosomes according to the Denver system of nomenclature (15)

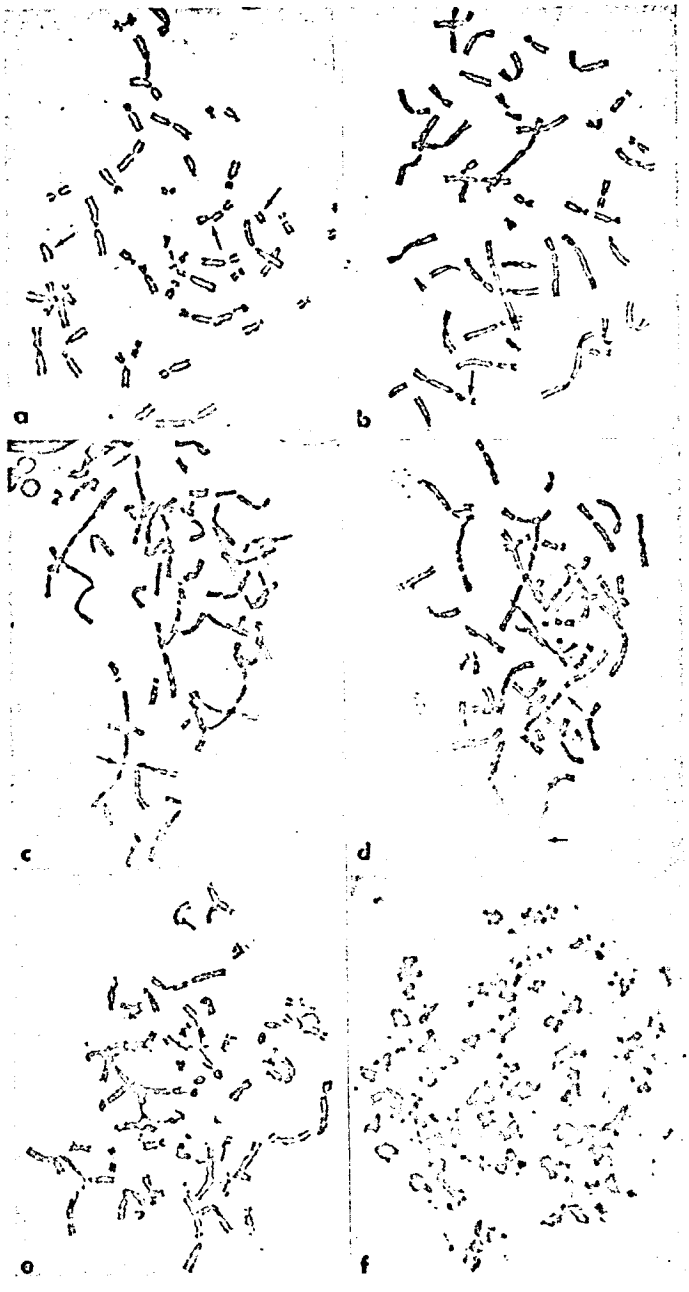


Fig. 1. Metaphase cells illustrating types of chromosomal damage due to SN. Cells a through e were treated with 0.01 μg/ml of SN for 24 hr, while cell f was treated with 0.1 μg/ml of SN for 6 hr. Arrows indicate type of chromosome damage: (a) chromatid break and acentric fragments; (b) isochromatid break; (c) 'translocation' cross, 'end-to-end' fusion, and chromatin strand; (d) centromere attenuation and uncoiled chromosome segment; (e) and (f) fragmentation and degeneration of chromosomes.

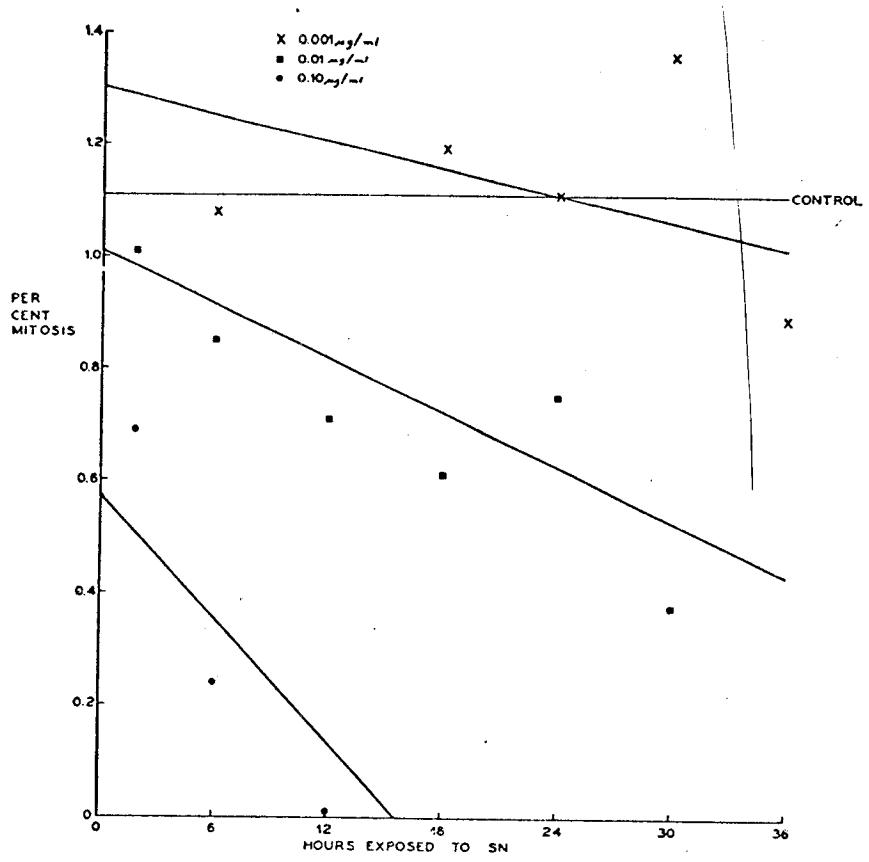


Fig. 2. The effect on mitotic rate with three concentrations of SN for varying lengths of exposure. The curves drawn are least square lines calculated by regression analysis; each point indicates the average mitotic rate for approximately 6,000 cells.

demonstrated an apparent nonrandom distribution according to length. If the drug were acting randomly per unit of chromatin then the distribution of breaks should be according to the size of the chromosomes. The analysis in Table I indicates that the chromosomal groups were not affected randomly. This observation was extended to the examination of intrachromosomal localization of abnormalities (20). Since it is known that ionizing radiation causes chromosomal breakage in a random fashion, a comparison between the effects of SN and X-rays was undertaken. In this study, chromosome selection was limited to the members of Group 1-3 in order to insure positive identification of individual chromosomes. An arbitrary total of 500 breaks among these three chromosomes constituted the sample size. The cells treated with X-rays were exposed to 50R or 75R delivered at a rate of 200R/minute calibrated as dose in air, while the SN produced abnormalities were derived from cells as treated above.

When a chromosome of Group 1-3 was found with a break, the following measurements were made using a calibrated ocular micrometer: (1) total length; (2) length of each arm; (3) distance from the centromere to the break.

TABLE I
'Goodness of Fit' test for random distribution of chromosome breaks induced by streptonigrin, based on unit chromosome lengths given in the Denver report

Chromosome group	1–3	4–5	6–12, X	13–15	16–18	19–20	21–22, Y	Total
Observed	111	59	152	29	30	2	5	388
Expected	96.68	47.63	140.54	39.46	33.69	17.79	16.21	388.00
χ^2	3.62	2.71	0.93	2.77	0.40	14.01	7.75	32.19

$P < 0.005$; df = 6.

The location of each break was then expressed as that percentage of the broken chromosome arm from the centromere to the break, for example,

$$\frac{\text{Distance from the centromere to break}}{\text{Length of the entire chromosome arm}} \times 100.$$

All measurements were made using the centromere as the origin and progressing along the chromosome arm in both directions. This percentage index eliminates the normal variation in chromosome size from cell to cell and allows the data to be pooled.

Upon collection of the samples, the breaks were assigned to 1 of 11 chromosomal segments, as shown in Fig. 3. The pericentromeric region (segment 0) included the centromere and 10% of the length of each chromosome arm. The remainder of the arm (90%) was divided into five equal segments. Chromosome breaks were then allocated to that segment of the arm encompassing the breakage point. Distribution of the breaks according to chromosome segments was tested for randomness by chi-square. The use of arm-ratio measurement allows the calculation of the total number of expected breaks per chromosome arm. After assigning 10% of the total number of observed breaks of each chromosome as the number expected in the pericentric region (segment 0), the expected number of breaks for each of the five segments of each arm was calculated from the remaining 90% of the observed breaks on the basis of arm ratios.

The array of breaks among chromosomes 1, 2 and 3 indicates a localized distribution after SN treatment ($P<0.001$). There was an excess of breaks in chromosome 1 with a corresponding deficiency in chromosome 3. X-ray induced breaks were distributed randomly among these chromosomes ($0.90 > P > 0.80$). A random distribution of breaks was also observed within each chromosome treated with X-ray. In the SN treated cultures, however, both chromosomes 1 and 2 showed significant deviations from the random distribution with an evident concentration of breaks in the centromeric regions (Fig. 4). In chromosome 1 the number of breaks caused by SN increases at both telomeric regions. The greatest concentration of breaks appears at the centromere and the secondary constriction which lies in segment 1 of the long arm. Chromosome 2 shows a different distribution pattern of breaks when SN is used. Again, the greatest number of breaks occurs in the centromeric region. However, there is a striking paucity of breaks in the short arm. There is also a decrease in the number of SN induced breaks in the telomeric region of the long arm of No. 2. In chromosome 3, although the distribution of breaks caused by SN

does not deviate significantly from randomness, there is a tendency for the telomeres to appear relatively resistant. These data suggest a marked difference in the response of the centromere regions of the three chromosomes to treatment with SN. The centromeric segments of chromosomes 1 and 2 seem highly susceptible to breakage in contrast to the centromere of No. 3. This study indicates that SN induces chromosome breaks in No. 1 more frequently than expected; the distribution of breaks within the chromosome is localized. Chromosome 2 breaks in proportion to its length; however, there is a non-random distribution to breaks within the chromosome. The short arm of No. 2 seems resistant to SN breakage. Although there is significant deficiency in the number of breaks in chromosome 3, these breaks are randomly distributed over the length of the chromosome.

Mitomycin C (MC)

MC is a chemically reactive antibiotic derived from *Streptomyces caepitosus*. This drug selectively inhibits DNA synthesis (21-29) and degrades cellular DNA, but does not affect the synthesis of RNA nor protein (30-32). MC induces bacteriophage production in lysogenic bacteria (33, 34), increases the rate of genetic recombination among mutant forms of *E. coli* (22, 29, 35), and possesses antitumor activity (22,35). In various other types of cell culture systems, MC inhibits mitosis, reduces cell viability and produces nuclear disorganization and giant cells (37). Several models for the mechanism of MC activity have been suggested. Reich *et al.* proposed that MC acts through scission of the DNA strands, thereby preventing replication (31). This implies that the template competence of the DNA is destroyed (30). The depolymerization of DNA and the accumulation of acid soluble fragments suggests that the DNA polymerase system is the possible target of MC activity (24, 31). Recently, it has been proposed that the primary action of MC is the 'cross-linking' of the complimentary strands of the DNA molecule, and that degradation of DNA may be of a secondary nature (38).

The effects of MC were tested on human leukocyte chromosomes similarly to that outlined for streptonigrin (39). Treatment with 5 μg/ml of MC resulted in total cell destruction. At 1 μg/ml, the mitotic rate was significantly lower than normal, and numerous chromosomal breaks and exchange figures were observed, while at the concentration of 0.1 μg/ml chromosome damage was less marked. At comparable molar concentrations, MC is less potent than streptonigrin: 2.0×10^{-7}M SN for 24 hours resulted in chromosome fragmentation and cell disintegration (19), whereas cultures treated with 1.8×10^{-6}M MC (1 μg/ml) contained some normal appearing chromosomes. MC did not break the chromosomes randomly in proportion to the relative length but, as with streptonigrin, showed a localized distribution of breakage. The largest deviation from randomness was found among chromosomes 1, 9, and 16. Figure 5 shows the distribution of MC induced chromosome breaks within these three chromosomes. As noted by many authors (40-43), the human chromosomes 1, 9, and 16 possess prominent secondary constrictions. These areas appear to be focal points of MC activity since high percentages of chromosome breaks occurred in these 'target areas.' Upon removal of the breaks found in these secondary constriction regions and subsequent retesting of the chromatin complement, the distribution of breaks was random ($0.50 > P > 0.25$).

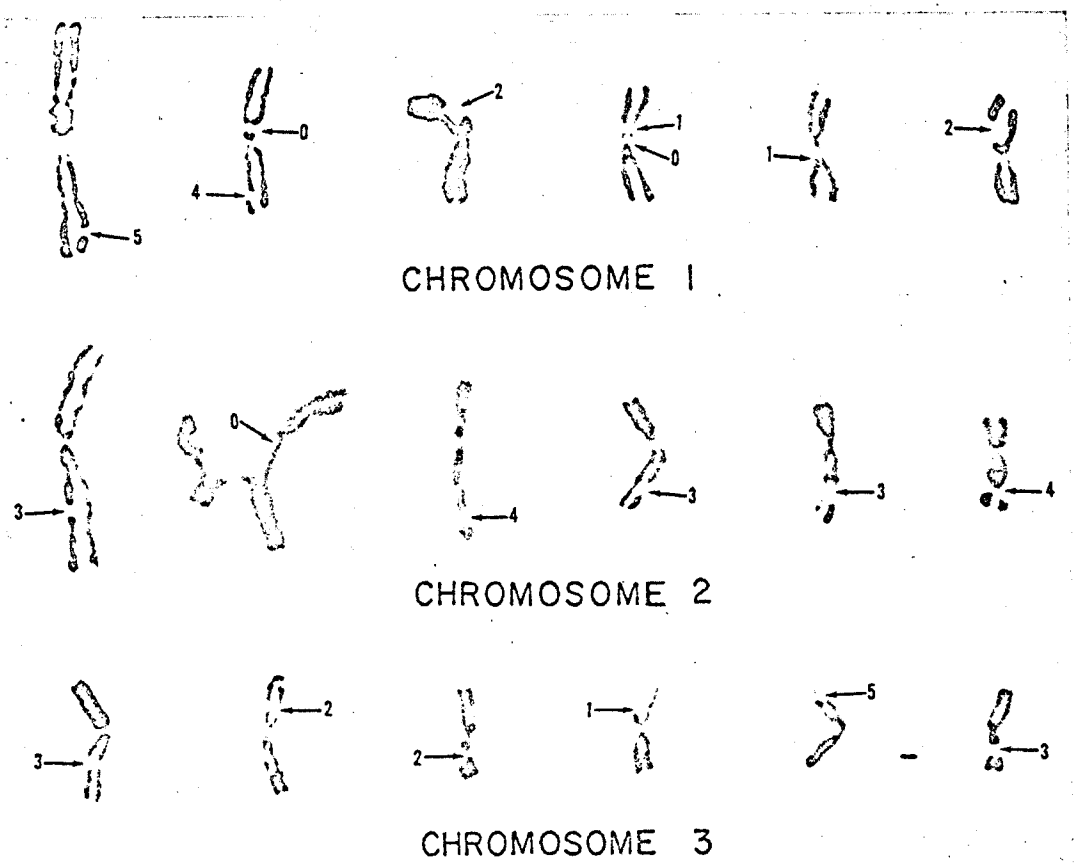


Fig. 3. Streptonigrin induced breaks in chromosomes 1, 2 and 3 are indicated by arrows. Numbers refer to chromosomal segment including the breakage point; S = short arm and L = long arm.

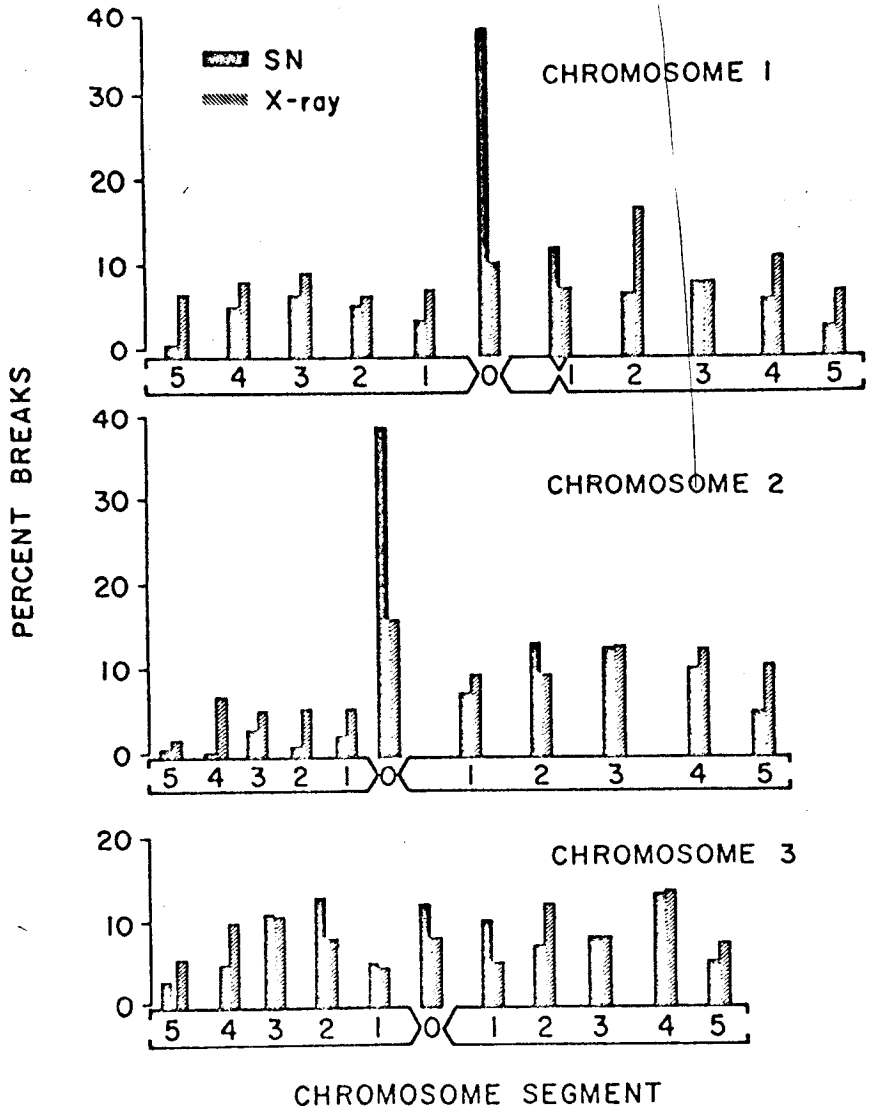


Fig. 4. The distribution of chromosomal breaks caused by SN and X-ray in chromosome Nos. 1, 2, 3.

More striking than the chromosome breaks, however, was the exaggerated appearance of the secondary constrictions after mitomycin treatment as indicated by Fig. 6. Other chromosomes of the complement, in addition to numbers 1, 9 and 16 in which heterochromatic regions have been described, were apparently not strongly affected by MC.

In some MC treated cells, chromosomal exchanges (quadriradial figures) were observed which resembled the pachytene cross configurations seen after a reciprocal translocation in meiosis. The frequency of these exchange configurations increased with MC concentration. Among 118 exchanges analyzed,

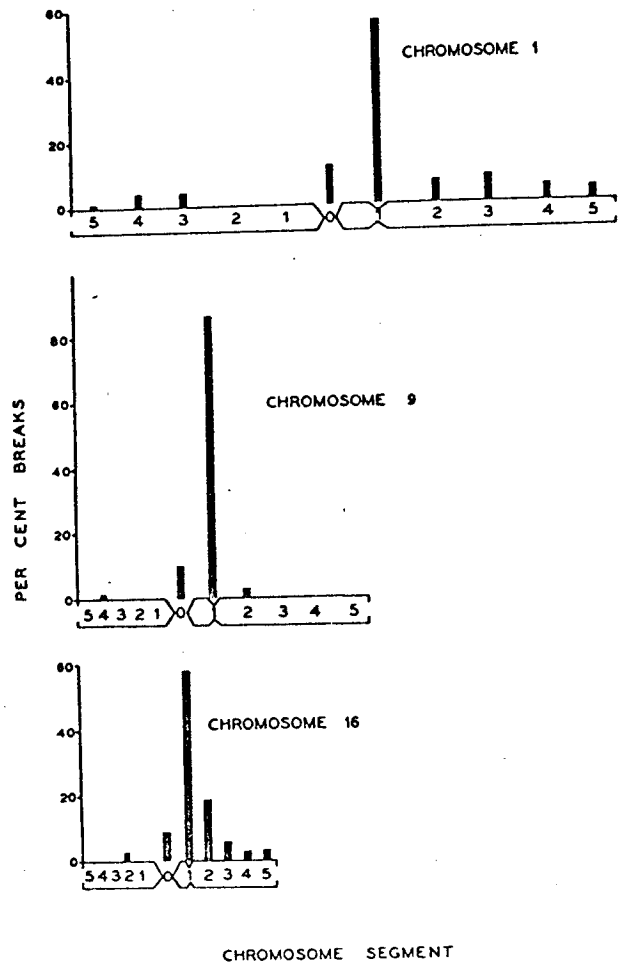


Fig. 5. The distribution of breaks within chromosomes Nos. 1, 9, and 16. The secondary constriction lies in segment 1 of the long arm in each chromosome.

60.2% of the figures apparently occur between homologs or apparent homologs. Similar figures have been observed following MC treatment (44) and they have been rarely observed in untreated leukocyte cultures (45). Therefore, a study was undertaken concerning the types of exchanges observed, the chromosomes involved, and the points of breakage in interchange. These data indicate that homologous exchanges occur far in excess of what might be expected by chance and that the genetic consequences of some of these exchanges may be analogous to somatic crossing over (46).

A sample of 238 exchange figures was obtained and representatives of such exchanges as shown in Fig. 7. These exchanges were classified into five types on the basis of homology and types of reunion. These are illustrated in Fig. 8. Classes I, II and III comprise homologous chromosomes, Classes I and II yielding symmetrical figures but differing in the arrangement of the centromeres,

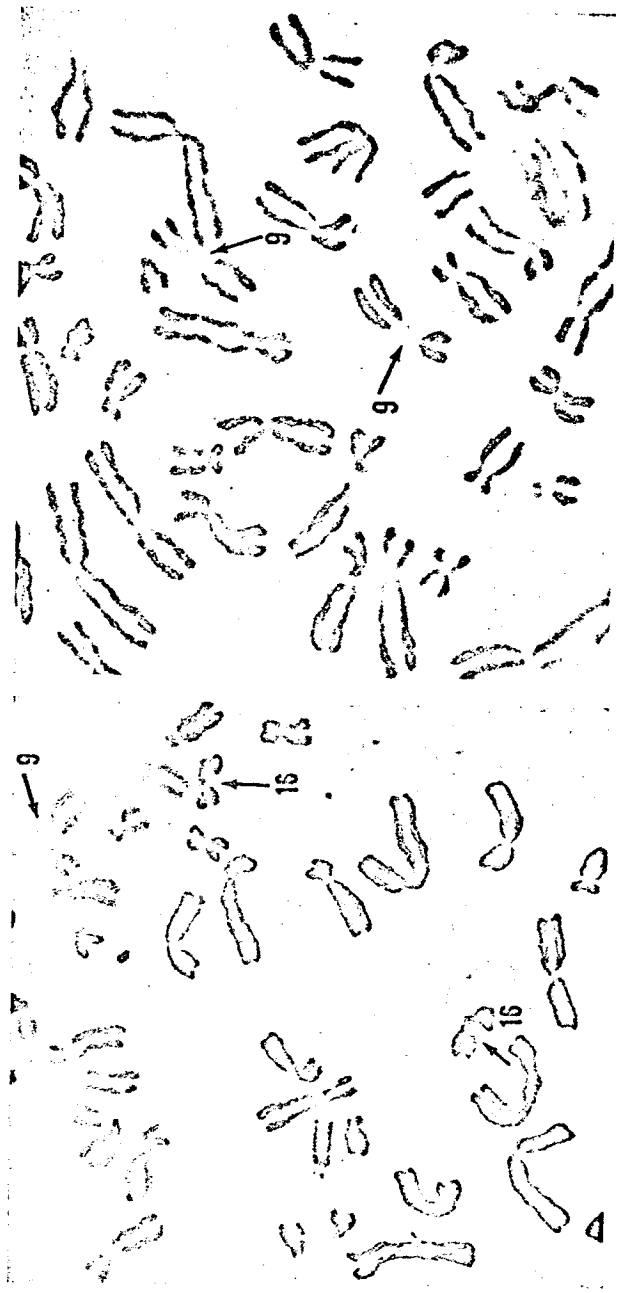


Fig. 6. Exaggerated secondary constrictions and breaks at secondary constrictions of chromosomes Nos. 9 and 16 induced by mitomycin C.

whereas Class III yields an asymmetrical figure. Class IV includes exchanges between chromosomes which are nonhomologous. The fifth type of classification involves exchanges including more than two chromosomes or complex exchanges. A total of 238 exchange figures was studied. As shown in Table II, 220 were simple two-chromosome exchanges while the remaining 18 were complex configurations involving more than two chromosomes. As can be seen from Table II, 110 of the 220 exchanges involved real or apparent homologs. This is a marked excess, not only of homologous exchanges in general, but of exchanges between certain pairs of chromosomes. Table III indicates the detailed breakdown of the types of exchanges observed. Of the 110 homologous exchanges, 36 involved the two No. 1 autosomes and 36 occurred in the No. 9 pair. It is also noteworthy that 14 of the nonhomologous exchanges occurred between Nos. 1 and 9. Several statistical tests were performed to show that the exchanges were not random with respect to: (1) individual chromosomes involved; (2) relative chromosomal length; and (3) frequency of breakage of specific sites with random reunion of the broken ends.

If all chromosomes had an equal probability of being involved in an exchange figure, then the probability that any two chromosomes drawn at random would be members of the same pair is 0.118 for the female complement and 0.130 for the male complement. Thus, an average value for the two sexes is 0.124 which asserts that, on the assumption of equal probability for each chromosome, there is less than one chance in eight that the two chromosomes chosen randomly will be real or apparent homologs. Clearly, 110 of 220 (50%) is a significant excess of homologous exchanges, and apparently chromosomal association in these exchanges is not by chance.

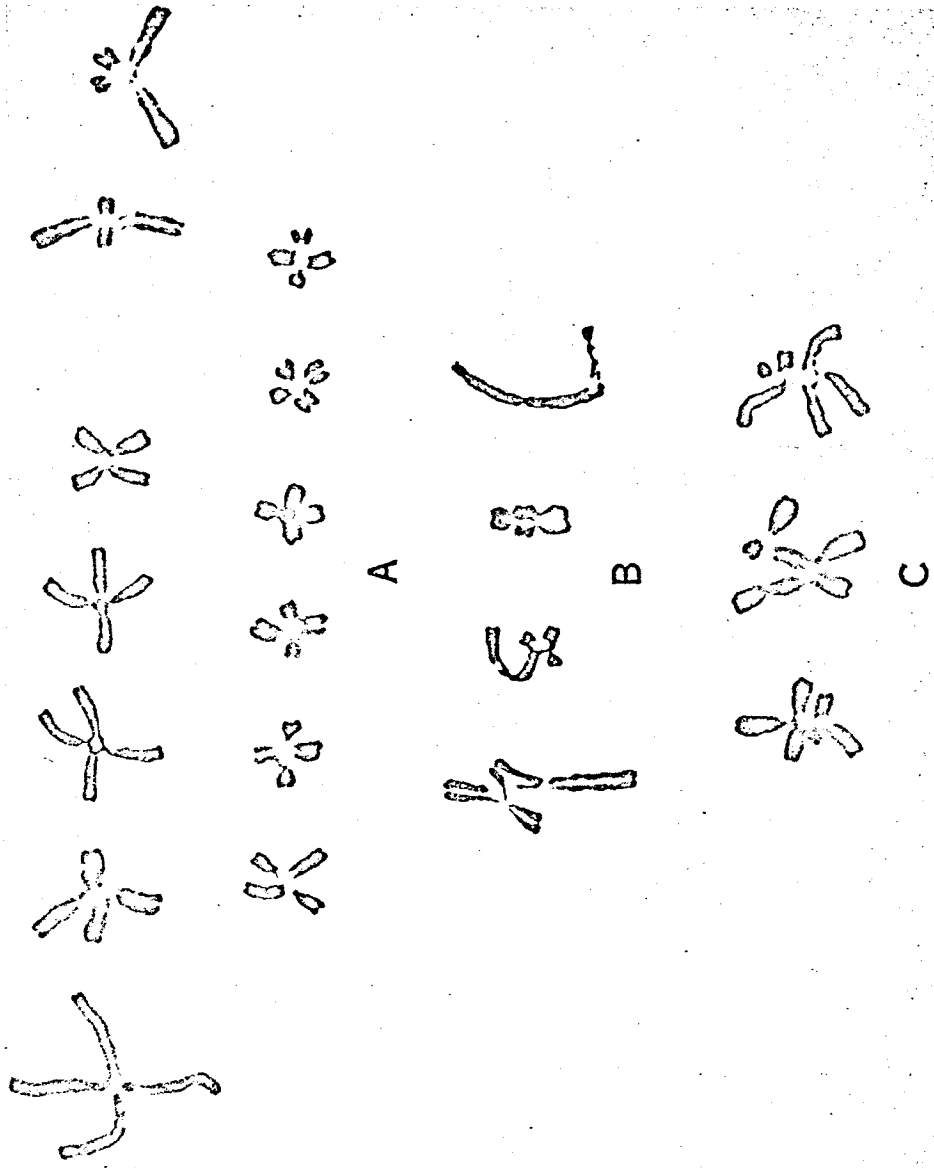
The probability that any chromosome is involved in an exchange could be related to the total chromatin length, if the drug acted uniformly throughout the chromatin material to produce exchange. Again, the data argue against such an interpretation. Using the measurements of the Denver nomenclature (15), it may be calculated that chromosome 1 constitutes approximately 9% of the length of the X-containing haploid complement. Thus, two No. 1 chromosomes would be expected to be exchanged less than 1% at a time (0.09^2). In fact, 16.4% of the two chromosome exchanges involve chromosome 1 which is greatly in excess of the prediction.

TABLE II

Frequencies of various classes of mitomycin induced chromosome exchanges

Class*	Number observed		
Simple exchanges:			
Class I	45	..	..
Class II	57	..	..
Class III	8	..	..
Class IV	110	..	..
Subtotal		220	
Complex exchanges:			
Class V	..	18	..
Total	..	..	238

*See Fig. 1 for definition of classes.


A
B
C

It is probable that the observed exchanges result from breakage and reunion without subsequent separation of newly formed chromosomes. It has also been shown that mitomycin C causes a nonrandom excess of breaks in chromosomes 1, 9 and 16, primarily in the heterochromatic regions of the long arms. If it is assumed that breakages are equal in 1 and 9 and that reunion of broken ends is random from cell to cell, then two thirds of the expected exchanges would involve 1 and 9 together, while one third of the exchanges would consist of the homologs (1/1 pair and 9/9 pair). In point of fact, only one sixth of the total of these three types of exchanges (14/86) involve one No. 1 and one No. 9. Much of this association may be due to a disproportionate contribution of the 'target chromosomes' (1 and 9) which are most frequently affected. Therefore, chromosomes 1 and 9 were omitted from consideration, and a recalculation of the break frequencies of the remaining chromosomes was obtained. Chi-square calculations demonstrated that these residual exchanges were still preferentially homologous, indicating that exchange figures are not due to random reunion of breaks, and another explanation is needed.

A possible explanation for such occurrence may be the existence of somatic pairing in human cells. If somatic pairing occurs normally, or if MC induces somatic pairing, then homologous regions of DNA would be in apposition and could interact as a result of treatment with MC. Alternatively, if the principal action of mitomycin C is to induce breakage of chromosomes of preferential sites during DNA synthesis, and if homolog synchrony occurs in the sequence of human chromosome replication, the interaction of homologs may be expected

TABLE III
Distribution of 220 mitomycin induced exchanges involving two chromosomes

Chromosome or group	Second chromosome or group involved												Total exchanges
	1	2	3	B	9	C	D	16	E	F	G	Y	
1	36	1	0	7	14	4	3	10	3	3	1	0	82
2	.	1	1	1	1	1	0	0	0	0	1	0	6
3	.	.	1	1	1	1	0	1	0	0	0	0	5
B	.	.	.	11	2	2	2	0	0	3	0	0	20
9	.	.	.	.	36	11	4	5	0	3	1	0	60
C	.	.	.	.	.	12*	4	2	0	0	5	0	23
D	.	.	.	.	.	.	2	1	0	1	1	0	5
16	.	.	.	.	.	.	.	5	0	0	0	0	5
E	.	.	.	.	.	.	.	.	2	1	0	0	3
F	.	.	.	.	.	.	.	.	.	8	2	0	10
G	.	.	.	.	.	.	.	.	.	.	1	0	1
Y	.	.	.	.	.	.	.	.	.	.	.	0	0
Total exchanges	36	2	2	20	54	31	15	24	5	19	12	0	220
Total chromosomes	118	8	7	40	114	54	20	29	8	29	13	0	440

*Five of these exchanges involved definite nonhomologs (e.g. 6 and 12).

Fig. 7. Chromosomal exchanges observed as 'translocation' configurations. Group A, exchanges between apparent pairs of homologues; second row demonstrates exchanges at secondary constrictions in chromosome pair No. 9; Group B, exchanges between non-homologous chromosomes; Group C, exchanges involving more than two chromosomes. This is a composite plate with variable magnifications.

without recourse to somatic pairing. The data lend credence to both theories and cannot discriminate between them. Furthermore, somatic pairing and homolog synchrony in DNA synthesis are not necessarily mutually exclusive events.

It might be argued that the Class I configuration (Fig. 8) represent mere chromosome association without actual breakage in the exchange of segments. Several cytological observations argue against this interpretation. If Class I figures were far in excess of Class II, then it might be concluded that pairing without breakage and reunion occur. In all the Class II figures, however, there is cytological evidence for breakage and exchange as shown by the possible emergence of dicentric chromosomes and acentric fragments (Fig. 9). It does not seem reasonable to presume, therefore, that breakage never occurs in Class I but always occurs in Class II exchanges. Figure 10 diagrams the three possible outcomes of breakage and reunion in two non-sister homologous chromatids. Random reunion of the four free chromosomal ends would result in equal frequencies of restitution, Class I and Class II figures. However, the restituted breaks should not remain in a cross configuration and would not be detected at metaphase. Therefore, the expected distribution of Class I and Class II exchange figures would approximate a 1:1 ratio. In other words, for every Class II exchange which is observed, there should be a corresponding Class I figure which resulted from breakage and reunion and not merely homologous pairing without exchange. In point of fact, there were 45 Class I figures and 57 Class

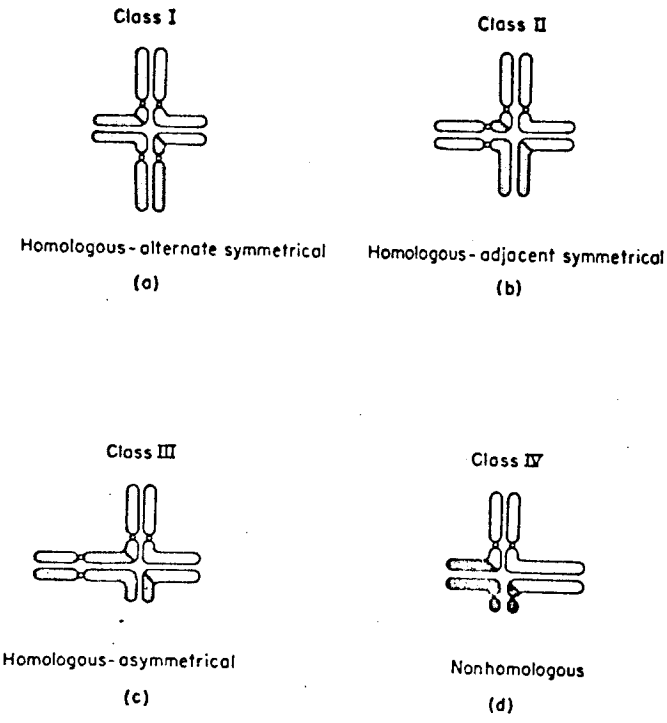


Fig. 8. Chromosome exchange Classes I to IV. Note that symmetry in (a) occurs in both vertical and horizontal planes while the axis of symmetry in (b) is only in one diagonal plane through the exchange points.

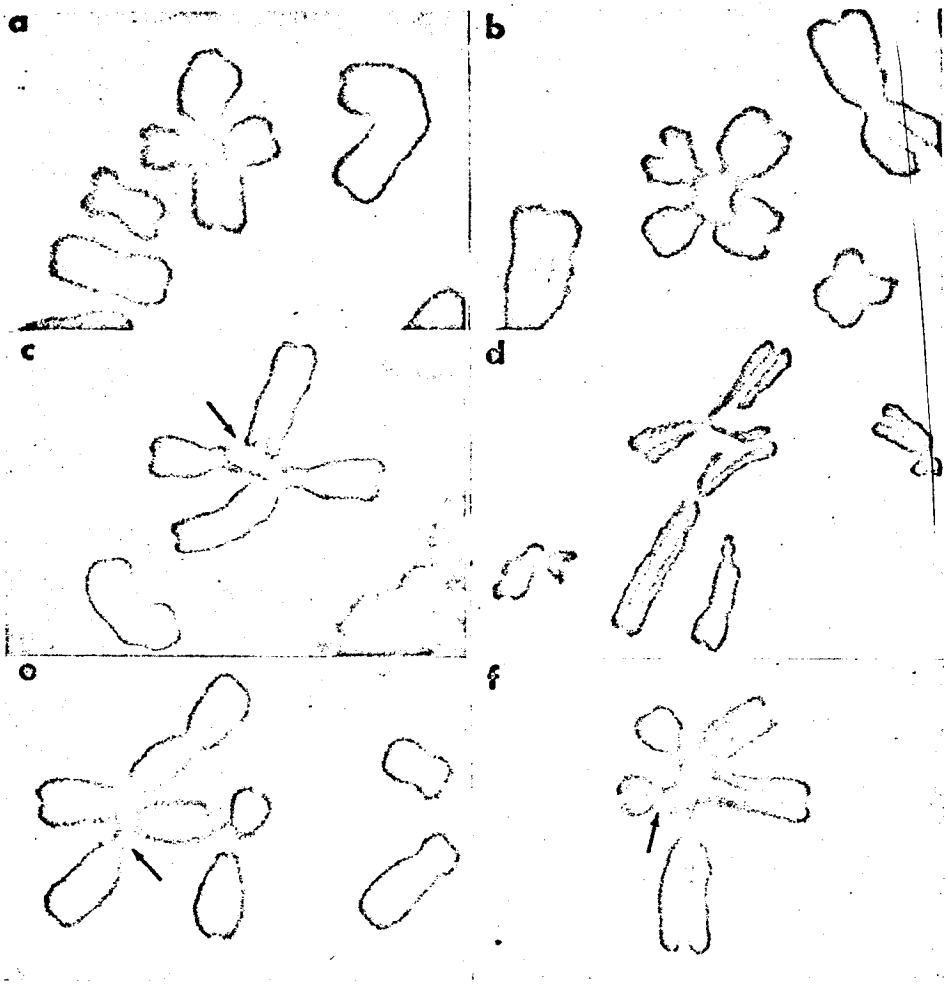


Fig. 9. Photomicrographs of chromosome exchanges induced by mitomycin C. (a) Class I; (b) Class II; (c) Class I (arrow indicates unhealed break at point of exchange); (d) Class IV (short arm of No. 2 exchanged with long arm of a C-group chromosome); (e-f) Class V (arrows point to acrocentric chromosomes involved in the exchange at their SAT-zones).

II figures which fit the expected 1:1 ratio ($0.30 > P > 0.20$). These observations suggest that probably all the Class I exchanges must represent a genetic event somewhat analogous to somatic crossing over. This study has illustrated that by combining the cytological evidence with logical arguments, the symmetrical homologous exchanges with opposite centromeres (Class I) may represent true exchange of genetic material and that mitomycin C may indeed be implicated in inducing or maintaining somatic crossing over. Critical proof, however, will be obtained, of course, only through the use of suitable genetic markers. If this phenomenon can be induced in diploid fibroblast cultures in vitro from tissues of individuals with informational genetic markers, then many of the problems in human somatic cell genetics may be amenable to experimental attack.

From consideration of the localization of the induced effects by both streptonigrin and mitomycin, an apparent nonrandom distribution of chromosomal breaks exists. Although the significance of such specificities is poorly understood, some general inferences may be drawn. In addition to these agents, most specific effects exhibited by other drugs as well have been localized in the heterochromatic regions of the chromosomes (e.g. the centromeres or secondary constrictions). These effects, however, may not be direct responses to the action of specific agents alone, since the enhancement, exaggeration and, possibly, breakage of heterochromatic secondary constrictions may also be intensified by various physical and chemical manipulations, e.g. fixation treatment (42), the use of calcium free medium (43), and cold shock (47-49). The similarity of effects induced by both nonspecific treatments and specific agents cast some doubt upon the specificity of certain agents.

That localization of specific chromosomal breakage may be a useful tool in the determination of chromosome structure may be particularly true for those agents whose chemical actions are well determined. Similar knowledge

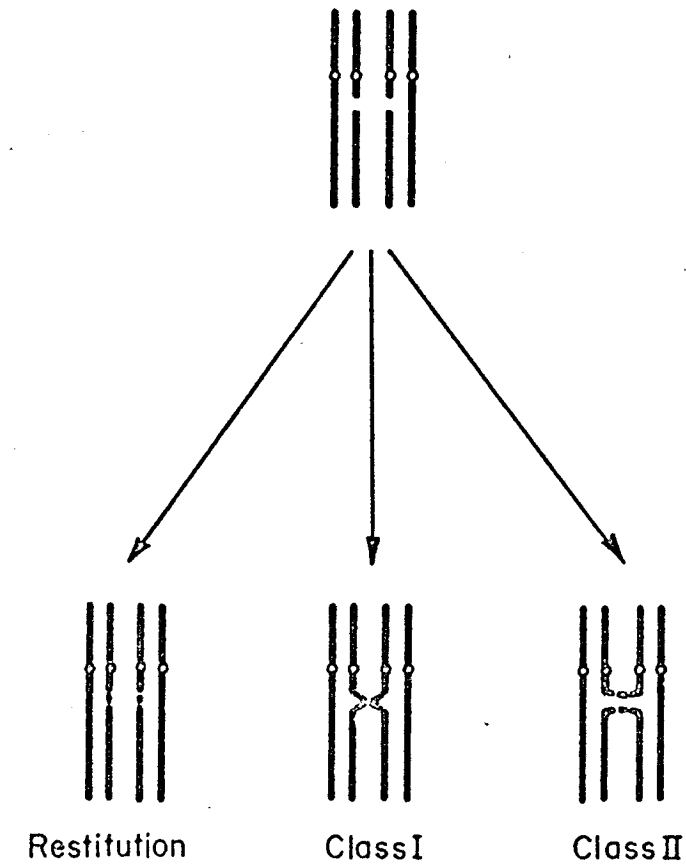


Fig. 10. Breakage in homologous regions of nonsister chromatids can result in three types of reunion. If reunion of the four broken ends occurs randomly by twos, then the three types will occur equally frequently. Restitution would probably not be detectable cytologically as an exchange.

concerning additional compounds manifesting specificities for different chromosomal regions (euchromatic) may lend insight into the structure of these regions. Although the agents discussed in this presentation appear quite exciting in experimental studies, one must exercise caution in the interpretation of their specificities. Many published reports describe gross morphologic chromosomal alterations which are quite removed from the structural or functional level of the genetic material. In addition, the observed effects are often overlapping and can be simulated by many agents capable of causing chromosome breakage. Since most of these agents affect only heterochromatic regions, one must consider the possibility that the specificity may not reside in the agent but rather may reflect the specific response of the chromosome to nonspecific environmental alteration. Therefore, the search for additional compounds which show localized affinities for euchromatin as well must be pursued. In this way, one might hope to find some agents which would increase our understanding of structural or functional regions of the chromosome.

Lysergic Acid Diethylamide (LSD-25)

To discuss the question of chromosomal breakage and the possible significance of those observations associated with ingestion of LSD at the current level of our understanding, and with an interpretation of the data at hand, is very difficult. A number of recent publications dealing with various aspects of this problem indicate that a definitive answer is not yet available and further investigative work is needed. Almost all the positive findings which implicate LSD as a potential excitant of genetic damage have been countered with negative findings utilizing almost the same test systems. I shall, therefore, attempt to review the current work on LSD pointing out some of the difficulties encountered in the various studies and possible reasons for the discrepancies in the published material.

In vitro experimentation utilizing six individuals (three males and three females) showed a marked suppression of mitosis (50). The mitotic rate in in vitro control cultures was 6.2% and a significant inhibition was apparent on the addition of the drug in most concentrations. However, the longer the exposure time to LSD, the greater the suppression of mitosis.

The induction of chromosomal damage by LSD in these in vitro experiments was also clearly demonstrated. There was no significant difference among the individuals nor between the sexes so the data were pooled. Among the controls, 3.9% of the cells exhibited chromosomal abnormalities, while among the treated cultures, the lowest frequency of abnormal cells was almost twice the control level (7.7%); it ranged to over four times the control values (17.5%). The vast majority of these abnormalities were simple chromatid and isochromatid breaks, although a few chromatid exchanges in dicentric chromosomes were observed (Fig. 11). It should be mentioned that all the morphologic structural rearrangements (dicentrics and quadriradial figures) were seen in the treated cultures, and none were seen in the controls. The distribution of chromosome breaks, both among and within the chromosome groups, appeared to be nonuniform. Such distributions were illustrated above with both streptonigrin and mitomycin. The centromeric regions of many chromosomes appear to be more vulnerable to LSD breakage than other regions as were the obvious heterochromatic secondary constrictions.

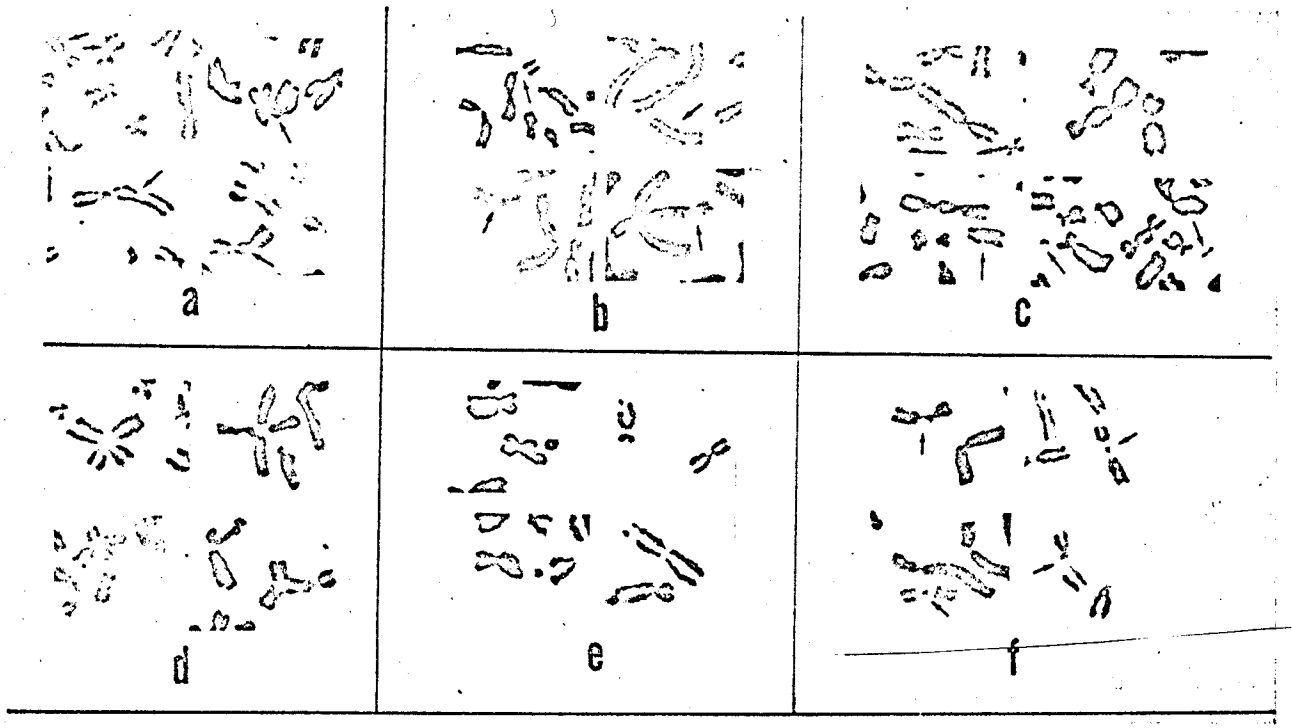


Fig. 11. Types of chromosomal abnormalities observed in both the in vitro experiments and from the leukocyte cultures derived from drug 'users' showing chromatid breaks (a), isochromatid breaks (b), dicentric chromosomes (c) (arrows indicate acentric fragments), exchange figures (d), chromatid breaks giving rise to terminal deletions and acentric fragments (e) and attenuation and breakage at the secondary constriction in chromosome No. 9 (f).

described who exhibited increased breakage as well as an increase in structural rearrangements (quadriradials). A series of five patients treated with pure LSD showed an elevated frequency of chromosomal breaks and gaps (63). Another series of patients receiving pure LSD for psychotherapy in known dosages has demonstrated a statistically significant difference in structural rearrangements as well as a transient increase in chromosomal damage between the LSD group and the controls (64). Similarly, the report by Sparkes *et al.* (62), which was considered negative by the authors, shows that all but one of the quadriradials and chromosomal interchanges was observed in the LSD 'users' or those medically treated with the drug.

A second major problem is the concommitant use of additional drugs. This difficulty is obvious in all the studies to date and deserves further investigation. We have tested several of these agents, notably some of the tranquilizers (various phenothiazines, diazepam and diazepoxide hydrochloride), both in vivo and in vitro and find no suggestion of a chromosome breaking potential (8). Additionally, environmental influences may play an important role. In evaluating those investigations utilizing 'hippy populations,' one must consider the overall physical status of the subjects. It is well known that these communal enclaves have high rates of venereal disease, viral infections, and general conditions of malnutrition. These may be contributing factors to the results obtained. However, insofar as LSD ingestion was a common selective factor for the individuals studied, it seems plausible that this agent may be, indeed, the incitant of chromosomal damage. However, the ultimate evaluation of the cytogenetic effects of LSD in man will require observations made under rigidly controlled circumstances.

The significance and potential dangers of chromosome breakage induced by agents such as those discussed above must be viewed from several aspects. First, there are the possible consequences to the individual exposed to the agent. From this point of view, we have no definitive evidence derived from man, but one may discuss the problem from our knowledge of analogous situations which could give rise to similar types of chromosomal breakage and rearrangement. Chromosomal abnormalities similar to those induced by the above agents have been noted to occur spontaneously in three diseases inherited in an autosomal recessive pattern: Bloom's syndrome (65), Fanconi's anemia (66), and ataxia telangectasia (67). Patients with these syndromes show a high propensity towards developing leukemia or other neoplasms. In this context, cells of neoplastic origin frequently illustrate a variety of chromosomal aberrations, many of which are not unlike those seen after exposure to various agents. Moreover, many of the agents known to produce similar aberrations such as radiation and benzene in man and other species as well as viruses in laboratory animals are known carcinogens. Whether or not chromosomal damage and genesis of neoplasia are causually related is not known; however, the association of chromosomal abnormalities in cancer is well documented. One of the obvious potential dangers, therefore, of exposure to chromosome breaking agents is the possible future increase in the incidence of leukemia and other neoplasms in the persons exposed. For example, the co-occurrence of chromosomal aberration and acute leukemia in LSD users has now been observed in two cases (68, 69).

In addition, other less evident dangers exist, such as the teratogenic potential of such chromosome breaking agents. This may be examined through the

production of congenital abnormalities and increased abortions in experimental animals studied. Both streptonigrin and mitomycin C possess potential for inducing teratogenic deformities in various test systems (70, 71), LSD, when injected into female rats, hamsters, or mice early in pregnancy results in a wide range of birth defects among the offspring (72-74). Recently, Alexander *et al.* have demonstrated the inherited nature of such abnormalities in LSD treated rats (75). Although the chromosomes of these malformed fetuses in experimental animals were usually not examined, the study of spontaneous abortions in man indicates that a high proportion of such abortuses do carry the chromosomal abnormalities (76); therefore, the possibility exists of chromosomal damage *in utero* leading to an increased number of abnormal offspring.

Perhaps the greatest danger of such agents lies in the potential chromosome damage to the gametes. Therefore, it is important to regard those drugs which are capable of causing such damage with extreme caution. If chromosomal damage similar to that observed in leukocytes and other somatic cells occurs in spermatogonial cells and in functional gametes, then the effects of these agents may be truly a genetic one. It has been shown with streptonigrin (77) and with LSD (53 to 55) that chromosomal damage in rodent meiotic cells may be induced. Ample evidence already exists in man that segregation of translocations results in chromosomal imbalance leading to fetal wastage in a high percentage of offspring with congenital abnormalities and mental retardation. Since the carriers of such balance translocations are usually clinically normal, as are many of their offspring, the consequences of this type of chromosomal abnormality may not be apparent for several generations.

This presentation has demonstrated the possibility of cytogenetic screening as an assay of activity for various types of drugs. Although data can be obtained indicating the type and severity of chromosomal damage induced by various agents, the implications and potential significance of such chromosomal breakage at present are still unknown. Although definitive answers to many of the questions raised by such research associated with the effects of exogenous agents are not yet available, the present status of our information demands a cautionary posture. Negative results regarding the mutagenic potential of a particular agent are not conclusive proof of its lack of effect, while positive results warrant testing for reproducibility by further investigation.

References

1. Lea, D. E. 1955. Actions of radiations on living cells. [2nd ed.] University Press, Cambridge.
2. Revell, S. H. 1953. Chromosome breakage by X-rays and radiomimetic substances in *Vicia*. *In* Symposium on chromosome breakage. Heredity (Suppl.) 6: 107.
3. Kihlman, B. A. 1961. Biochemical aspects of chromosome breakage. Advan. Genet. 10:1, 51.
4. Kihlman, B. A. 1966. Actions of chemicals on dividing cells. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
5. Ostertag, W. 1966. Chemische mutagenese an menschlichen Zellen in Kultur. Abhandl. Saech. Akad. Wiss. Leipzig Math. Naturw. Kl. 1:5.
6. Cohen, M. M., and Shaw, M. W. 1965. Specific effects of viruses and antimetabolites on mammalian chromosomes. pp. 50-66. *In* C. J. Dawe [ed.] In vitro: the chromosome: structural and functional aspects, Vol. 1. Waverly Press, Baltimore, Maryland.
7. Stenchever, M. A., Frankel, R. Jarvis, J. A., and Veress, K. 1968. Some effects of Diazepam (Valium) on human cells in vitro. Am. J. Obstet. Gynecol. (In press).
8. Cohen, M. M., Hirschhorn, K., and Frosch, W. A. Cytogenetic effects of several tranquilizing drugs in vivo and in vitro. J. Am. Med. Assoc. (In press).

9. Carr, D. H. 1967. Chromosomes after oral contraceptives. Lancet **2**: 830.
10. Wuu, K. D., and Grant, W. F. 1966a. Induced abnormal behavior in a barley plant (Hordeum vulgare, L.) with the herbicide Lorox. Phyton **23**: 63.
11. Wuu, K. D., and Grant, W. F. 1966b. Morphological and somatic chromosomal aberrations induced by pesticides in barley (Hordeum vulgare). Can. J. Genet. Cytol. **8**: 418.
12. Fetner, R. H. 1962. Ozone-induced chromosome breakage in human cell cultures. Nature **194**: 793.
13. Tough, I. M., and Court Brown, W. M. 1965. Chromosome aberrations and exposure to ambient benzene. Lancet **1**: 684.
14. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. **20**: 613-616.
15. The Denver Report. 1963. A proposed standardized system of nomenclature of human mitotic chromosomes. Lancet **1**: 1063.
16. Levine, M., and Borthwick, M. 1963. The action of streptonigrin on bacterial DNA metabolism and on induction of phage production in lysogenic bacteria. Virology **21**: 568, 574.
17. Levine, M. and Borthwick, M. 1963. The action of streptonigrin on genetic recombinations between bacteriophages. Proc. 11th Intern Congr. Genet.
18. Radding, C. M. 1963. Uptake of tritiated thymidine by K12 (λ) induced by streptonigrin. Proc. 11th Intern. Congr. Genet.
19. Cohen, M. M., Shaw, M. W., and Craig, A. P. 1963. The effects of streptonigrin on cultured human leukocytes. Proc. Nat. Acad. Sci. U.S. **50**: 16-24.
20. Cohen, M. M. 1963. The specific effects of streptonigrin activity on human chromosomes in culture. Cytogenetics **2**: 271-279.
21. Ben-Porat, T., Reissig, M., and Kaplan, A. 1961. Effect of mitomycin C on synthesis of infective virus and DNA in pseudorabies virus infected rabbit kidney cells. Nature **190**: 33.
22. Iijima, T., and Hagiwara, A. 1960. Mutagenic effect of mitomycin C on *Escherichia coli*. Nature **183**: 395.
23. Otsuji, N. 1962. DNA synthesis and lambda phage development in a lysogenic strain of *E. coli* K12. Biken's J. **5**: 9.
24. Reich, E. 1961. Bactericidal action of mitomycin C. Biochim. Biophys. Acta **53**: 132.
25. Sekiguchi, M., and Takagi, Y. 1959. Synthesis of deoxribonucleic acid by phage infected *Escherichia coli* in the presence of mitomycin C. Nature **183**: 1134.
26. Sekiguchi, M., and Takagi, Y. 1960. Effect of mitomycin C on the sythesis of bacterial and viral DNA. Biochim. Biophys. Acta **41**: 434.
27. Shiba, S., Tirawaki, A., Taguchi, T., and Kawamata, J. 1959. Selective inhibition of formation of DNA in *E. coli* by mitomycin C. Nature **183**: 1056.
28. Shiba, S., Tirawaki, A., Taguchi, T., and Kawamata, J. 1958. Studies on the effect of mitomycin C on nucleic acid metabolism in *E. coli*. Biken's J. **1**: 179.
29. Yuki, S. 1962. The effect of mitomycin C on recombination in *E. coli* K12. Biken's J. **5**: 47.
30. Reich, E., and Franklin, R. M. 1961. Effect of mitomycin C on the growth of some animal viruses. Proc. Nat. Acad. Sci. **47**: 1212.
31. Reich, E., and Tatum, E. L. 1969. Bacteriocidal action of mitomycin C. Biochim. Biophys. Acta **45**: 608.
32. Sekiguchi, M., and Takagi, Y. 1960. Effect of mitomycin C on the synthesis of bacterial and viral DNA. Biochem. Biophys. Acta **41**: 434.
33. Levine, M. 1961. Effect of mitomycin C on interactions between temperate phages and bacteria. Virology **13**: 493.
34. Otsuji, N. 1962. DNA synthesis and lambda phage development in a lysogenic strain of *E. coli* K12. Biken's J. **5**: 9.
35. Szybalski, W. 1958. Special microbial systems: II. Observations on chemical mutagenesis in microorganisms. Ann. N.Y. Acad. Sci. **73**: 475.
36. Kuroda, Y., and Furuyoma, J. 1963. Physiological and biochemical studies of effects of mitomycin C on strain HeLa cells in cell culture. Cancer Res. **23**: 682.
37. Reich, E., Franklin, R. M., Shatkin, A. J., and Tatum, E. L. 1961. Effect of mitomycin on mammalian cells in culture. Federation Proc. **20**: 154.
38. Iyer, V. N., and Szybalski, W. 1963. A molecular mechanism of mitomycin action: Linking of complementary DNA strands. Proc. Nat. Acad. Sci. **50**: 355.

39. Cohen, M. M., and Shaw, M. W. 1964. Effects of mitomycin C on human chromosomes. J. Cell Biol. **23**: 386-395.
40. Ferguson-Smith, M. A. 1962. The identification of human chromosomes. Proc. Roy. Soc. Med. **55**: 471.
41. Moorhead, P. S., and Defendi, V. 1963. Asynchrony of DNA synthesis in chromosomes of human diploid cells. J. Exp. Biol. **16**: 202.
42. Saksela, E., and Moorhead, P. S. 1962. Enchancement of secondary constructions and the heterochromatic X in human cells. Cytogenetics **1**: 225.
43. Sasaki, M. S., and Makino, S. 1963. The demonstration of secondary constrictions in human chromosomes by means of a new technique. Am. J. Human Genet. **15**: 24.
44. Nowell, P. C. 1964. Mitotic inhibition and chromosome damage by mitomycin in human leukocyte cultures. Exp. Cell Res. **33**: 445.
45. German, J. L. 1964. Cytological evidence for crossing-over *in vitro* in human lymphoid cells. Science **144**: 298.
46. Shaw, M. W., and Cohen, M. M. 1965. Chromosome exchanges in human leukocytes induced by mitomycin C. Genetics **51**: 181-190.
47. Darlington, C. D., and La Cour, L. F. 1938. Differential reactivity of the chromosome. Ann. Botany (London) **2**: 615-625.
48. Darlington, C. D., and La Cour, L. F. 1940. Nucleic acid starvation of the chromosomes in Trillium. J. Genet. **40**: 185-213.
49. Hampel, K. E., and Levan, A. 1964. Breakage in human chromosomes induced by low temperature. Hereditas **51**: 315-343.
50. Cohen, M. M., Marinello, M. J., and Back, N. 1967. Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. Science **155**: 1417-1419.
51. Cohen, M. M., Hirschhorn, K., and Frosch, W. A. 1967. *In vivo* and *in vitro* chromosomal damage induced by LSD-25. New England J. Med. **277**: 1043-1049.
52. Cohen, M. M., Hirschhorn, K., Verbo, S., Frosch, W. A., and Groeschel, M. M. 1968. The effect of LSD-25 on the chromosomes of children exposed in utero. Pediatric Res. **2**: 486-492.
53. Skakkeback, N. E., Phillip, J., and Rafaelsen, O. J. 1968. Studies on meiotic chromosomes of mice injected with LSD-25. Science **160**: 1246-1248.
54. Peterson, K., Legator, M., and Jacobson, C. Meiotic studies on LSD treated rats. Mammalian Chromosome Newsletter (In press).
55. Cohen, M. M., and Mukherjee, A. B. 1968. Meiotic chromosomal anomalies in mice injected with LSD-25. Nature **219**: 489-490.
56. Jarvik, L. F., and Kato, T. 1968. Is lysergide a teratogen? Lancet **1**: 250.
57. Irwin, S., and Egozcue, J. 1967. Chromosomal abnormalities in leukocytes from LSD-25 users. Science **157**: 313-314.
58. Egozcue, J., and Irwin, S. 1968. Chromosomal damage in LSD-25 users. J. Am. Med. Assoc. **204**: 214-218.
59. Zellweger, H., McDonald, J. S., and Abbo, G. 1967. Is lysergic acid diethylamide a teratogene? Lancet **2**: 1066-1068.
60. Loughman, W. D., Sargent, T. W. and Israelstam, D. M. 1967. Leukocytes of humans exposed to lysergic acid diethylamide: lack of chromosomal damage. Science **158**: 505-510.
61. Bender, L., and Sankar, D. V. 1968. Chromosome damage not found in leukocytes of children treated with LSD-25. Science **159**: 749.
62. Sparkes, R. S., Melnyk, J., and Bozzetti, L. P. 1968. Chromosomal effect in vivo of exposure to lysergic acid diethylamide. Science **160**: 1343-1345.
63. Nielsen, J., Friedrich, U., and Tsuboi, T. 1968. Chromosome abnormalities and psychotropic drugs. Nature **218**: 488-489.
64. Hungerford, D. A. Taylor, K. M., Shagass, C., LaBadie, G. U., Balaban, M. A. Paton, G. R. 1968. Cytogenetic effects of LSD therapy in man. J. Am. Med. Assoc. **206**: 2287.
65. German, J., Archibald, R., and Bloom, D. 1965. Chromosomal breakage in rare and probably genetically determined syndrome of man. Science **148**: 506-507.
66. Bloom, G. E., Warner, S., Gerald, P. S., and Diamond, L. K. 1966. Chromosome abnormalities in constitutional aplastic anemia, New England J. Med. **274**: 8-14.
67. Hecht, F., Koler, R. D., Rigas, D. A., Dahnke, G. S., Case, M. P., Tisdale, V., and Miller, R. W. 1966. Leukaemia and lymphocytes in ataxia-telangiectasia. Lancet **2**: 1193.

68. Grossbard, L., Rosen, D., McGilvray, E., DeCapoa, A., Miller, O. J., and Bank, A. 1968. Acute leukemia with ph' -like chromosome in an LSD user. J. Am. Med. Assoc. **205:** 791-793.
69. Warburton, D. 1968. (personal communication).
70. Warkany, J., and Takacs, E. 1965. Congenital malformations in rats from streptonigrin. Arch. Pathol. **79:** 65-79.
71. Kury, G., and Craig J. M. 1967. The effect of mitomycin C on developing chicken embryos. J. Embryol. Exp. Morphol. **17:** 229-237.
72. Alexander, G. J., Miles, B. E., Gold, G. M., and Alexander, R. B. 1967. LSD: injection early in pregnancy produces abnormalities in offspring of rats. Science **157:** 459-460.
73. Geber, W. F. 1967. Congenital malformations induced by mescaline, lysergic acid diethylamide and bromolysergic acid diethylamide in the hamster. Science **158:** 265-267.
74. Auerbach, R. and Rugowski, J. A. 1967. Lysergic acid diethylamide: effect on embryos. Science **155:** 1325-1326.
75. Alexander, G. J., Machiz, S., Alexander, R. B. 1968. Inherited abnormalities in 3 generations of offspring of LSD treated rats. Federation Proc. **27:** 220.
76. Carr, D. H. 1967. Chromosome anomalies as a cause of spontaneous abortion. Am. J. Obstet. Gynecol. **97:** 283-293.
77. Jagiello, G. 1967. Streptonigrin: effect of first meiotic metaphase of the mouse egg. Science **157:** 453.