INDUCED AND SPONTANEOUS CHROMOSOMAL ABERRATIONS IN CULTURED HUMAN LEUKOCYTES

by

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We accept this thesis as conforming to the required standard.

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I ABSTRACT

The frequency of chromosome breaks was increased in replicate cultures from each of ten individuals when lysergic acid diethylamide at a concentration of 1 ug/ml of culture was added 24 hours prior to the harvest of the cells. The differences between the control and treated cultures ranged from +3.00 to +7.93 with a mean of +4.63, indicating no variation in response between individuals. The breaks were randomly distributed among the seven groups of chromosomes of the complement.

No significant difference in either the number of cells with aberrations, or the number of breakage events was observed between cells cultured from a patient with Fanconi's anaemia before and 24 hours after treatment with 250 ug. of growth hormone. Both were significantly increased over the control. After treatment with growth hormone, the number of breaks per aberrant cell was decreased, and the distribution of frequencies of specific types of aberrations was changed. Non-homologous exchange figures were the only two break events observed in cultures from the patient. None were observed in control cells. The distribution of breaks among the seven groups of chromosomes was random.

The frequency of chromosome aberrations was increased in cultures from a single individual when treated with 1 ug/ml of mitomycin-C for one hour at the beginning of the culture period. In the treated cultures, 181 breaks were observed in 64 of the 100 cells examined, whereas only 5 breaks were observed in three of the 100 cells scored in the control samples. Forty-seven

exchange configurations were observed in the treated cultures, 42.56% of these being non-homologous exchanges. No marker or dicentric chromosomes were observed.

Breaks were randomly distributed among the seven groups of chromosomes of the complement.

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V INTRODUCTION

The association of chromosomal aberrations with congenital malformations and malignancy has stimulated an increased interest in the study of the effects of a wide variety of agents on the chromosomes. Such studies have a potential practical, as well as academic, value as they provide a means of identifying cytotoxic and possibly teratogenic or carcinogenic agents.

Some of the agents which induce chromosomal aberrations were first identified by plant cytogeneticists. Extensive review articles outlining the effects of a variety of chemical and physical agents on the chromosomes have been published (Darlington and Koller, 1947; Oehlkers, 1952; Revell, 1952).

Plant cytogeneticists had access to material in which a large number of cell divisions were naturally occurring. However, the mammalian cytogeneticist was hindered by the technical difficulty of obtaining adequate numbers of dividing cells for analysis. Consequently, mammalian cytogenetics and particularly human cytogenetics, remained a restricted field of study until the middle nineteen fifties when a satisfactory *in vitro* system was devised.

The discovery that phytohemagglutinin (PHA) stimulated division of circulating human white blood cells, made it possible to obtain adequate numbers of rapidly dividing cells in culture (Nowell, 1960). Refinements were soon made to the *in vitro* culture technique providing a convenient method of chromosome preparation suitable for detailed analysis (Moorhead *et al.* 1960).

With the advent of a technique that enabled researchers to study the morphology of the human chromosome complement, a system for the classification of the chromosomes that would become universally recognized was established. This was effected in 1960 by a "Proposed System of Nomenclature of Human Mitotic Chromosomes" (Denver Report, 1960). This was subsequently revised in London and Chicago (Chicago Conference, 1966).

Aside from being a useful diagnostic aid in determining chromosome abnormalities responsible for some congenital malformations, the three day PHA stimulated leukocyte culture of a few drops of human blood provides a rapid and convenient system for studying the effects of a great variety of agents on the chromosomes.

The study presented here was carried out to investigate the frequency and type of chromosomal aberrations observed in human peripheral leukocytes exposed in vitro to (a) lysergic acid diethylamide, and (b) mitomycin-C. A comparison of the frequency and types of chromosomal aberrations in samples obtained from a patient with Fanconi's Anaemia before and after treatment with growth hormone is also included.

A. Effects of Lysergic Acid Diethylamide (LSD) In Vitro

Little work has been reported on the effects of LSD in vitro in comparison to the number of reports published on in vivo studies; only two reports, both by Cohen and his associates, have appeared in the literature (Cohen, Marinello, and Black,

1967; Cohen, Hirschhorn, and Frosch, 1967). They reported finding a marked depression of mitoses in dividing cell polutions, and an increase in chromosomal aberrations when leukocyte cultures were treated with LSD. Most the chromosome aberrations observed in these studies were chromatid and isochromatid breaks, although apparent exchange figures and dicentric chromosomes were also observed.

The exchange configurations observed by Cohen et al. (1967) in the LSD treated cultures resembled those in cultures exposed to mitomycin-C (Nowell, 1964; Cohen and Shaw, 1964) and those found in two autosomal recessive diseases, Fanconi's anaemia (Bloom et al. 1966; German and Crippa, 1966) and Bloom's syndrome (German, Archibald, and Bloom, 1965). The frequency of aberrations is, however, much lower in LSD treated cultures.

Neither the mechanism nor the phase of the mitotic cycle at which LSD is effective has been investigated.

B. Effects of LSD In Vivo

The considerable use of LSD by young people in the last few years had led researchers to investigate not only its effects on chromosomes but also its possible mutagenic and teratogenic effects.

At the present time, several authors have reported finding an increased level of chromosome damage in 'users' of LSD (Cohen, Hirschhorn, and Frosch, 1967; Hirschhorn and Cohen, 1967; Irwin and Egozcue, 1967; Egozcue, Irwin and Maruffo, 1968).

Cohen et al. (1967) observed that the frequency of chromosomal aberrations ranged from 5.3% to 25.1% in the leukocytes from 18 LSD 'users'. In a series of drug free controls, the frequency of chromosomal aberrations ranged from 2.3% to 5.5%, indicating a three to four times increase in damage in treated cultures.

Irwin and Egozcue (1967) observed an increased level of chromosome breakage in 6 out of 8 LSD 'users'. Control breakage frequencies ranged from 7 to 25% whereas patients ingesting LSD demonstrated a 12 to 38% breakage rate.

Egozcue et al. (1968) in a later series of drug free controls and LSD 'users', observed control breakage rates to vary from 6.0 to 16.5% while individuals who had ingested LSD demonstrated breakage values ranging from 8 to 45%. A two fold increase in chromosome damage was observed in LSD 'users'.

Neither Cohen et al. (1967) nor Irwin and Egozcue (1967) observed a relationship between the frequency of chromosome breakage and the number of doses taken, the amount of the dose, or the period of time which had elapsed between the last dose and the time at which the study was undertaken.

Hungerford et al. (1968) reported finding a transient increase of chromosomal abnormalities when LSD was injected intraveneously, with a return to control frequencies one month after administration of the final dose.

A number of researchers have reported finding no increase in chromosome damage in individuals ingesting LSD (Loughman et al. 1967; Bender and Sankar, 1968; Sparkes, Melnyk, and Bozzetti, 1968).

Loughman et al. (1967) reported that leukocytes from 8 patients receiving large doses of LSD (4,000 ug.) failed to show a significantly higher breakage rate than in the control cultures. Bender and Sankar (1968) reported that seven children treated for periods of from 5½ to 35 months, failed to show a significant increase in chromosome breakage. Chromosome studies were not performed until 20 to 48 months after the last dose. Sparkes et al. (1968) also found no significant increase in chromosome damage in a group of 'users' and medically treated individuals over the controls.

The reported findings of chromosome damage caused by ingestion of LSD, are conflicting, and a definitive statement as to whether LSD does or does not increase the frequency of chromosome damage is not possible at this time.

C. Effects of LSD In Utero

The effects of LSD on the chromosomes of children whose mothers had taken the drug during pregnancy have been reported (Cohen, Hirschhorn, and Frosch, 1967; Egozcue et al. 1968; Zellweger et al. 1967; Cohen et al. 1968). Cohen et al. (1968) observed a high frequency of chromosome damage (13 and 19%) in two of four children exposed to LSD in utero during the third and fourth months of pregnancy. Egozcue et al. (1968) also studied four children whose mothers took LSD during pregnancy. Three of these showed a high frequency of chromosome breaks. (range 22-28%) Cohen et al. (1968) reported finding an increased frequency of breakage among children exposed to LSD in utero when

compared to matched controls.

Although there is a great deal of controversy over the possible teratogenic effects of in-utero exposure to LSD, only one report of congenital malformations in a child whose mother ingested LSD during pregnancy has appeared. Zellweger and his colleagues (1967) described a case of a child born with lower leg deformities, and it was known that the mother had ingested LSD early in her pregnancy. The authors felt that it was not unreasonable to assume a casual relationship between the LSD and the limb deformities.

Sato and Pergament (1968) found no foetal abnormalities in a child born to a mother who had ingested LSD early in the pregnancy.

Teratogenic effects of LSD in rats and hamsters have been reported (Alexander et al. 1967; Geber, 1967; Auerbach and Rugowski, 1967). The most common abnormalities have been those of the brain, spinal cord and liver, with abortion and underdeveloped offspring also prevalent.

Warkany and Takacs (1968) reported finding no significant increase in abnormalities when LSD was injected into 55 pregnant rats.

The mutagenic effects of LSD in Drosophila have been reported by Browning (1968). However, Grace et al. (1968) found no evidence of chromosome breakage or mutations.

D. Lysergic Acid Diethylamide - Effects on Meiotic Chromosomes

Damage to meiotic chromosomes of mice injected with LSD

has been reported. Skakkebaek et al. (1968) reported finding several breaks, gaps, and unidentifiable fragments in treated animals, but with few exceptions, not in the controls. Cohen and Mukherjee (1968) observed a ten fold increase in chromosome damage in spermatogonial cells and bone marrow cells of mice treated with LSD in comparison to the controls.

E. Fanconi's Anaemia

Fanconi's anaemia is a syndrome of multiple congenital malformations and progressive pancytopenia which is thought to be inherited as an autosomal recessive. Cytogenetically the condition is characterized by a variety of chromosomal anomalies consisting of chromatid and isochromatid gaps and breaks, fragments, and exchange configurations. (Schmid et al. 1965; Bloom et al. 1966).

Schmid et al. (1964) were the first to recognize the chromosomal damage in patients with Fanconi's anaemia.

Bloom et al. (1966) studied the peripheral leukocytes of 12 patients with this disorder. A variety of structural exchanges, endoreduplications and other chromosomal aberrations were observed in ten of the twelve patients. In the leukocyte cultures, 315 cells, (19.43%) of the 1,621 cells scored had a breakage event of one type or another. In bone marrow preparations from one of the patients, 18% of the cells examined possessed a breakage event. Seventy-eight chromatid exchanges were observed in the leukocyte cultures, and two were observed in the cells of the

bone marrow.

A large number of control cells (approximately 15,000) were studied to determine the frequency of aberrations in patients without this form of constitutional aplastic anaemia. Eight chromatid exchanges and only a few endoreduplications were observed.

Swift and Hirschhorn (1966) studied two patients with this disorder. In both cases the peripheral leukocytes and fibroblasts were studied, and in one case the bone marrow. In the leukocyte cultures of both cases, 49% of the cells were abnormal. It was also observed in the fibroblast cultures of case I that 41% of the cells were abnormal while in case II, 36% of the cells were aberrant. Chromatid and isochromatid gaps and breaks, fragments, exchange figures and dicentric chromosomes were observed in leukocyte and fibroblast cultures. Ten percent of the bone marrow cells contained abnormalities, including chromatid and isochromatid breaks and gaps, fragments and exchange figures but no ring or dicentric chromosomes.

To date, there has been no apparent success in determining the underlying causes responsible for the degenerative aplasia or the associated congenital malformations observed in this defect.

F. Effects of Mitomycin-C In Vivo and In Vitro

Wakaki et al. (1958) first isolated mitomycin-C as a distinct fraction of the mitomycin group of antibiotics. The drug was subsequently used for the treatment of neoplastic diseases, but because of its toxic effect on the bone marrow, and the subse-

quent thrombocytopenia and leukopenia, its use was discontinued (Watne, Moore, and Bedrettia, 1967; Jones, 1959).

Mertz (1961) was the first to observe the effect of this compound on the chromosomes. Using root tip chromosomes of Vicia faba, he observed chromatid aberrations when cells were exposed to a solution of 0.001% mitomycin-C for one hour. He also observed a marked inhibition of mitosis. Few division figures were evident 24 hours after treatment with the drug, but at 72 hours the frequency of chromatid aberrations was greatest. This high frequency of isochromatid aberrations was still evident 96 hours after exposure. The data indicated that most of the interphase stages were sensitive to the action of the mitomycin.

Mitomycin-C has been reported to be an effective breaking agent of human chromosomes in vitro by Nowell (1964), Cohen and Shaw (1964), and Shaw and Cohen (1965). By adding mitomycin-C to leukocytes in vitro at a concentration of 1 ug/ml for the first hour in culture, Nowell observed that the drug induced chromatid breaks as well as a large number of exchange configurations. Large and small acentric fragments were also found, but ring and dicentric chromosomes were uncommon. It was further observed that if cells were exposed to the drug for one hour at the 24th, 52nd, 62nd or 68th to 72nd hour, the number of aberrations decreased quite markedly. Treatment of cells with mitomycin-C for 24 hours or more completely inhibited subsequent mitotic activity.

Cohen and Shaw (1964) and Shaw and Cohen (1965) reported treating cells in culture with 0.1, and 1.0 ug/ml for the last 24 hours of culture. Again, large numbers of breaks and exchange figures were produced, notably at the secondary construction region of chromosomes #1, #9, and #16.

The most unusual effect of mitomycin-C was the large number of exchange figures that were produced. Nowell (1964), Cohen and Shaw (1964), and Shaw and Cohen (1965), all noted the structural rearrangements frequently involved members of apparent homologous pairs of chromosomes.

Experiments carried out on E. coli indicate that mitomycin-C causes selective inhibition of DNA (Shiba et al. 1959) while RNA and protein synthesis appear to be unaffected. (Sekiguchi and Takagi, 1960).

Iyer and Szbalski (1964) attributed the inhibition to cross linking of DNA which interfered with replication.

VI MATERIALS AND METHODS

A. Experimental Procedures

All experiments were carried out on cultured human leukocytes using a modification of the technique used by Moorhead et al. (1960), adapted for micro-amounts of whole blood. (For complete technique see Appendix A.)

In each of the experiments performed, 0.25 cc of whole blood was added to 5 ml. of culture medium. Replicate cultures were set up for each of the treated and control experiments, and these cultures were then incubated for approximately 72 hours at 37°C. One and a half to two hours before the cells were harvested, Colcemid was added at a concentration of 0.02 ug/ml of culture, to arrest cell division. At the end of the incubation period, cells were treated with a hypotonic solution and fixed in 3:1 absolute alcohol and glacial acetic acid. Flame dried slides were then prepared from each of the cultures.

1. Experiments Using Lysergic Acid Diethylamide (LSD)

Ten volunteers, five males and five females were used for in vitro experiments with LSD. Nine of the ten volunteers were students, and one was a professor. These individuals were interviewed and asked whether they had received x-rays of any kind for diagnosis or for treatment, and whether they had recent viral infections. The two patients who had received recent x-rays are identified in Table I.

Three cc's of whole blood were obtained from each individual, and 12 replicate cultures were established.

Lysergic acid diethylamide, (Delysid, LSD-25, Sandoz Ltd.) was obtained in sealed glass vials containing 0.1 mg LSD in 1.0 ml of aqueous solution. The solution was diluted with sterile distilled water to a concentration of 20 ug/ml, and 24 hours prior to the termination of the cultures, 0.25 cc (5 ug) was added to six of the replicate cultures yielding a final concentration of 1 ug/ml. An equal volume of sterile distilled water (0.25 cc) was added to each of the remaining six cultures.

2. Treatment of Leukocytes from a Patient with Fanconi's Anaemia with Growth Hormone

Blood was obtained from a patient immediately before treatment with growth hormone, and eight replicate cultures (0.25 cc whole blood/5 cc of medium) were established.

Twenty four hours after treatment with 250 ug. of growth hormone, another sample of blood was taken and set up according to the procedure outlined above.

A sample from a normal boy, incubated in the same media at the same time, was used as a control primarily to ensure that environmental factors did not contribute to the breakage frequency in cultures from the patient.

3. Experiments Using Mitomycin-C

A 3 cc sample of whole blood was obtained from a single individual, and 0.25 cc of whole blood was added to each of twelve replicate cultures.

Mitomycin-C obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio was dissolved in sterile distilled water to a concentration of 20 ug/ml. At the beginning of the incubation period, 0.25 cc (5 ug.) of the solution was added to six of the replicate cultures to yield a final concentration of 1 ug/ml. An equal volume of sterile distilled water (0.25 cc) was added to each of the remaining six replicate cultures.

After one hour of incubation cells in all cultures were washed twice in culture medium, resuspended in fresh media, and replaced in the incubator for the remainder of the 72 hour culture period.

4. Blinding and Coding of Slides

In each study (LSD, Mitomycin-C and Fanconi's anaemia,) the ten best slides from each of the control and treated samples were selected and coded to ensure the examiner could not identify the source.

5. Microscopy

The slides were examined and photographed on a Zeiss automatic photomicroscope equipped with phase contrast condenser and objective lenses. Good, well spread metaphase figures were selected under low power (x16 objective) and once a cell was selected it was included in the study. Wherever possible, 10 cells on each of 10 slides were selected to yield a total of 100 cells for each control or treatment.

Once a cell was selected it was analyzed under oil immersion (x100) for structural aberrations. The cells were then photographed

on high contrast, fine grain film (Adox 135, KB-14), and reanalyzed.

B. Criteria Used to Define Chromosome Aberrations

All cells were analyzed for chromosome number and structural aberrations, including gaps, breaks, acentric fragments, deletions, dicentrics, marker chromosomes and exchange configurations.

A break was defined as a discontinuity in an arm in which the acentric fragment was displaced from its alignment with the proximal or centric part of the arm. Achromatic segments in which the distal fragment remained in alignment with the proximal arm were scored as gaps, and were not considered to be breakage events.

Configurations that involved more than one chromosome were presumed to result from chromatid interchanges. Throughout this study, these configurations will be referred to as 'exchanges'.

Breaks, acentric fragments and deletions were scored as single break events, while dicentric chromosomes and exchanges were scored as two break events.

C. Determination of the Distribution of Breakage Events Among the Groups of Chromosomes

A measure of whether or not the breakage events were randomly distributed throughout the chromosome complement was obtained by comparing the distribution of observed aberrations with the number expected on the basis of the relative length of the chromosomes in each group. The relative lengths of the chromosomes used are those established at the Denver and London Conferences (Chicago Conference, 1966).

VII RESULTS

A. Lysergic Acid Diethylamide - In Vitro Study

The results of chromosome analysis for treated and control cultures from each of the ten subjects are presented in Appendix B. These results are summarized in Table I.

A total of 1010 control cells and 1094 cells exposed to LSD were analyzed. An average of 3.91% of the cells in the control cultures contained aberrations whereas 7.89% of the cells in the treated cultures were similarly affected. This indicates a significant increase in the total number of cells demonstrating a breakage event. (X^2 test. $p < 0.001$)

A comparison of the total number of breaks in the treated and control cultures indicated a significant increase in the treated cultures. The number of breaks per 100 cells observed in the control cultures ranged from 0 to 15.12 with a mean of 4.72. In the cultures treated with LSD, the number of breaks per 100 cells ranged from 4.0 to 18.70 with a mean of 9.37.

Aneuploid levels in both the treated and control cultures were similar, with 90.03% of the treated cells and 89.23% of the control cells having a modal number of forty-six chromosomes.

As control values vary considerably from individual to individual, a more accurate measure of damage associated with LSD was obtained by analysis of deviations between the treated and control sample for each individual. The deviations and paired t-test values for single break events, two break events, total break events and gaps are presented in Table II.

TABLE I

Summary of the Results of Chromosome Analysis in Treated and Control Cultures

	Sex	Total No. Cells		Total No. Gaps per 100 Cells		Total No. Cells with Abberations		Breaks per 100 Cells		
		C	T	C	T	C	T	C	T	
J.A.	m	86	123	12.79	19.51	11.63	15.45	15.12	18.70	*
K.A.	f	120	123	22.50	39.02	2.50	7.32	4.17	8.94	
P.H.	f	104	136	21.15	21.32	1.92	5.15	1.92	5.15	
E.M.	f	100	122	12.00	18.03	6.00	9.02	6.00	13.93	
G.R.	m	100	100	9.00	27.00	4.00	10.00	4.00	10.00	*
S.S.	m	100	100	29.00	32.00	00.00	4.00	00.00	4.00	
G.T.	f	100	100	13.00	23.00	3.00	7.66	5.00	9.00	
J.C.	f	100	90	23.00	21.11	3.00	7.00	4.00	10.00	
W.G.	m	100	100	25.00	32.00	3.00	6.00	3.00	6.00	
T.B.	m	100	100	17.00	22.00	4.00	8.00	4.00	8.00	
TOTAL	10	1010	1094	184.44	254.99	39.05	79.60	47.21	93.72	

* Received chest or dental x-rays within six months of testing.

TABLE II

Deviations Between Treated and Control Cultures

	Gaps	Single B. E.	Two Break Events	Total Breaks
1.	+ 6.72	+ 2.06	+ 1.62	+ 3.58
2.	+ 16.52	+ 3.15	+ 1.62	+ 4.67
3.	+ 0.17	+ 3.23	00.00	+ 3.23
4.	+ 6.08	+ 4.65	+ 3.29	+ 7.93
5.	+ 18.00	+ 6.00	00.00	+ 6.00
6.	+ 3.00	+ 4.00	00.00	+ 4.00
7.	+ 10.00	+ 6.00	- 2.00	+ 4.00
8.	- 1.89	+ 3.55	+ 2.44	+ 6.00
9.	+ 7.00	+ 3.00	00.00	+ 3.00
10.	+ 5.00	+ 4.00	00.00	+ 4.00
Mean difference	+ 7.00	+ 3.96	+ 0.697	+ 4.64
T value	3.49	9.86	1.44	9.51
Prob.	<0.01->0.001	<0.001	<0.2->0.1	<0.001

In each of the ten cultures treated with LSD, there was an increase in the number of single break events and the total number of break events, with the amount of deviation ranging from 2.06 to 6.00 breaks per 100 cells for single break events, and from 3.00 to 7.93 breaks per 100 cells for total break events. The number of two break events was increased in four individuals, decreased in one, and no difference was observed in five. (Table II)

A paired t-test analysis indicated a significant increase in single break events and total break events, but not in two break events.

An analysis of specific types of aberrations is presented in Table III, and representative figures are presented in Fig. 1.

1. Single Break Events

Single break events comprised the greatest number of aberrations in both the treated and control cultures, 88.46% of the total number of break events in the treated and 91.30% of the total number of breaks in the control being of this type.

Chromatid breaks (Fig. 1-D, 1-E, and 1-F) were the most common of the single break events scored. In the treated cultures, 64.33% of the total number of break events were of this type, whereas 54.35% of the total number of break events in the controls were chromatid breaks. However, 6.12 chromatid breaks per 100 cells were observed in the treated cultures, to 2.47 per 100 cells in the control.

TABLE III

Frequency of Single and Two Break Events
Observed in Treated and Control Cultures

		SINGLE BREAK EVENTS					TWO BREAK EVENTS						
	Total No. of Cells	Total chromatid breaks	Acentrics	Deletions	No. of Aberrations	No. of B. E.	Rings	Dicentrics	Exchanges	No. of Aberrations	No. of B. E.	TOTAL NO. OF ABERRATIONS	TOTAL NO. OF B. E.
T.	1094	67 6.12 /100	24 2.19 /100	1 0.09 /100	92	92	0	2	4	6	12	98	104
C.	1010	25 2.47 /100	16 1.58 /100	1 0.099 /100	42	42	0	0	2	2	4	44	46

X^2 single break = 61.47
$p < 0.01$

FIGURE 1.

Representative Types of Chromosomal Aberrations Observed in Control Cultures and in Cultures Exposed to Lysergic Acid Diethylamide

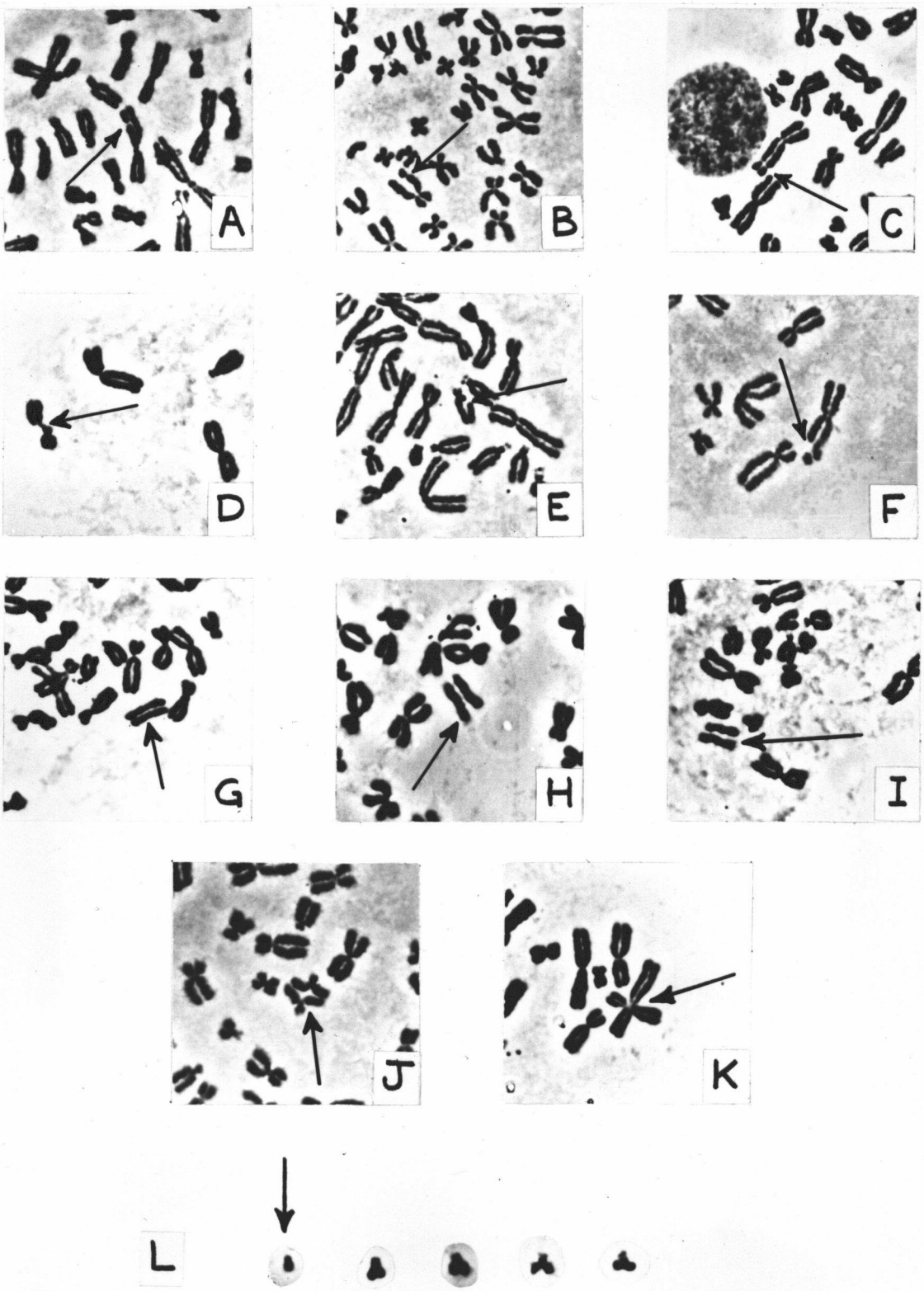
A and B - chromatid gaps
C - isochromatid gap

D, E, and F - chromatid breaks

G, H, and I - acentric fragments

J - exchange figure from treated culture
K - exchange figure from control culture

L - deleted G group chromosome


A
B
C
D
E
F
G
H
I
J
K
L
10μ

Acentric fragments (Fig. 1-G, 1-H, 1-I) were observed in both treated and control cultures. The frequency of this type of aberration was increased from 1.58 per 100 cells in the control to 2.19 acentric fragments per 100 cells in the treated cultures.

Only two deletions that could be definitely identified were observed. One was found in the treated cultures and one in the control. These deletions were observed as centric fragments, and were scored as single break events (Fig. 1-L).

2. Two Break Events

Two break events were rarely observed, and there was no significant increase between the treated cultures and controls. Six were observed in the treated cultures and 2 in the controls. The two observed in the control cultures, and four of the six observed in the treated cultures were exchange figures (Fig. 1-J and 1-K). Two dicentric chromosomes were also observed in the treated cultures.

In the ten replicate cultures two break events were observed in both treated and control cultures from one individual, and in three individuals they were observed only in the treated replicates.

3. Gaps

The difference in the frequency of gaps between the treated and control cultures (Table II) varied from -1.89 to +18.00, with a mean difference of 7.05 and a standard deviation of 6.39. This represents a significant difference (t-test. $p < 0.01 - > 0.001$) in the gaps in the treated and control cultures.

TABLE IV

Distribution of Identifiable Breaks Among the Chromosome Groups

LSD TREATED MALES

	A	B	C	D	E	F	G
OBSERVED	13	3	8	8	3	1	2
EXPECTED	8.99	4.64	13.74	3.86	3.34	1.77	1.64

X^2 total = 9.60 $p < 0.25 - > 0.10$ Not sig. at 0.05 level

LSD TREATED FEMALES

	A	B	C	D	E	F	G
OBSERVED	8	7	14	4	1	0	1
EXPECTED	8.19	4.19	13.99	3.49	3.02	1.60	1.16

X^2 total = 5.16 $p < 0.75 - > 0.50$ Not sig. at 0.05 level

LSD CONTROL MALES

	A	B	C	D	E	F	G
OBSERVED	4	5	4	1	1	0	0
EXPECTED	3.55	1.83	5.43	1.52	1.32	0.70	0.65

X^2 total = 7.51 $p < 0.5 - > 0.25$ Not sig. at 0.05 level

LSD CONTROL FEMALES

	A	B	C	D	E	F	G
OBSERVED	6	1	6	1	0	0	0
EXPECTED	3.25	1.68	5.36	1.40	1.21	0.64	0.46

X^2 total = 5.24 $p < 0.75 - > 0.50$ Not sig. at 0.05 level

4. Distribution of Breakage Among Chromosome Groups

The distribution of observed break events among the seven groups of chromosomes for treated and control males and females are presented in Table IV. Chi-square analysis indicated no significant deviation from random.

B. Effects of Growth Hormone on a Patient with Fanconi's Anaemia

This study was carried out on a patient with Fanconi's anaemia who had been assessed annually at the Health Centre for Children, and who had not required any treatment other than the administration of growth hormone. Previous chromosome studies indicated a high frequency of chromosome aberrations (Corey and Andrews, 1968).

In this study, blood samples were obtained immediately before, and twenty four hours after the administration of growth hormone.

A normal boy of approximately the same age was used as a 'standard'.

The detailed results of this study are presented in Appendix C. These results are summarized in Table V.

When the number of aberrant cells were compared in the cultures before and after treatment with growth hormone, there was a significant increase both before (25.49%) and after treatment (28.03%) over the standard (10.98%), but no significant difference was observed between the two samples taken from the patient with Fanconi's anaemia.

Although the total number of breakage events per 100 cells was increased in both samples from the patient, there was a decrease after the treatment with growth hormone. In the cells observed prior to the treatment with the hormone, 46.07 breaks per 100 cells were observed, whereas in the sample after treatment, 35.51 breaks per 100 cells were observed.

TABLE V

Summary of Results of Chromosome Analysis Before and After Treatment with Growth Hormone, and in a 'Standard'

	Total No. Cells	Total No. Gaps	Total No. Breaks	Total No. Cells with Aberrations	Br. freq. per aberr. Cell
B.G.H.	102	58 56.86/100	47 46.07/100	26 25.49/100	1.80
A.G.H.	107	46 45.10/100	38 35.51/100	30 28.03/100	1.26
'Standard'	91	21 23.07/100	11 12.08/100	10 10.98/100	1.10

This is reflected in the number of break events per aberrant cell. (The ratio of breakage events to the number of aberrant cells.) In the cultures examined prior to growth hormone treatment, 1.80 breaks per aberrant cell were observed, whereas a breakage frequency of 1.26 breaks per aberrant cell was observed after treatment, suggesting that the growth hormone may have an effect on the number of aberrations found in each of the aberrant cells.

It was further observed before treatment with growth hormone that individual cells demonstrated up to and including four breaks per cell, but after treatment with growth hormone, no more than two breaks per cell were observed. The distribution of 0, 1, 2, 3, and 4 breaks per cell in the cultures before treatment did not fit a poisson distribution of rare events (X^2 test, $p < 0.005$). However, after treatment, the distribution of cells with up to two breaks per cell did not deviate from random (X^2 test. $p < 0.25- > 0.10$).

A comparison of the different types of aberrations observed before and after treatment with growth hormone is presented in Table VI and representative figures are presented in Fig. 2. There was a significant difference in the distribution of single break events (X^2 test. $p < 0.005$) in the two samples from the patient. The number of single break events and two break events was elevated in both the samples from the patient in comparison to the 'standard'.

TABLE VI

Frequency of Single and Two Break Events Observed in Cultures Before and After Treatment with Growth Hormone, and in a Standard

		SINGLE BREAK EVENTS					TWO BREAK EVENTS						
	Total No. Cells	Total No. Chromatid Breaks	Acentrics	Deletions	No. of Aberrations	No. of B.E.	Rings	Dicentrics	Exchanges	No. of Aberrations	No. of B.E.	TOTAL NO. OF ABERR.	TOTAL NO. OF B.E.
BGH	102	16 15.68 /100	17 16.66 /100	2 1.96 /100	35	35	0	0	6 5.88 /100	6	12	41	47
AGH	107	29 27.10 /100	3 2.80 /100	2 1.86 /100	34	34	0	0	2 1.86 /100	2	4	36	38
'S'	91	7 7.69 /100	3 3.29 /100	1 1.09 /100	11	11	0	0	0	0	0	11	11

X^2 single = 21.44. $p < 0.005$

X^2 break events = 6.06
$p < 0.025 -> 0.010$

FIGURE 2.

Representative Types of Chromosomal Aberrations Observed Before and After Treatment with Growth Hormone in a Patient with Fanconi's Anaemia

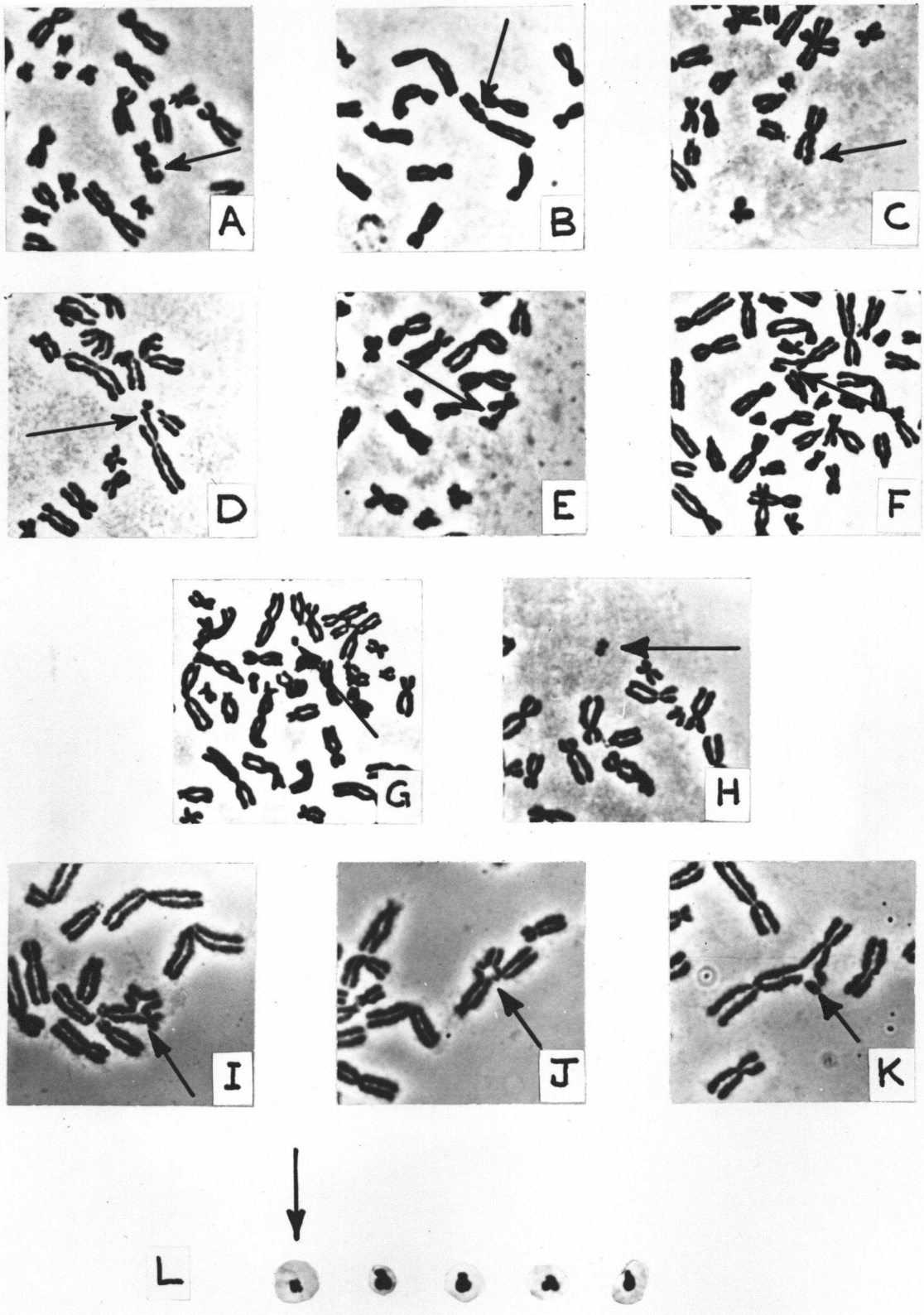
A, B, and C - chromatid gaps

D, E, and F - chromatid breaks

G and H - acentric fragments

I, J, and K - non-homologous exchange figures

L - deleted G group chromosome


A
B
C
D
E
F
G
H
I
J
K
L
10μ

1. Single Break Events

Although the total number of single break events was similar in both cultures from the patient, the distribution of types of aberrations was significantly different due to the change in the relative frequencies of chromatid breaks and acentric fragments. Before treatment, breaks and acentric fragments occurred with approximately equal frequencies, whereas after treatment, chromatid breaks occurred with a much higher frequency than acentric fragments.

Deletions were rare and occurred with equal frequency in both cultures.

2. Two Break Events

Exchange configurations were the only two break events observed in the cells cultured before or after treatment with growth hormone. The chromosomes involved in each of the exchange configurations are presented in Table VII. No two break events were observed in the 'standard'.

Six exchange figures were observed in the cells cultured before hormone treatment while only two exchanges were observed in the cultures after administration of the hormone.

The number of break equivalents resulting from two break events was decreased after treatment with the growth hormone.

3. Gaps

Prior to growth hormone treatment, 56.86 gaps per 100 cells

TABLE VII

Identification of Chromosomes Involved in Exchange Figures

BGH	AGH	'Standard'
1xC	CxG	None
3xB	1xC	
2xB		
1xB		

2 not identifiable

were observed, while 45.10 gaps per 100 cells were observed after treatment. The frequency of gaps was decreased after the treatment with the hormone but both the cultures from the patient with Fanconi's anaemia demonstrated a higher frequency of gaps in comparison to the 21 gaps observed in the cells from the 'standard'.

4. Distribution of Breaks Among Chromosome Groups

In the cells observed prior to, and after treatment with growth hormone, the expected distribution of identifiable breaks based on the relative lengths of the chromosome groups did not deviate from random. (Table VIII)

TABLE VIII

Distribution of Identifiable Breaks Before and After Treatment with Growth Hormone

	A	B	C	D	E	F	G
B.G.H.	11	5	7	1	0	0	0
	5.68	2.93	8.69	2.44	2.11	1.12	1.04

X^2 total = 11.87 $p < 0.10 - > 0.05$ Not sig. at 0.05 level

	A	B	C	D	E	F	G
A.G.H.	11	4	12	4	1	0	1
	7.81	4.03	11.94	3.35	2.90	1.54	1.43

X^2 total = 4.33 $p < 0.75 - > 0.50$ Not sig. at 0.05 level

C. Mitomycin-C

An increase in the number and types of chromosomal aberrations was observed after exposure of human leukocytes in vitro for one hour to a low concentration of mitomycin-C (1 ug/ml). The detailed results of this study are found in Appendix D and are summarized in Table IX. Representative figures are presented in Fig. 2.

In 64 of the 100 cells exposed to this drug, 181 breakage events were observed, whereas only 5 breakage events were observed in 3 of the 100 control cells examined.

A detailed analysis of the types of aberration in the treated and control cultures is presented in Table X. Of the 181 breakage events observed after treatment, 85 were single break events and 96 were two break events. The 5 breakage events observed in the control were all single break events.

Aneuploid levels differed markedly with 94% of the control cells and 61% of the treated cells having 46 chromosomes.

1. Single Break Events

Chromatid breaks were the most common single breakage events found in the treated cultures; 65 aberrations or 35.91% of the total number of break events being of this type.

In the treated sample, 18 acentric fragments were observed. Five were found in the control, and this represented the total number of breakage events.

TABLE IX

Summary of the Results of Chromosome Analysis in Treated and Control Cultures

	Total No. Cells	Total No. Cells with Breaks	Total No. Gaps	Total Breaks
TREATED	100	64	41	181
CONTROL	100	3	17	5

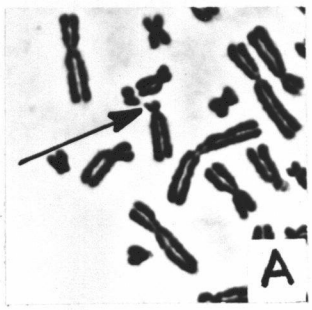
FIGURE 3.

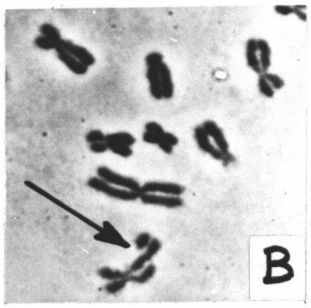
Representative Types of Chromosomal Aberrations Observed in Cultures Exposed to Mitomycin-C.

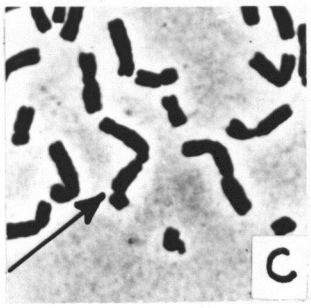
A, B, and C - chromatid breaks

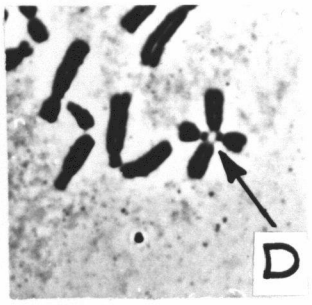
D, E, F. G, H, and I - homologous exchange figures

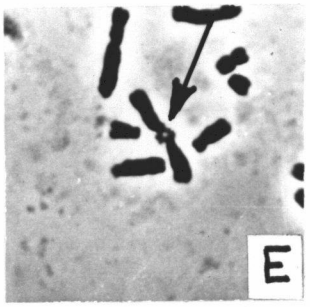
I, J, K, L, M, and N - non-homologous exchange figures

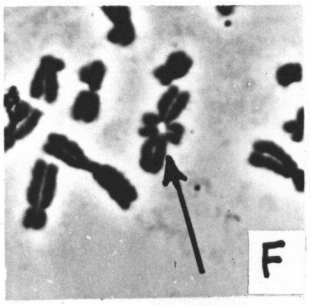


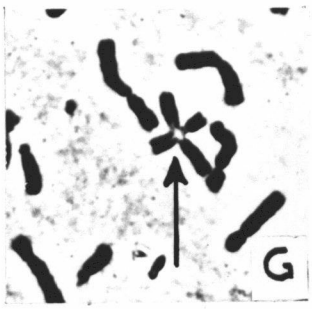


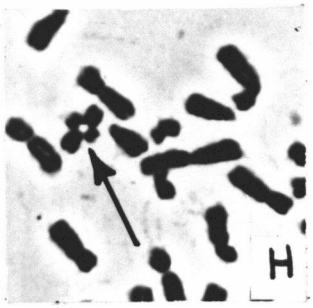


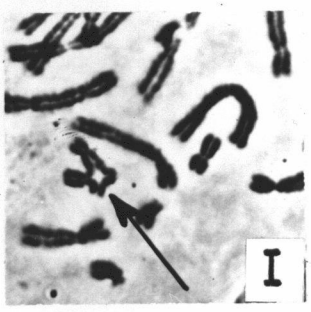


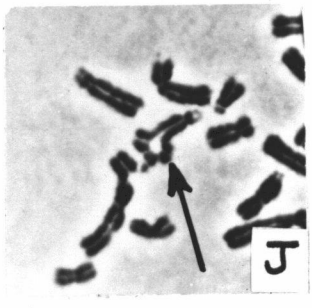


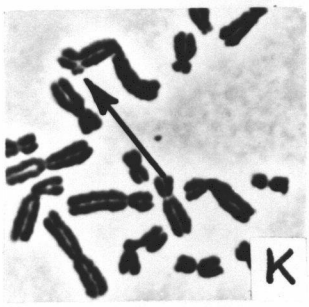


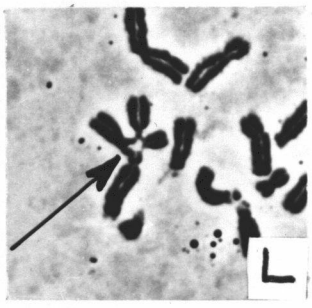


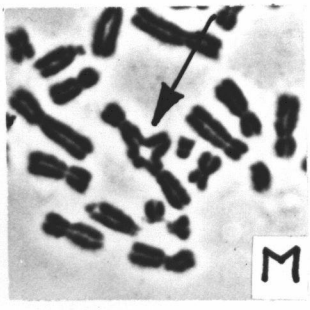


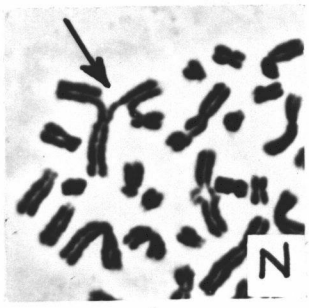












10μ

TABLE X

Frequency of Single and Two Break Events in Treated and Control Cultures

	SINGLE BREAK EVENTS						TWO BREAK EVENTS						
	Total No. Cells	Total Chromatid Breaks	Ac.	Del.	No. of Aberr.	No. of B. E.	Rings.	Dic.	Exch.	No. of Aberr.	No. of B.E.	TOTAL NO. OF ABERR.	TOTAL NO. OF B.E.
TREATED	100	65	18	2	85	85	0	0	48	48	96	133	181
CONTROL	100	0	5	0	5	5	0	0	0	0	0	5	5

Two deletions that could be definitely identified were found in the treated cultures; none being observed in the controls.

2. Two Break Events

The most striking observation in cultures treated with mitomycin-C was the high frequency of exchange figures. Forty-seven exchange configurations were observed in the treated cultures; none were observed in the controls.

The 47 exchange figures represented 96 break events or 53.04% of the total number of breakage events observed. The exchange figures are listed in Table XI. Twenty-seven or 57.44% of the exchange figures were between apparent homologous chromosomes (Fig. 3., 3D-H.) while 20 or 42.56% were between chromosomes of different groups (Fig. 3., 3-H to 3-N). One unusual exchange figure appeared to involve 4 chromosomes and was scored as a 4 break event. The chromosomes involved in this particular exchange were tentatively identified as CxCxDxG.

Chromosomes of the C group were most frequently involved in the exchange figures, 59.57% of the exchanges involving one or more chromosomes from this group. Chromosomes from the G group were rarely involved in exchanges, only 6.38% involving chromosomes from this group.

3. Gaps

Gaps were more frequent than breaks in the control cultures and less frequent than breaks in the treated cultures.

TABLE XI

Identification of Chromosomes Involved in Exchange Figures in Treated Cultures

A. Exchanges Involving Apparent Homologous Chromosomes

3 (1x1) 1 (BxB) 18 (CxC) 2 (DxD)

2 (ExE) 1 (FxF)

SUBTOTAL = 27

B. Exchanges Involving Members of Different Chromosome Pairs

2 (1xB), 1 (3xB), 1 (FxB), 4 (1xC), 1 (BxC),

2 (ExC), 1 (DxC), 1 (FxC), 1 (1xF), 1 (FxE),

1 (1xG), 1 (GxE), 1 (DxE), 1 (DxG), 1 (CxCxDxG)

SUBTOTAL = 20

TOTAL NUMBER OF EXCHANGES = 47

The increase from 17 gaps in the control to 41 in the treated cultures indicated that the drug significantly increased the frequency of this type of event.

4. Distribution of Break Events Among Chromosome Groups

The distribution of identifiable breaks among the seven groups of chromosomes did not deviate from random (Table XII) indicating that the drug did not preferentially cause an excess of breaks in any particular group of chromosomes.

TABLE XII

Distribution of Identifiable Breaks Among Chromosome Groups

	A	B	C	D	E	F	G
OBSERVED	32	16	66	13	19	11	6
EXPECTED	38.58	19.88	58.96	16.54	14.32	7.60	7.04
X^2_{sub}	1.12	0.75	0.84	0.75	1.52	1.53	0.15

$X^2_{TOTAL} = 6.66$ $P_{0.05}$ df = 6 = 12.6

$p < 0.50 - > 0.25$

Not significant at 0.05 level

VIII DISCUSSION

A. Lysergic Acid Diethylamide

Cohen, Marinello, and Black (1967), and Cohen, Hirschhorn and Frosch (1967), observed that LSD at concentrations from 0.001 to 10 ug/ml of culture, with exposure times from 4 to 48 hours prior to the harvest of the cells, increased the frequency of chromosome aberrations. The highest concentration of 10 ug/ml brought about greater damage in a shorter exposure time. The same effect was observed at a concentration of 1 ug/ml with an exposure time of 24 hours. However, at a concentration of 0.001 ug/ml, more chromosome damage was observed at longer exposure times, while an exposure of four hours at the same concentration produced few breaks. Among the treated cultures, the lowest breakage frequency (7.7%) was almost twice the control value of 3.9%, and the breakage frequency in treated cultures ranged to over four times the control values (17.5%).

The manner in which the results are presented by Cohen et al. (1967), yields no information concerning possible variation in response. Since the same authors found that not all indivuals had an increase in breakage after ingesting LSD, information concerning possible variability in individual response in vitro would appear to be valuable.

In the present study, one of the in vitro experiments reported by Cohen, Marinello, and Black (1967) was repeated but with an experimental design which permitted direct comparison

of samples from the same individual, cultured with and without LSD. The effects of the drug were measured by the difference between the samples rather than using the breakage frequency, and the variation in difference was used as a measure of variation in response.

The concentration of 1 ug/ml for 24 hours was selected because previous experiments (Cohen, Marinello, and Black, 1967) indicated increased breakage values, and yet the dosage was consistent with survival of sufficient cells for analysis. The culture technique and scoring system used were similar to those used by Cohen, Marinello and Black (1967).

The average frequency of breakage in the control cultures, and the range of breakage in the treated cultures reported here are very similar to those reported by Cohen, Marinello, and Black (1967), and Cohen, Hirschhorn, and Frosch (1967). In the present study it was observed that the breakage frequency in treated cultures ranged from 4.0 to 18.70 with a mean of 9.57. It was further observed that although there was a wide range of values after treatment, there was an equally wide range before treatment, but the differences ranged only from 3.00 to 7.93 with a mean of 4.64, indicating very little variation in response from individual to individual.

While it is true that the breakage frequency in treated cultures increased 1.24 to 4 times, this is relative to the breakage frequency in the control cultures and is not a reflection of variation in the amount of damage. In fact, the 1.24 times increase represented a change from 15.12 to 18.70, and

the 4 times increase represented an increase of from 0 to 4 breaks. Thus these two extremes represent increases of 3.58 and 4 breaks respectively.

In the study presented here, the overall results were similar to those in other published results. However, some minor differences in the types of abnormalities were observed. Cohen, Hirschhorn, and Frosh (1967) observed a 'high frequency of small acentric fragments' whereas in this study, the number of acentric fragments was not significantly increased in the treated cultures when compared to the controls. This may be due to the fact that Cohen and his associates included terminal breaks with acentric fragments.

Cohen and his coworkers reported finding two break events only in cultures treated with LSD. However, in the present study, two break events were observed in both treated and control cultures and were not significantly increased in the treated replicates. Although two break events are very rare, they have been observed in normal individuals (Bloom et al. 1966).

As they were observed in low frequency accompanied by an increase in single break events by Cohen et al. and in the present study, it seems unlikely that LSD specifically increases this type of aberration.

B. Fanconi's Anaemia

Reports of high frequencies of chromosomal aberrations are sufficiently consistent to be considered part of the syndrome

known as Fanconi's anaemia (Swift et al. 1966; Bloom et al. 1966; Varela and Sternberg, 1967). Cell death due to chromosome breakage has been suggested as a possible cause of the progressive pancytopenia (Bloom et al. 1966).

At the present time, patients with this disease are treated with steroid hormones such as testosterone and cortisone, as this form of treatment appears to retard the progressive bone marrow aplasia (Shahadi and Diamond, 1959). However, the literature contains no information of the effects of treatment on the frequency of chromosomal breakage.

The availability of a patient in whom the anticipated bone marrow aplasia had not progressed sufficiently to warrant treatment, provided an opportunity to observe the frequency and type of aberrations both before onset of the bone marrow aplasia and before treatment, and to further study the possible effects of treatment on the frequency and type of aberrations.

A previous study had indicated that a high frequency of breakage was evident in this patient two years prior to the present study (Corey and Andrews, 1968). It was observed at that time that 34% of the cells demonstrated a breakage event; in the present study, 25.5% of the cells before, and 28.04% after treatment with the hormone were similarly affected. Between the study conducted on this patient two years ago, and the present one, the patient had been given a course of growth hormone treatment.

In the initial study, and in the present one, the frequencies and types of aberrations were similar to those reported elsewhere. Bloom et al. (1966) reported that in 1,621 leukocytes from patients with Fanconi's anaemia, 16.8% of the cells demonstrated a breakage event. The most frequent aberrations were chromatid and isochromatid breaks, acentric fragments and exchange configurations. In the present study, the number of chromatid breaks observed was increased from 16 in the untreated cultures to 29 after treatment with the growth hormone; the number of acentric fragments was decreased from 17 in untreated cultures to 3 after exposure to growth hormone. Bloom et al. (1966) also observed 78 exchange figures in the 1,621 cells scored yielding a frequency of 0.048 for this type of event. The frequency of exchanges in the patient studied here prior to growth hormone treatment is consistent with the results of Bloom et al., the frequency of these two break events being 0.059; after treatment, the frequency of exchanges was 0.019.

Although the number of cells with aberrations was unchanged in samples obtained 24 hours after growth hormone treatment in comparison to the samples taken before, changes in the frequency and types of aberrations were observed in that there was a reduction of (1) the number of breaks per aberrant cell, (2) the number of acentric fragments, and (3) the number of exchange figures.

Due to the limited nature of the study, it is impossible to assess the significance of the results, but the suggested reduction in the amount of damage per aberrant cell and the reduction in two break events encourages further investigation of growth hormone as a treatment. This is further emphasized by the fact that treatment with testosterone and cortisone has undesirable side effects such as masculinization of the patient and an inhibition of the defence mechanisms of the body. Growth hormone, however, may have the desired effect of stimulating the growth of these dwarfed patients, and if it did decrease the chromosome damage and retard somewhat the progression of the disease, it would be a more desirable treatment.

C. Mitomycin-C

The experiment with mitomycin-C was undertaken as a pilot project to develop an induced chromosome breakage system which could, in future, be used to assess the effects of steroids and other hormone used to treat patients with Fanconi's anaemia. Mitomycin-C was used because it not only has been reported to cause a tremendous increase of breakage events *in vitro* (Nowell, 1964; Cohen and Shaw, 1964; Shaw and Cohen, 1965), but the aberrations such as chromatid breaks, acentric fragments and exchange figures are similar to those found in Fanconi's anaemia.

Although mitomycin-C has been reported to be effective at a variety of times and concentrations (Cohen and Shaw, 1964), the one hour treatment of 1 ug/ml of mitomycin-C at the beginning of the culture period reported by Nowell (1964)

resulted in the greatest number of chromosomal aberrations with the highest mitotic index.

The treatment method of exposing leukocytes in vitro to 1 ug/ml of mitomycin-C during the first hour of culture has several advantages for an in vitro model system in that (1) it is a breakage system where the breaking agent can be removed from the culture system before treatments such as growth hormone in the experiment with Fanconi's anaemia are added, (2) it is a system in which the cells exposed to the drug go through more than one cell division, so that an attempt can be made to measure the survival of cells, and (3) the drug increases the variety as well as the frequency of chromosome and chromatid aberrations.

Many of the chromosome aberrations observed with treatment with mitomycin-C were unusual. Apart from chromatid and isochromatid breaks, and acentric fragments, a large number of exchange configurations were observed. Cohen and Shaw (1964) observed 76 exchanges in 114 cells yielding a mean number of exchanges per cell of 0.67. In the present study, the mean number of exchanges per cell in treated cells was observed to be 0.47. None were observed in the control cultures.

Different types of exchange configurations may have different consequences in subsequent cell divisions. Therefore, examination of chromosome aberrations after treatment with mitomycin-C can provide a measure of cell survival by comparison of the frequencies and types of exchange configurations with the

types and frequencies of aberration after the cells have proceeded through several divisions.

Exchanges between chromosomes of different groups, or situations in which chromosomes of the same groups have undergone unequal exchange to yield asymmetrical figures were considered as non-homologous exchange configurations. In contrast to Fanconi's anaemia where all of the exchanges observed were non-homologous, mitomycin-C treatment resulted in both homologous and non-homologous exchanges. Shaw and Cohen (1965) observed 46.36% of the exchanges in mitomycin-C treated cultures to be homologous exchanges; in the present study, 57.44% were of this type.

Homologous exchanges would not produce detectable karyotype changes in subsequent cell divisions. Non-homologous exchanges, like those found in Fanconi's anaemia and in cells treated with mitomycin-C should have resulted in balanced translocations, duplications and deficiencies, and marker chromosomes, as well as acentric fragments and dicentric chromosomes. No dicentric chromosomes or marker chromosomes were observed in mitomycin treated cultures, but acentric fragments and deletions were found. Acentric fragments and deletions could also have been produced by single break events rather than being the end results of non-homologous exchange configurations. Nowell (1964) reported that 'dicentric and ring chromosomes were extremely rare', and Cohen and Shaw (1964) and Shaw and Cohen (1965) make no mention of finding dicentric chromosomes. However as they treated cells late in the culture period,

presumably in the last cell division, no two break events would have been expected.

The absence of marker and dicentric chromosomes suggests that cells with non-homologous exchanges cannot proceed through another cell division, or, if the division is complete chromosome imbalances in the daughter cells may be responsible for the death of the cells. This suggestion is tenative in that the cultures were terminated at 72 hours and there is no indication whether or not these aberrations occur in the first division after treatment.

IX SUMMARY

A. Lysergic Acid Diethylamide

The frequency and types of chromosomal aberrations were observed in replicate cultures from each of ten individuals cultured with and without the addition of 1 ug/ml of lysergic acid diethylamide during the last 24 hours of the culture period. Approximately 100 cells from each of the replicates were analyzed and the difference between untreated and treated replicates was used as a measure of the effects of the treatment.

The frequency of breaks in untreated replicates ranged from 0 to 15.12 breaks per 100 cells, with a mean of 4.72. In the treated replicates, the frequency of breaks per 100 cells ranged from 4.00 to 18.70 with a mean of 9.37.

The difference between untreated and treated cultures ranged from +3.00 to +7.93 with a mean of +4.65.

A paired t-test analysis indicated a significant increase in both total break events and single break events, but not in aberrations due to two break events.

The chromosome breaks were randomly distributed among the seven groups of chromosomes of the complement.

B. Fanconi's Anaemia

The frequency and types of chromosome aberrations were observed in a patient with Fanconi's anaemia immediately before and 24 hours after administration of 250 ug. of growth hormone,

and in a control. Approximately 100 cells from each sample were examined.

Forty-seven breaks in 102 cells were observed in untreated cultures, 38 breaks in 107 cells being observed after exposure to the hormone. Eleven breaks in 91 cells were observed in cells of the 'standard'.

There was no significant difference in the number of aberrant cells in the cultures from the patient, but the number of breaks per aberrant cell were decreased from 1.80 in the cultures before treatment to 1.26 in the cells after exposure to the hormone.

Single break events were the most common aberrations in both samples from the patient, but a significant difference in the distribution of single break events between the two cultures was observed.

Exchange configurations were the only two break events observed in both samples with the number of break events being decreased from 12 to 4 after treatment. No two break events were observed in the cells from the 'standard'.

Chromosome breaks were randomly distributed among the seven groups of chromosomes in all samples.

C. Mitomycin-C

An increase in the frequency and types of chromosomal aberrations was observed after exposure of human leukocytes to a low concentration of mitomycin-C for one hour at the

beginning of the culture period when compared to the untreated cultures.

One hundred and eighty one breaks in 64 of the 100 cells exposed to the drug were observed. Only 5 breaks were observed in 3 of the 100 control cells examined.

Single break events represented 46.96% of the total number of break events observed in the treated cultures. The five break events observed in the control cultures were all single break events.

Exchange figures constituted all of the two break events in the treated cultures; 27 homologous and 20 non-homologous exchanges being observed. No two break events were observed in the control cultures.

The chromosome breaks were randomly distributed among the seven groups of chromosomes of the complement.

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APPENDIX A.

LEUKOCYTE CULTURE TECHNIQUE

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1. Cultures were grown in sterile roller tubes containing 5 cc of GIBCO Chromosome Medium 1A. 0.25 cc of heparinized venous blood was added to each tube, and the cultures were then incubated at 37°C for approximately 72 hours.

2. Colcemid in a final concentration of 0.02 ug/ml was added to the cultures approximately two hours prior to the harvest of the cells.

3. At the end of the incubation period, the cells were centrifuged at 800 rpm for 8 minutes. The supernatant was removed and discarded, and the cells were suspended in 5 cc of hypotonic solution (5:1 distilled water: fetal calf serum), and incubated at 37°C for 15 minutes.

4. A drop of fixative (3:1 absolute ethyl alcohol: glacial acetic acid) was added, and the cultures were centrifuged at 800 rpm for 8 minutes.

5. The supernatant was discarded leaving a large drop over the cells. 4 to 5 cc of 3:1 fixative was slowly added and the cells gently agitated.

6. The tubes were then stoppered and refrigerated at 4°C for approximately half an hour and then left at room temperature for one hour or longer.

7. The cells were then centrifuged at 800 rpm for 8 minutes and the supernatant removed and discarded. 0.5 to 1.0 cc of fresh fixative was then carefully added so as not to disturb the button of cells. After two minutes the excess fixative was removed and replaced with fresh 3:1 fix. This procedure was repeated several times.

8. The cells were finally suspended in fresh fixative and a drop of the suspension was placed on precleaned cold wet slide. The slides were then flamed, air dried and stored until required.

Staining of Slides

A few drops of aceto-orcein (2% in 60 cc glacial acetic acid) were placed on a slide, and a clean coverslip was applied.

The slide was left this way for 1-2 minutes, and then the excess stain was removed by placing the slide between a few layers of paper towelling and applying pressure to the coverslip. The coverslip was then sealed with paraffin.

APPENDIX B.

DETAILED RESULTS FOR LSD 1-10

	T							C					
NO. OF CELLS	123							86					
TYPES OF GAPS AND BREAKS	G	TG	Iso g.	B	TB			G	TG	Iso g.	B	TB	
L. S. D. - 1 CHROMOSOME GROUPS A	3	3	0	6	2			2	1	0	3	0	
B	7	0	0	2	0			1	0	0	2	0	
C	7	0	1	1	1			3	4	0	1	1	
D	2	0	0	3	0			0	0	0	0	0	
E	1	0	0	1	1			0	0	0	0	0	
F	0	0	0	0	0			0	0	0	0	0	
G	0	0	0	0	0			0	0	0	0	0	
TOTAL	20	3	1	13	4			6	5	0	6	1	
ID. GAPS ID. BREAKS	24			17				11			7		
NON-IDENTIFIABLE EXCHANGES	0							0					
IDENTIFIABLE EXCHANGES	0							0					
DELETIONS	0							0					
ACENTRICS	4							6					
DICENTRICS	1							0					
TOTAL BREAKS AND GAPS	23/24							13/11					
CELLS WITH ABERRATIONS	19							10					
TOTAL BREAKAGE FREQUENCY	0.1870							0.1512					

	C					T					
NO. OF CELLS	120					123					
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB	
L. S. D. - 2 / CHROMOSOME GROUPS A	7	4	0	2	0	12	9	1	2	1	
B	7	1	0	0	0	4	2	0	2	0	
C	4	1	0	1	0	11	1	0	2	0	
D	2	0	0	0	0	3	1	0	0	0	
E	0	1	0	0	0	2	0	0	0	1	
F	0	0	0	0	0	0	0	0	0	0	
G	0	0	0	0	0	1	1	0	0	0	
TOTAL	20	7	0	3	0	33	14	1	6	2	
ID. GAPS	27					48					
ID. BREAKS	3					8					
NON-IDENTIF. EXCHANGES	0					0					
IDENTIFIABLE EXCHANGES	0					0					
DELETIONS	0					0					
ACENTRICS	2					1					
DICENTRICS	0					1					
TOTAL BREAKS AND GAPS	5/27					11/48					
CELLS WITH ABERRATIONS	3					9					
TOTAL BREAKAGE FREQUENCY	0.0416					0.0894					

	T					C				
NO. OF CELLS	136					104				
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB
L. S. D. - 3 CHROMOSOME GROUPS A	10	5	0	1	0	4	3	0	1	0
B	7	0	0	2	0	3	0	0	0	0
C	3	1	0	0	0	5	3	0	0	0
D	1	0	0	0	0	0	0	0	0	0
E	0	2	0	0	0	1	0	0	0	0
F	0	0	0	0	0	3	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0
TOTAL	21	8	0	3	0	16	6	0	1	0
ID. GAPS	29			3		22			1	
ID. BREAKS										
NON-IDENTIF. EXCHANGES	0					0				
IDENTIFIABLE EXCHANGES	0					0				
DELETIONS	0					0				
ACENTRICS	4					1				
DICENTRICS	0					0				
TOTAL BREAKS AND GAPS	7/29					2/22				
CELLS WITH ABERRATIONS	7					2				
TOTAL BREAKAGE FREQUENCY	0.0514					0.0192				

C					T					
100					122					NO. OF CELLS
TB	B	Iso g.	Tg	G	TB	B	Iso g.	Tg	G	TYPES OF GAPS AND BREAKS
1	0	0	4	1	0	0	0	4	6	A
0	1	0	1	3	0	2	0	1	1	B
0	2	0	0	1	0	4	0	0	8	C
0	0	0	0	0	0	4	0	0	1	D
0	0	0	0	2	0	0	0	0	1	E
0	0	0	0	0	0	0	0	0	0	F
0	0	0	0	0	0	0	0	0	0	G
1	3	0	5	7	0	10	0	5	17	TOTAL
4		12			10		22			ID. GAPS ID. BREAKS
0					1 = 2B					NON-IDENTIF. EXCHANGES
0					1					IDENTIFIABLE EXCHANGES
0					0					DELETIONS
2					5					ACENTRICS
0					0					DICENTRICS
6/12					17/22					TOTAL BREAKS AND GAPS
6					11					CELLS WITH ABERRATIONS
0.0600					0.1393					TOTAL BREAKAGE FREQUENCY

L. S. D. - 4

CHROMOSOME GROUPS

C					T					
100					100					NO. OF CELLS
TB	B	Iso g.	Tg	G	TB	B	Iso g.	Tg	G	TYPES OF GAPS AND BREAKS
0	0	0	2	1	1	2	0	7	3	A
0	0	0	2	0	0	0	3	1	1	B
0	1	0	1	1	2	0	0	6	3	C
0	0	0	1	0	0	2	0	3	0	D
1	0	0	0	0	0	0	0	0	0	E
0	0	0	0	0	0	0	0	0	0	F
0	0	0	1	0	0	0	0	0	0	G
1	1	0	7	2	3	4	3	17	7	TOTAL
2		9			7		27			ID. GAPS ID. BREAKS
0					0					NON- IDENTIF. EXCHANGES
0					0					IDENTIFIABLE EXCHANGES
0					0					DELETIONS
2					3					ACENTRICS
0					0					DICENTRICS
4/9					10/27					TOTAL BREAKS AND GAPS
4					10					CELLS WITH ABERRATIONS
0.04					0.100					TOTAL BREAKAGE FREQUENCY

L. S. D. - 5

CHROMOSOME GROUPS

C					T					
100					100					NO. OF CELLS
G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB	TYPES OF GAPS AND BREAKS
3	3	0	0	0	4	1	4	1	1	A
3	0	0	0	0	5	1	0	0	0	B
11	3	0	0	0	8	3	1	0	0	C
3	1	0	0	0	3	0	0	1	0	D
0	0	2	0	0	2	0	0	1	0	E
0	0	0	0	0	0	0	0	0	0	F
0	0	0	0	0	0	0	0	0	0	G
20	7	2	0	0	22	5	5	3	1	TOTAL
29			0		32			4		ID. GAPS ID. BREAKS
0					0					NON- IDENTIF. EXCHANGES
0					0					IDENTIFIABLE EXCHANGES
0					0					DELETIONS
0					0					ACENTRICS
0					0					DICENTRICS
0/29					4/32					TOTAL BREAKS AND GAPS
0					4					CELLS WITH ABERRATIONS
0.00					0.04					TOTAL BREAKAGE FREQUENCY

L. S. D. - 6

CHROMOSOME GROUPS

	T							C						
	100							100						
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB			G	Tg	Iso g.	B	TB		
L. S. D. - 7 / CHROMOSOME GROUPS A	6	1	0	1	1			3	2	0	1	0		
B	0	0	0	1	0			1	0	0	0	0		
C	3	9	0	4	0			4	2	0	1	0		
D	4	0	0	0	0			1	0	0	1	0		
E	0	0	0	0	0			0	0	0	0	0		
F	0	0	0	0	0			0	0	0	0	0		
G	0	0	0	0	0			0	0	0	0	0		
TOTAL	13	10	0	6	1			9	4	0	3	0		
ID. GAPS	23			7				13			3			
ID. BREAKS														
NON-IDENTIF. EXCHANGES	0							0						
IDENTIFIABLE EXCHANGES	0(1)							1						
DELETIONS	0							1						
ACENTRICS	2							1						
DICENTRICS	0							0						
TOTAL BREAKS AND GAPS	9/23							5/13						
CELLS WITH ABERRATIONS	7							3						
TOTAL BREAKAGE FREQUENCY	0.09							0.05						

	T							C						
NO. OF CELLS	90							100						
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB			G	Tg	Iso g.	B	TB		
L. S. D. - 8 / CHROMOSOME GROUPS A	6	0	0	2	0			4	1	0	1	0		
B	2	0	0	0	0			5	0	0	0	0		
C	7	1	1	4	0			7	2	1	1	0		
D	0	0	0	0	0			1	0	0	0	0		
E	1	0	0	0	0			1	1	0	0	0		
F	0	0	0	0	0			0	0	0	0	0		
G	1	0	0	0	0			0	0	0	0	0		
TOTAL	17	1	1	6	0			18	4	1	2	1		
ID. GAPS ID. BREAKS	19			6				23					3	
NON-IDENTIF. EXCHANGES	0							0						
IDENTIFIABLE EXCHANGES	2							1						
DELETIONS	1							0						
ACENTRICS	2							1						
DICENTRICS	0							0						
TOTAL BREAKS AND GAPS	9/19							4/23						
CELLS WITH ABERRATIONS	7							3						
TOTAL BREAKAGE FREQUENCY	0.10							0.04						

C					T					
100					100					NO. OF CELLS
TB	B	Iso g.	Tg	G	TB	B	Iso g.	Tg	G	TYPES OF GAPS AND BREAKS
0	1	0	2	5	0	0	2	3	7	A
0	0	0	2	2	0	1	0	0	1	B
0	1	0	2	8	0	1	0	3	9	C
0	0	0	1	1	0	0	0	0	1	D
0	0	1	0	0	0	0	0	0	3	E
0	0	0	0	0	0	1	0	1	1	F
0	0	0	1	0	0	1	0	0	1	G
0	2	1	8	16	0	4	2	7	23	TOTAL
2		25			4		32			ID. GAPS ID. BREAKS
0					0					NON-IDENTIF. EXCHANGES
0					0					IDENTIFIABLE EXCHANGES
0					0					DELETIONS
1					2					ACENTRICS
0					0					DICENTRICS
3/25					6/32					TOTAL BREAKS AND GAPS
3					6					CELLS WITH ABERRATIONS
0.03					0.06					TOTAL BREAKAGE FREQUENCY

L. S. D. - 9

CHROMOSOME GROUPS

	T					C				
NO. OF CELLS	100					100				
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB
L. S. D. - 10 / CHROMOSOME GROUPS A	6	4	1	0	0	4	0	0	0	0
B	3	0	0	0	0	2	0	0	1	2
C	4	1	1	3	0	6	1	0	0	0
D	1	00	0	1	1	0	3	0	0	1
E	1	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0
G	0	0	0	1	1	0	0	1	0	0
TOTAL	15	5	2	5	2	12	4	1	1	3
ID. GAPS	22			7		17			4	
ID. BREAKS										
NON-IDENTIF. EXCHANGES	0					0				
IDENTIFIABLE EXCHANGES	0					0				
DELETIONS	0					0				
ACENTRICS	1					0				
DICENTRICS	0					0				
TOTAL BREAKS AND GAPS	8/22					4/17				
CELLS WITH ABERRATIONS	8					4				
TOTAL BREAKAGE FREQUENCY	0.08					0.04				

APPENDIX C.

DETAILED RESULTS OF BEFORE AND AFTER TREATMENT WITH GROWTH HORMONE ON A PATIENT WITH FANCONI'S ANAEMIA

		BGH								AHG								STANDARD							
NO. OF CELLS		102								107								91							
TYPES OF GAPS & BREAKS		G	TG	Iso g.	B	TB	G	TG	Iso g.	B	TB	G	TG	Iso g.	B	TB	G	TG	Iso g.	B	TB	G	TG	Iso g.	B	TB
FANCONI'S ANAEMIA	A	11	3	0	7	4	7	4	0	6	5	3	2	0	0	0	3	2	0	0	0	2	0	0	0
	B	5	1	0	5	0	9	2	0	1	3	2	2	0	0	1	2	2	0	0	1	2	0	0	1
	C	21	10	3	7	0	12	2	0	11	1	8	1	0	0	2	1	1	0	0	2	1	0	0	2
	D	1	0	0	1	0	3	0	1	3	1	2	0	0	0	1	0	0	0	0	0	1	0	0	0
	E	1	1	0	0	0	3	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	TOTAL	39	16	3	20	4	36	9	1	23	10	16	5	0	1	5	2	5	0	1	5	2	0	1	2
ID. GAPS ID. BREAKS		58			24		46			33		2			7										
NON-IDENTIFIABLE EXCHANGES		2 = 48					0					0													
IDENTIFIABLE EXCHANGES		4					2					0													
DELETIONS		2					2					1													
ACENTRICS		17					3					3													
DICENTRICS		0					0					0													
TOTAL BREAKS & GAPS		47/58					38/46					11/21													
CELLS WITH ABERRATIONS		26					30					10													
TOTAL BREAKAGE FREQUENCY		0.461					0.355					0.121													
BREAK FREQ/ ABERRANT CELL		1.80					1.26					0.110													

APPENDIX D.

DETAILED ANALYSIS OF TREATMENT WITH MITOMYCIN-C

	M-C									C								
NO. OF CELLS	100									100								
TYPES OF GAPS AND BREAKS	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB	G	Tg	Iso g.	B	TB
MITOMYCIN - C / CHROMOSOME GROUPS A	10	2	1	26	5	5	0	0	0	0										
B	3	1	1	13	3	2	0	0	0	0										
C	12	2	2	60	6	4	2	0	0	0										
D	3	0	0	9	4	0	1	0	0	0										
E	1	1	0	15	4	0	0	0	0	0										
F	2	0	0	9	1	0	2	0	0	0										
G	0	0	0	5	1	1	0	0	0	0										
TOTAL	31	6	4	137	24	12	5	0	0	0										
ID. GAPS	41									17										
ID. BREAKS	161									0										
NON-IDENTIF. EXCHANGES	0									0										
IDENTIFIABLE EXCHANGES	48									0										
DELETIONS	2									0										
ACENTRICS	18									5										
DICENTRICS	0									0										
TOTAL BREAKS AND GAPS	181/41									5/17										
CELLS WITH ABERRATIONS	64									3										
TOTAL BREAKAGE FREQUENCY	1.81									0.05										