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Chromosomal Aberrations in Users of Psychoactive Drugs

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Peripheral blood leukocytes from 56 users of psychoactive drugs and 16 non-drug-users were cultured and examined for the presence of structural chromosomal abnormalities. None of the subjects admitted recent exposure to x-rays, other irradiation, or viral infection. Drug-users were divided into five groups: those who smoked marihuana lightly; psychiatric patients treated with phenothiazine; and one group each of heavy users of two or more combinations of marihuana, heroin, and amphetamine. With the exception of the controls and the light users of marihuana, all groups showed elevated incidences of chromosomal aberrations. Increases were not general in any one group, but were largely accounted for by a few individuals within each group with more than one aberration each. Although it may be that any or all of the drugs could damage chromosomes in this way, it seems more likely that some other factor or factors common to drug-users might be responsible.

OUR INTEREST in this subject originated with the report of Cohen et al[1] that subjects who had taken lysergic acid diethylamide (LSD) showed a higher incidence of leukocyte chromosomal aberrations than normal controls. Since all their LSD-users had also taken one or more other psychoactive drugs, the possibility existed that LSD might not be the only drug implicated in chromosomal damage. This possibility was also raised by their finding of an elevated incidence of aberrations in a small group of subjects who had taken such other drugs but not LSD.

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We therefore planned a retrospective study which would concentrate on users of psychoactive drugs other than LSD. A prospective study, in which chromosome analyses would be made before and after taking the drug, was not ethically possible for most of the drugs we considered. Two prospective studies on LSD have now been published.[2,3]

Methods

Controls and drug-users were chosen from patients and staff of the Psychiatric Division of Bellevue Hospital, students of New York University, patients in Manhattan State Hospital, New York city, and persons attending the Drug Addiction Service at Morris J. Bernstein Institute, New York city. Histories were obtained by a questionnaire, filled out either by medical personnel during an interview and from records or by the subject himself. Details were requested, as precise as possible, of dosages and numbers of occasions of use of named drugs in eight categories: mild tranquilizers such as chlordiazepoxide (Librium); strong tranquilizers such as the phenothiazines; amphetamines; barbiturates; marihuana or hashish; hallucinogens; opiates and cocaine; and others. Relevant items of the medical history were exposure to x-rays or other irradiation, affliction with a long-term disease, and recent viral infections. Subjects were excluded if they had a history of diagnostic x-ray exposure more extensive than simple chest or dental x-rays in the preceding 12 months, or of therapeutic irradiation or radioisotope exposure at any time, or of viral disease within one month prior to examination. Some of the subjects excluded because of irradiation were, however, tested on a random and blind basis, as a test of the efficacy of our methods in picking up chromosomal aberrations. Ten-milliliter samples of heparinized peripheral blood were drawn, identified by code number only so as to maintain blind conditions, and treated by a modification of Moorhead's original method,[4] with leukocytes being cultured for two instead of three days.[5] Leukocytes were incubated for 46 to 50 hours prior to addition of vinblastine sulfate (Velban) and the air-dry method of slide preparation was used.

From each subject we scored 50 cells, selected under low power (× 120) on the basis of intactness and quality of chromosome spreading. Under oil immersion (× 1,200), the chromosome number was determined, and any aberrations present were scored. Single-chromatid and isochromatid breaks were distinguished from gaps on the basis of displacement of the distal chromatid fragments. Centromere breaks, representing splitting at the centromere, were scored separately. Dicentrics and rings were scored as such, whether the rings were centric or acentric. Fragments, whether minute or large acentrics, were scored as such, but were not recorded if they accompanied a dicentric or ring. Translocations were detected as elongated chromosomes and pericentric inversions by an obvious shift in centromere position. Deletions were scored if they were obvious as a shortening, and chromatid exchanges were also recorded.

Scoring was done directly under the microscope, and cells with definite or suspected aberrations (other than single-chromatid or isochromatid gaps and breaks) were photographed, and a formal karyotype prepared. All aberrations were seen and agreed upon by at least two examiners.

Results

Chromosome studies were carried out on 56 drug-users (27 men and 29 women) and 16 non-drug-users as controls (7 men and 9 women), as well as on seven subjects excluded from the main investigation because of their irradiation history. Twelve of the controls had never used any of the drugs considered, while the other four had made extremely light use of either amphetamines or marihuana more than one year previously. The drug-users were grouped into five categories before the results of the microscopic analysis were considered. The first two groups of subjects used essentially only one drug. Group 1 consisted of 13 individuals, students or staff, who were generally similar to the controls in history, except that they smoked marihuana lightly.

Most used it not more than once per month, and a few about twice per month. Only two of them had ever used another psychoactive drug: this was a mild tranquilizer (chlordiazepoxide) taken occasionally. Group 2 consisted of 11 hospitalized schizophrenic patients under continuous therapy with various phenothiazine drugs. Five had received medium or large doses (300 to 900 mg of chlorpromazine [Thorazine] daily, or its equivalent) for 7 to 12 years, and six had received smaller doses for shorter periods. All but one were still on the drug when bled. Very few used other drugs: these were either antiparkinsonian drugs or mild tranquilizers. There were two autistic children aged 5 and 8, and nine adult schizophrenics. It was necessary to obtain blood from these patients as long as possible after their last intake of phenothiazines so as to minimize the inhibitory effect of these drugs on mitosis in the leukocyte cultures.[6] In practice, they were usually bled just before the second drug dose of the day.

Nearly all the other 32 subjects used two or more drugs, at least one of them heavily. There were three principal patterns of heavy drug use discernible: mainly amphetamines; mainly heroin; or mainly marihuana with or without LSD. From these, we made three further groupings. Group 3 consisted of ten amphetamine-users. Six of them took it for weight control under medical supervision, usually on a regime of two weeks on and two weeks off over a long period of time. They used it either alone or in combination with barbiturates or mild tranquilizers. Also included in the amphetamine group are four amphetamine-dependent subjects seen as psychiatric patients. They regularly took large doses of amphetamine and varying but smaller amounts of barbiturate. One of them also used marihuana moderately, and another had taken LSD 25 times in three years and occasionally used heroin. Group 4 consisted of 11 subjects addicted to heroin, who stated that they injected from 3 to 25 "bags" daily. In our experience, this probably represents a daily dose of 10 to 500 mg of pure heroin. Seven of them also used cocaine and a few had recently begun methadone (Dolophine) treatment. Use of other drugs varied from none to one or more of amphetamine, barbiturate, marihuana (moderate or heavy), and phenothiazine. Group 5 consisted of 11 subjects who were not primarily addicted to or dependent upon any one drug, but used a variety of drugs of which only marihuana was common to all. This they used heavily, on more than ten occasions per month, often daily, and often smoking several "joints" per occasion. Seven of them also used LSD in amounts varying from three times in the previous month to more than 100 times over two years. Use of other drugs was never more than light or moderate, and involved one or more of the amphetamines with or without barbiturates, phenothiazines, or heroin. Five of this group were students, and six were seen as psychiatric patients.

A further group, considered for comparative purposes only, was composed of seven subjects with a history of considerable irradiation. If not excluded, four of them would have been in the controls, and one each in the light marihuana, amphetamine, and heavy marihuana groups. For some of the results, we also calculated totals separately for the eight subjects, three students and five psychiatric patients, who took LSD with other drugs. Seven of these were from the heavy marihuana and mixed group, and one from the amphetamine group. The latter subject, a patient, took barbiturates and heroin also, but not marihuana.

A summary of the microscopic findings by groups is given in Table 1. The percent ages are based on the numbers of cells with one or more aberrations, and not on the numbers of aberrations. Gaps are not listed since the incidence in controls (6.1%) was higher than in any other group (3.23% to 5%). The light marihuana-users were clearly not different from controls, showing a similar low incidence of some aberrations. For purposes of this paper, therefore, we do not consider them as "drug-users," which term we shall apply henceforth only to the heavy drug-users making up the other four groups.[2-5] All categories of aberrations, except chromatid exchanges, showed increased incidences in one or other group of drug users. Single-chromatid breaks (SCB) were increased in groups 2, 4, 5, and perhaps in LSD-users. The main increases of chromosome-type breaks (CB) were in groups 3 and 4, while chromosome-type rearrangements (CR) occurred only in groups 2, 3, and 5. In almost all cases, however, the increases were appreciably smaller than those seen in the irradiated subjects.

We did not apply tests of significance to the increases; χ^2 tests of group totals of abnormal and normal cells are inappropriate since the 50 cells examined per subject are not independent. Further, the data are not sufficient for statistical tests by individual subjects. Examination of the results for individuals showed, however, that the increases were not general in any class of drug-users, but were almost entirely accounted for by a few individuals in each group with two or more aberrant cells each, out of 50 examined. This level of aberrations was chosen because no individual among the controls or the light marihuana-users had more than one aberrant cell. As an illustration of the effect of the multi-aberrant individuals on the group totals, we have recalculated the group results without them. As may be seen in Table 2, after this exclusion the differences of the drug groups from controls become extremely small, and provide no clear evidence for any general effect

Table 1.—Chromosomal Aberrations in Drug-Users, Controls, and Irradiated Subjects

Study Group	No. of Cases	No. of Cells Examined	% of Cells With One or More Chromosomal Aberrations of Type*									
					Chromosome-type Breaks			Chromosome-type Rearrangements				Total All Types of Aberrations
			CX	SCB	ICB	CeB	Ace	Rings	T+Inv+De	Total CB	Total CR	
Controls	16	771	0.13	0.26	0.00	0.00	0.13	0.00	0.00	0.13	0.00	0.52
1) Light marihuana	13	650	0.15	0.31	0.00	0.15	0.15	0.00	0.00	0.31	0.00	0.77
2) Phenothiazines	11	600	0.00	1.25	0.33	0.00	0.00	0.17	0.17	0.33	0.33	2.00
3) Amphetamines	10	500	0.00	0	0.20	0.80	0.40	0.00	0.20	1.40	0.20	1.60
4) Heroin	11	650	0.15	1.08	0.46	0.31	0.15	0.00	0.00	0.92	0.00	2.15
5) Heavy marihuana ± LSD	11	550	0.00	1.64	0.00	0.36	0.00	0.00	0.18	0.36	0.18	2.18
2-5) All heavy drug-users	43	2,300	0.04	1.04	0.26	0.35	0.13	0.04	0.13	0.74	0.17	2.00
Irradiated	7	400	0.00	2.25	0.25	0.25	1.50	0.25	1.50	2.00	1.75	6.00
LSD†	8	400	0.00	0.75	0.00	0.50	0.00	0.00	0.00	0.50	0.00	1.25

* CX, chromatid exchange; SCB, single-chromatid break; ICB, isochromatid break; CeB, centromere break; Ace, free fragment(s); T+Inv+De translocation + inversion + deletion.
† Includes some subjects from groups 3 and 5 (see text).

Table 2.—Chromosomal Aberrations in Drug-Users and Controls*

Study Group	No. of Cases	No. of Cells Examined	% of Cells With One or More Aberrations			
			Chromatid-type Breaks (SCB+CX†)	Chromosome-type Breaks	Chromosome-type Rearrangements	Total of All Types of Aberrations
Controls	16	771	0.39	0.13	0	0.52
1) Light marihuana	13	650	0.46	0.31	0	0.77
2) Phenothiazines	8	450	0.22	0.44	0	0.67
3) Amphetamines	7	350	0.00	0.57	0	0.57
4) Heroin	7	350	0.29	0.86	0	1.14
5) Heavy marihuana ± LSD	9	450	0.67	0.22	0	0.89
2-5) All heavy drug-users	31	1,600	0.31	0.50	0	0.81
LSD	8	400	0.75	0.50	0	1.25

* Excluding subjects with more than one aberrant cell.
† SCB, single-chromatid break; CX, chromatid exchange.

on aberration incidence of any one drug or pattern of drug use.

We can thus confine further discussion to the 12 multi-aberrant subjects. Three of them occurred in the phenothiazine group. Since they are the only three in the group at the highest dosage levels, there is a suggestion that heavy use of phenothiazine may produce chromosomal aberrations. These three are, however, also among the five long-term schizophrenics. Two of them are the oldest in the group (47 and 41), and have been hospitalized for schizophrenia 21 and 20 years, while the third, although only 28, has been schizophrenic since childhood and hospitalized 12 years. The two long-term schizophrenics without multiple aberrations are aged 39 and 34 and have been in hospitals 10 and 11 years. They were maintained on lower doses of phenothiazines (450 or 300 mg of chlorpromazine daily) than the affected patients, and one is now on chlordiazepoxide alone. The other patients in the phenothiazine group are generally younger, are only intermittently ill, and thus live out of the hospital much of the time, and are on lower drug doses. The occurrence of multiple aberrations in three subjects seems, therefore, to be associated both with high phenothiazine dosage and with unknown factors involved in particularly long periods of severe psychotic illness requiring hospitalization. One possibility is long-term subclinical infection. Another is unreported irradiation expo-

sure, particularly since the aberrations seen in two of these subjects —a ring and a deletion—are often found in irradiated subjects but are uncommon in normals. There was, however, no mention of irradiation in the hospital records of the long-term patients, going back 10 to 21 years. Our evidence concerning trihexyphenidyl hydrochloride (Artane), an antiparkinsonian drug, is scanty. Two long-term schizophrenics in the group were receiving this drug: one had multiple aberrations and the other none.

There are nine subjects with multiple aberrations still to be considered, three in the amphetamine group, four heroin addicts, and two heavy marihuana-users, neither of whom used LSD. They all used more than one drug; thus, six used amphetamines, six barbiturates, five marihuana heavily, five heroin, two cocaine, and none LSD. It is striking that seven of the above nine subjects were heavily dependent on drugs, and were leading highly abnormal lives. Four were long-term heroin addicts and three were seen as psychiatric patients. The last three had histories of many years of drug abuse, two being primarily amphetamine-dependent while the third used marihuana very heavily and heroin, amphetamines, and barbiturates less heavily. In contrast, only two of the nine multi-aberration subjects were leading fairly normal lives, one a young student whom we had placed in our heavy marihuana group, since he smoked about ten times each month and used amphetamines lightly; the other a girl using an amphetamine-barbiturate combination for weight reduction. Their loads of multiple aberrations were milder than most: respectively, two SCBs and two centromere breaks.

Comment

Each group of drug-users showed increases in the incidences of some types of structural chromosomal aberrations over both controls and light users of marihuana. Our control values were low compared with those reported for controls in most recent studies on drug-users,[1-3,7] but accord well with those found in the extensive work of Court-Brown et al.[8] We found no dicentrics at all, and no increase in chromatid exchanges (CX) in drug-users. The overall frequency of the latter in our study, 3 per 3721 or $<0.1\%$, is similar to that found in a large series of normals.[8] Both dicentrics and chromatid exchanges were especially mentioned in some previous reports[1,7,9] as the type of rearrangement figures occurring in LSD-users.

We used a macromethod of leukocyte culture, which, as pointed out by Tjio et al,[2] probably leads less easily to artificially produced chromosomal aberrations than the micromethods sometimes used.[1,7,9] Our method was nevertheless capable of demonstrating the occurrence of numerous aberrations of almost all types following a known chromosome-damaging procedure, irradiation. These findings confirm those previously reported,[6] and reiterate the potential danger of some common diagnostic x-ray procedures. In our seven subjects these included four cases of rather extensive skeletal x-rays up to five years previously, two recent gall-bladder series, and one case of intravenous pyelography.

Since increased aberration incidences were found in each of the four heavy drug-use groups, it might be concluded that any or all of the drugs could damage chromosomes in this way; or alternatively, some other factor or factors common to drug-users might be responsible. The latter view seems preferable in view of our observation that the increases were not general throughout the groups, but were largely accounted for by a few subjects with more than one aberration each. In the case of the phenothiazine group there is a suggestion that either high drug dosage or other factors associated with long-continued psychotic illness could be responsible for the aberrations found in three subjects. These positive findings do not conflict with the negative findings of Cohen et al,[10] since their phenothiazine-treated subjects were all short-stay patients treated with medium or low doses. Our data are too sparse to allow any conclusion as to whether one phenothiazine rather than another might cause chromosomal damage, as recently reported by Nielsen et al.[11] The three affected patients in our study were receiving respectively chlorpromazine, thioridazine (Mellaril), or perphenazine (Trilafon), while most of the others were receiving chlorpromazine.

The nine other subjects with multiple aberrations were found in three different drug-use groups, but were heavily concentrated among the subjects who were abusing drugs and leading abnormal lives. It thus seems clear that these three groupings—into users of mainly one or another drug—did not permit identification of any particular drug as a chromosome-damaging agent. It might have been more relevant to ask whether the subjects were addicted or dependent, or not and whether they were seen as psychiatric patients, or were living fairly normally. The increased incidence of structural chromosomal aberrations may, therefore, have resulted from unknown factors associated with a life of heavy drug abuse, usually with addiction or dependence, rather than from the drugs themselves. Viral infections, including hepatitis, are one obvious possibility. Although no subject was included in the investigation who admitted to past hepatitis or recent other viral infection, we recognize that we could not be sure of getting reliable histories from the addicted or dependent subjects.

In several recent studies, attention has been concentrated on the possible role of LSD in producing

chromosomal aberrations in vivo, although it was rarely possible to examine subjects who were not multiple-drug-users. Our finding of chromosomal aberrations among an appreciable proportion of multi-drug-using subjects not using LSD throws considerable doubt on the validity of the claims made for a specific in vivo effect of LSD on chromosomes.

We have encountered many of the difficulties involved in evaluating drug effects of human chromosomes in vivo. In this double-blind but retrospective study of drug-users, it proved to be impossible to be sure of screening out the contaminating effects of radiation exposure and viral infection. The prospective form of study is to be preferred, in which samples are obtained just prior to, and at different intervals after, the administration of the drug.[2,3] In this way, the only variable is the drug. With most drugs of abuse this ideal approach is obviously not ethically possible. We believe that in any further retrospective studies, large numbers of persons using a given drug must be examined, and as many as 200 cells per person must be scored, if the observed aberration frequency is low. From our experience, this will be a formidable task.

All the structural chromosomal aberrations described in drug-users by ourselves and others have been sporadic in occurrence. No case has been reported of the initiation of a clone or subpopulation of cells all containing the same abnormality, as is sometimes the case following irradiation.[12] There is thus no evidence that lymphocyte precursors are affected in drug-users.

The occasional aberrations we have described among drug-users were seen only in somatic cells, and no inferences can reasonably be drawn, on the basis of their presence, about meiotic chromosome effects or transmission to progeny. Similarly, the presence of isolated structural rearrangements has no proven clinical significance for the host, and while associations may be drawn, there is no direct evidence in man of their deleterious effects.

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