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# Chromosomal Analyses of Bone Marrow and Peripheral Blood in Subjects With a History of Illicit Drug Use

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*Cytogenetic and clinical data were obtained from 22 former illicit-drug-users and 22 controls. Subjects were grouped into four categories depending on main drug of choice: opiates, psychedelics, amphetamines, and barbiturates. No relationship or difference was ascertained between abnormal metaphases and drug type, time off drugs, years on drugs, or life style. Drug users showed a disparity in the chromosome damage of the bone marrow and peripheral blood, since their peripheral blood lymphocytic cells exhibited a statistically significant number of abnormal metaphases, while the bone marrow was essentially normal. Karyotype analyses of marrow and blood metaphases did not reveal clones of cells. Although the chromosome damage is statistically different ($P < 0.001$) between the former illicit-drug users and controls, there is no proof of direct causality between drugs, drug abuse, and damaged chromosomes.*

ONE OF THE principal concerns of the cytogenetics of former drug abusers has been the possible production of residual chromosomal damage which could be transmissible. Specific objectives have been to determine those drugs which produce chromosome damage and the manner in which the body handles affected cells with chromosomal aberrations.

The literature concerning chromosomal breaks by chemical agents is notoriously controversial and unsettled. A computer-generated bibliography for 1969 on chemical mutagenesis contains 967 citations[1] of their effects on plants and animals. An excellent review of chemicals tested on human cells only, is that of Margery Shaw[2] which lists approximately 200 agents proved or suspected of breaking chromosomes (visible) or causing mutations (submicroscopic).

These two citations, in addition to those listed subsequently, are illustrative of the dilemma and conflicting interpretations. Suffice to say that the papers have been selected as representative in that they serve to delineate the area of interest or controversy cited.

The existence of this controversy points to the fact that nondrug variables may contribute to the production of chromosomal damage. For instance, poor health and nutrition and chronic fatigue, usual concomitants of drug intoxication, may also be involved in previous findings. Also, previous reports have described data on the circulating lymphocyte, thus restricting the data to one cell. Data on the possible chromosome damage of bone marrow cells from humans after prolonged nontherapeutic use of psychotropic drugs is lacking.

The present study was designed to evaluate the effects of illicit drug abuse more completely in a population where some of the other stresses were controlled. Former illicit-drug-users living in a therapeutic residential house were studied. The data were obtained from the chromosome constitution of the subjects' bone marrow, comparison of this to their peripheral blood and that of a control population, and other clinical observations at the time of taking. The results demonstrated significant changes in the peripheral blood and not the bone marrow.

## Methods

**Subjects and Controls.**—The 22 subjects were former illicit-drug-users residing at a special therapeutic institution for a minimum of three months. Urine monitoring for the detection of illicit use of opiates, barbiturates, and amphetamines was conducted twice a week. Supervision of the patients by a staff composed of ex-drug addicts further ensured that the subjects had been drug-free for the duration of their residence. The communal milieu afforded the opportunity to maintain sufficient bed rest, nutritional adequacy, and medical supervision. Informed consent was obtained from the subjects. The 22 volunteers who served as controls were selected from the technical staff of the Chicago Lying-in Hospital. In selecting the controls, we made every effort to recruit those interested in the project who were aware that it concerned illicit-drug-users, but who had no history of illicit drug abuse.

Subjects were grouped into four sections based on their main drug of dependence: opiates, psychedelics, amphetamines, and barbiturates. However, these groupings are not exclusive since 20 subjects had taken more than one drug type and 21 had a history of septic parenteral drug administration. At the time of each test a subject's bone marrow aspiration was performed, two peripheral blood cultures for each subject and its replicate control were prepared, routine laboratory analyses were performed for the subjects, including blood chemical determinations, urinalysis, complete blood count (CBC), sedimentation rate, and platelet determinations.

**Study Protocol.**—*Bone Marrow Aspiration.*—A direct technique for studying the marrow chromosomes without prior in vitro culture was a modification of the method of Tjio and Whang.[3] This method has no stimulatory effect on cell division. Smears and sections of marrow particles were prepared for and interpreted by the Division of Hematology.

*Peripheral Blood.*—The 72-hour culture procedure was a modification of a whole blood micromethod previously described.[4] The 72-hour cultures were assessed for the number of cell cycles using tritiated thymidine. Utilizing this procedure, no cells were found to be in a second mitotic division. Two culture flasks were set up for each subject and replicate control. Four slides were prepared from each flask, giving eight for each subject and control.

*Scoring.*—A minimum of 100 intact cells from each peripheral blood cul-

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ture and bone marrow preparation were counted. All slides were coded and the observer was unaware of any relationship to patients or controls. To strengthen the impartiality no observer scored slides from both bone marrow and peripheral blood of the same subject. All eight slides were scored to obtain the 100 cells.

Classification and identification of the chromosome aberrations followed the International (Denver) System[5] as modified by Patau[6] and clarified by the London Conference (1963)[7] and the Chicago Conference (1966).[8] This standardization enabled us to delineate cells with a normal chromosome number not necessarily indicative of a normal chromosome construction. We elected to score and include chromatid gaps in our tabulations of chromosome damage since this achromatic area is a visible structural aberration of the chromosome, and varies in frequency from case to case. The aberrations scored were:

GAPS.—A paled or achromatic area of chromatid whose length is not greater than the width of the chromatid.

CHROMATID BREAKS (cb).—A portion or fragment of one chromatid completely separated from the chromosome.

ISOLOCUS BREAKS (ace).—Acentric homologous chromatid portions of a chromosome.

EXCHANGE FIGURES (ex).—Translocated combinations of two or more chromosomes often forming quadriradial and triradial figures.

DICENTRIC (dic).— Functionally single chromosome with two kinetochores and formed by the fusion of portions of two chromosomes.

ISOCHROMOSOME (i).—Chromosome formed by a horizontal fission of the kinetochore.

RING (r).—Single or chain-linked depending on the number of chromatid ends joined with each other.

TRANSLOCATION (t).—Fusion of a chromosome fragment to another intact chromosome.

Gg −.—A small Philadelphia-like chromosome appearing as a group G chromosome with 40% of the long arms deleted.

MARKER (mar).—An atypical unidentifiable chromosome shorter or longer than those of the normal complement and caused respectively by a deletion or translocated addition of chromatin.

INVERSION (INV).—Reversal of a segment within a chromosome.

*Blood Chemistry.*—The sodium and potassium were measured by emission flame photometer, chloride by Cotlove Chlorodometer, and uric acid by the uricase method.

*Hematology.*—The white blood cell (WBC), red blood cell, hemoglobin, and hematocrit values were obtained using the Coulter Counter Model "S." Differential counts were obtained by microscopic analysis of stained blood smears counting each type of WBC in 100 cells scored.

*Urinalysis.*—A routine urinalysis included: (*a*) visual examination for type and appearance; (*b*) pH reaction using a Universal Indicator; (*c*) specific gravity using a refractometer; (*d*) protein and reduction tests using the Uristix method; and (*e*) microscopic examinations for casts, organisms, and other sediments on the slide.

Calculation of Data.—The statistical analysis of the data was evaluated by a one-way analysis of variance

$$[DFB = M\text{-}1, DFW = \sum_{i=1}^{M} (N_i - 1),$$

$$FR = MSB/MSW]$$

and a two sample test of significance using the Welch Procedure which approximates the "Student's"-*t* for cases of unequal variances ("Student's"-*t* assumes equal variance). The calculations for the analysis of the data are stored in a PDP/81 computer.

## Results

The number, type, and percent of aberrations in this study are summarized in the Table. All types of chromosome damage as listed in *Methods* were noted. The aneuploidy was both hypodiploid and hyperdiploid. The chromosome damage seen in the peripheral blood of the subjects illustrates the marked disparity with the chromosome profiles of the subjects bone marrow.

The magnitude and range of the chromosome complements in the bone marrow cells of the subjects did not show appreciable deviations from the normal number of 46 chromosomes. No difference was noted when this series of marrow was compared to a control population (Propp and Amarose, unpublished data).

The chromosomal aberrations in the 2,269 scored bone marrow cells were unexpectedly low, with a total of 102 abnormal metaphases (4.5%) when including cells containing only chromatid gaps, or 32 (1.4%) if we exclude those cells with only gaps.

Disparity of abnormal metaphases between the subjects' bone marrow and peripheral blood was apparent. Peripheral blood from the subjects had 2,237 cells scored, with a total of 449 (20.1%) of all abnormal metaphases or 313 (14.0%) if we exclude cells with only chromatid gaps. Even if cells with only chromatid gaps were excluded, the one-way analysis of variance showed the number of abnormal metaphases in the subjects to be significantly different in their peripheral blood and bone marrow at a level of confidence of $P < 0.001$.

The 2,217 cells scored from the controls' peripheral blood had a total of 259 abnormal metaphases (11.7%), or 173 (7.8%) when cells with only chromatid gaps were excluded. All chromosome aberrations in the peripheral blood of the controls totalled 324, as compared to 622 for the subjects. The aberration types which were not higher in the subjects were exchange figures and inversions. The one-way analysis of variance comparing the abnormal metaphases of the controls and subjects showed the number to be significantly different at a level of confidence of $P < 0.001$.

Although the two classifications of abnormal metaphases are significantly different between the peripheral blood of controls and subjects, there was no specific difference between the four groups of subjects relative to drug of choice. The average age of all subjects and controls was similar, while that of the subjects differed when grouped by drug of choice: opiate group, 30.3 years; psychedelic group, 19 years; amphetamine group, 24.5 years; and

20 years for the barbiturate group. This is not an unexpected finding when consideration is given to the changing lifestyles of illicit-drug-users which has influenced the history of drug abuse and the types of drugs used.

The average number of years on drugs for all subjects was 7.3, but the average for the opiate group was 13.2 years, 3 years for the psychedelic, 6.8 years for the amphetamine, and 6 years for the barbiturate group. The small number of persons in each group makes an interpretation difficult with respect to months off drugs.

The subjects' time off drugs was compared to the percent of abnormal metaphases in the bone marrow and peripheral blood. No correlation was evident to account for the discrepancy between their bone marrow and peripheral blood. Our data show that 14 subjects in residence for one to six months were on drugs for an average of 22.8 years, had an average of 14.1% abnormal metaphases in the peripheral blood and 1.3% in the bone marrow; eight subjects in residence six to 13 months were on drugs for an average of 9.6 years, had 13.8% abnormal metaphases in the peripheral blood, and 2.1% in the bone marrow.

Although the subjects were asymptomatic at the time of study, the marrow of four showed an absence of iron stores, six had low iron stores, ten had normal iron stores, and two had unscorable iron stores. While this may reflect a poor nutritional status, the cause of the low iron stores was not systematically investigated in this study. The clinical chemistry and hematologic data showed no consistent pattern in the deviations from the normal values. (Specific values are available upon request.)

Although we conducted extensive screening interviews in selecting our control group one control returned six months later and stated that a total of 60 oral LSD ingestions were taken in a one-year period immediately prior to this study. The chromosome profile of this subject did not show evidence of residual chromosome damage.

A second control showed an unusually high percent of abnormal metaphases. In a follow-up interview it was found that, in 1959 and 1963, this control subject had two acute episodes of pancreatitis which has been treated with a prescribed daily therapeutic regimen of pancreatic enzymes and hydrochloric acid substitution since 1963.

**Summary of Chromosome Profiles**

| | | Counted Cells | Abnormal Metaphases | | Type and Number of Chromosome Aberrations | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Gaps | cb | ace | ex | dic | i | r | t | Gq- | mar | inv |
| Bone marrow | No. | 2,269 | 102* | 32† | 90 | 26 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| (Subjects) | % | | 4.5 | 1.4 | 4.0 | 1.1 | 0.3 | 0 | — | — | — | — | — | 0.04 | — |
| Peripheral blood | No. | 2,237 | 449 | 313 | 194 | 170 | 128 | 5 | 17 | 8 | 6 | 61 | 12 | 18 | 3 |
| (Subjects) | % | | 20.1 | 14.0 | 8.7 | 7.6 | 5.7 | 0.2 | 0.8 | 0.4 | 0.3 | 2.7 | 0.5 | 0.8 | 0.1 |
| Peripheral blood | No. | 2,217 | 259 | 173 | 96 | 127 | 48 | 6 | 14 | 4 | 1 | 24 | 2 | 9 | 3 |
| (Controls) | % | | 11.7 | 7.8 | 4.3 | 5.3 | 2.2 | 0.3 | 0.6 | 0.2 | 0.05 | 1.1 | 0.09 | 0.4 | 0.14 |

* Includes abnormal metaphases containing only chromatid gaps.
† Abnormal metaphases excluding those with only chromatid gaps.

## Comment

Modifications in number and morphology of mammalian chromosomes have been related to a number of factors. An illustrative, but not inclusive, list of alleged causes are: diagnostic x-rays,[9,10] internal and external irradiation,[4,11-19] marrow disease states,[20-26] viruses,[27-29] cytostatic chemicals,[30-32] chemical analogues of nucleic acid bases,[33] antibiotics,[34-43] teratogenic agents,[44] lysergic acid diethylamide,[45-51] psychotropic drugs,[58-64] and artificial sweeteners.[65,66]

The diversity and disparity of opinions relevant to chromosome damage and hallucinogenic drugs began in 1967 when three different approaches were used in the research. In the first study, Cohen et al[45] examined human lymphoblasts treated in vitro with varied concentrations of LSD and compared the results with control data. They concluded that LSD had a severe chromosome damaging effect. Loughman et al[55] did not see a difference in chromosomal damage between LSD-treated in vitro cultures and controls when using a dose equivalent to the 100$\mu$g or lowest dose used by Cohen et al.

The second approach has been to examine the chromosome profiles of the small lymphocytes obtained from persons using illicit LSD. Irwin and Egozcue[46] claimed a significant amount of abnormal chromosome damage, while Loughman et

al[55] disclaimed evidence of severe chromosome damage in eight illicit-drug-users.

Third, the psychotherapeutic use in humans of LSD afforded the opportunity to analyse cultured lymphoblasts obtained from such patients and compare the data to controls who received no medication. Bender and Siva Sankar[56] found no difference between five psychotic children who had received LSD and five who had not. Some of the children were given the drug every day for as long as two to three years. Sparkes et al[57] compared 12 people —four who were treated with LSD, four who took illicit LSD, and four controls. They did not see a significant difference between the control and LSD groups.

Negative findings with LSD were also reported by Hungerford et al,[52] Tjio et al,[53] and Dorrance et al.[54] Kato and Jarvik[48] reported chromosome breakage in vitro with LSD, but not in vivo. Corey et al[49] reported no cytogenetic evidence of chromosome damage with LSD administered therapeutically but in vitro use of LSD showed an increased frequency of chromosome aberrations. Psychotropic tranquilizers were reported as negative for chromosome damage by Stenchever and Jarvis,[64] Schmid and Staiger,[61] Cohen et al,[62] and Staiger.[63] Friedrich and Nielsen[59] reported negative in vitro data with lithium carbonate although chromosome damage was seen in blood cultures obtained from patients treated therapeutically.

Our data extend previous work in this area. The combined use of peripheral blood and bone marrow preparations in these studies provide insight in understanding effects of illicit-drug-abuse. For the first time in man, cells of the lymphopoietic and hemopoietic systems have been simultaneously compared.

Our data demonstrate that a population of individuals who had used excessive quantities of certain pharmacologic agents showed significantly greater residual chromosome damage in the peripheral blood relative to the controls. The changes noted differ from findings reported after irradiation,[4,9-18] in that cells did not deviate markedly with respect to the normal number of 46 chromosomes. Also, levels of aneuploidy and polyploidy were low in both peripheral blood and bone marrow. Damage to the chromosomes was random and affected all groups. In no instance did we find a marker or standard karyotype in any culture from any subject or control.

An attempt was made to correlate the variance between the normal metaphases of the subjects' bone marrow and peripheral blood. When we obtained the bone marrow and peripheral blood we assumed that the time the subjects had been in the therapeutic community would be instrumental in this discrepancy. Such was not the case.

There appear to be four possible explanations for this discrepancy: (1) the circulating lymphocytes in the peripheral blood may be exposed to a significantly higher concentration of the drug than bone marrow cells; (2) or, given the same concentration in the organism there may be differential sensitivity of the two cell types; (3) the bone marrow had time to recover if chromosome damage ever was present; and (4) we must remember that the lymphocytes were induced to replicate in vitro for a 72-hour period; whereas the bone marrow was neither antigenically challenged into mitosis nor submitted to an in vitro physiologic state.

With regard to stem cell changes, it would be extremely unlikely that a number of, or even a few, stem cells would have exactly the same changes which would give rise to identical abnormalities of chromosomes in all daughter cells. A more likely occurrence would have the stem cells with chromosomal injury continue to produce a specific response to that injury.

The lifestyles of the 22 subjects in this study did not demonstrate a single instance of aseptic administration and use of their drugs. There is no way we can prove the purity of the preparations. Conceptually, it is possible that the chronic and septic use of drugs could have damaged the detoxifying potential of the liver to an extent that the small lymphocytes became infected. It is interesting to note that the control who subsequently reported 60 oral LSD ingestions did not show greater chromosome damage when compared to both subjects and controls.

Thus, the damaged chromosomes we saw in excess of those found in "normal" controls could be a result of the pharmacologic agents or the inability of these cells to repair the damaged chromosomes prior to the induction of mitosis. The cells, in vitro, thus enter a mitotic suicide since the type of chromosome damage precludes the complete and normal formation of two daughter cells. In the organism, these cells could harbor the residual damaged chromosomes for long periods of time with no observable harm to the individual until shunted into the circulating blood where their function might be impaired.

The small lymphocyte is an immunologically competent cell capable of responding to antigenic challenge. These mature cells with a long circulating lifespan are not ordinarily in division in circulating blood and residual chromosome damage is elicited only by antigenic induction of cell division.

These studies have demonstrated the incidence of chromosome aberrations without recognizable biologic sequellae. Chromosome breakage, including the most bizarre arrangements associated with psychotropic drugs, is no accurate indicator of a neoplastic potential. There is a risk in drawing any conclusions about chromosomal abnormalities in any given clinical state, such as leukemia, unless the aberration is in the

form of a marker carried over the years and in numerous different cases.

The absence of clinical sequellae and the presence of statistically significant numbers of abnormal metaphases indicate that these subjects, like those who have had extensive radiation therapy[4,18] are able to tolerate these cells with no apparent immediate adverse effects.

Many investigators have related the high frequency of chromosomal aberrations from psychotropic drugs to a possible future occurrence of leukemia or other forms of cancer. The knowledge thus far obtained from such studies, while significant, is insufficient to conclude that the chromosome damaged somatic noncancerous cells represent a diagnostic indicator of future cancer from these very same cells. The data simply illustrate the cellular response to internal stimuli and reflect the degree of chromosome damage relative to the lifestyle when the subject was a drug abuser. The final determination of the biological consequences of drug induced chromosome alterations awaits intensive longitudinal studies of individuals showing residual chromosome damage.

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## Nonproprietary and Trade Names of Drugs

Lithium carbonate—*Lithane, Lithonate, Eskalith.*

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