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INCREASED FREQUENCY OF SISTER-CHROMATID EXCHANGES IN ALCOHOLICS

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Summary

The frequency of SCEs was significantly increased in the alcoholics analyzed ($10.6 \pm \text{SD } 0.66$) when compared to the frequency of a control group ($8.4 \pm \text{SD } 0.51$). Statistical analysis of the data obtained showed that the increase was not apparently related to age, sex, cigarette smoking, duration in years of alcohol abuse, nutritional status or type of alcoholic beverage commonly consumed by the individual.

Alcoholics recovering for at least one year from alcohol abuse were examined and the frequency of SCEs was found to be equal to the SCE frequency in the control group.

There was no statistical significance between the age, sex of the individual, smoking history and years of abstinence from alcohol abuse with respect to the frequency of SCEs. Therefore, one year of abstinence appears sufficient to allow the SCE frequency to return to that found in the control group.

In order to keep extraneous factors at a minimum and to analyze the effect of a particular factor, such as alcohol, on the number of SCEs, a careful medical history and screening program was followed. However, more information is needed to determine which factors play a role in causing genetic damage and inducing SCEs and to determine the significance SCEs may have with respect to genetic information and function.

Sister-chromatid exchanges (SCE) are considered indicators of genetic damage (Latt, 1974; Lambert et al., 1978) and have been used to investigate the effect that common factors found in the environment such as cigarette smoking (Lambert et al., 1978; Hollander et al., 1978) to chemotherapeutic agents such as chlorambucil (Solomon and Bobrow, 1975) may have on genetic stability. Several chemical mutagens and carcinogens increase the frequency in mammalian cells in in vitro (Perry and Evans, 1975) and in vivo tests (Vogel and Bauknecht, 1976).

One chemical that has been implicated in chromosome changes is alcohol (Obe et al., 1977). Obe and Herha (1975) found increased chromosome aberrations in alcoholics. In further investigations, Obe and Ristow (1977) found that acetaldehyde, a metabolic derivative of alcohol, induced SCEs while SCE induction did not occur with ethanol in in vitro studies.

Later, Obe et al. (1979) found increased SCEs in bone-marrow cells of mice that were exposed to ethanol and acetaldehyde in in vivo studies. Inadequate data are available with regard to SCEs in alcoholics, therefore, evidence compiled from 11 alcoholic, 10 control and 9 recovering alcoholic subjects is presented which indicated an increased SCE frequency in alcoholics compared with a control group.

Materials and methods

All subjects in this study were equally questioned about previous diseases, nutritional status, medical treatment, and X-ray investigation, as well as exposure to environmental and occupational hazards. Their smoking history and alcohol consumption (drinking history, type of beverages, amount and frequency) were also recorded.

10 control subjects with no history of significant illnesses, and no medication or X-rays within the past 6 months were selected (Table 1). They were not knowingly exposed to environmental or occupational hazards and their nutrition was adequate. 2 subjects smoked cigarettes (20 per day). Each control subject denied ingesting alcohol on a regular basis at present or in the past and admitted to consuming less than one drink per week.

11 alcoholics were selected for this study (Table 2). These subjects were equally questioned with the control group. No subject was on medication nor recently exposed to X-rays or other known environmental hazards and the nutritional status was adequate, judged on appearance and history, in 5 of the 11 subjects. 10 subjects smoked cigarettes with an average of 20 cigarettes per day and a range of 10–45 cigarettes per day. Alcohol had been heavily ingested for 6–30 years by these alcoholics with an average of 17.1 years.

9 recovering alcoholics were included in this study (Table 3). The criteria for a subject to be selected for this group included the abstention from alcohol use for a minimum of one year and successful completion of an alcohol-dependency program. The average time of recovery was 4.4 years with a range of 1–10 years. Previously, the subjects had an average duration of alcohol abuse of 14.6 years with a range of 7–35 years. No significant abnormalities in their medical history were found including the use of medication and exposure to X-rays and environmental hazards. Their nutrition was adequate. 7 subjects smoked cigarettes with an average of 24 cigarettes per day and a range of 7–60 cigarettes per day.

Peripheral blood (0.4 ml) was added to basal medium—Eagle's containing 20% fetal calf serum, antibiotics, and 20 μ M 5-bromodeoxyuridine. Phytohemagglutinin-M (Gibco) was added and the cultures were incubated at 37°C in complete darkness. At 69 h, colcemid (Gibco) was added and the cells harvested at 72 h then treated with hypotonic saline and fixed in 3 : 1 methanol—acetic acid. Air-dried slides were stored in darkness for 24 h then stained with the fluorescence plus Giemsa (FPG) technique (Perry and Wolff, 1974). The slides were analyzed for SCEs and the SCE frequency for each subject was based on the number of SCEs in a minimum of 15 cells.

Results and discussion

The average SCE frequency in the alcoholic subjects analyzed was 10.6 while the frequency in the control subjects was 8.4 (Table 4). The Mann Whitney *U* test was applied to the SCE data and a significant difference was found ($p < 0.001$). Because of this significant difference between the number of SCEs of the alcoholic and control subjects, SCEs from recovering alcoholic subjects of at least one year duration were analyzed. The average number of SCEs found in this group was 8.4 which equaled the SCE frequency in the control group (Table 4). Hence, the SCE frequency in this situation may change within individuals and once a mutagenic agent, such as alcohol, is removed from the environment then the number of SCEs appears to return to the level observed before the mutagenic exposure.

In order to identify a possible cause of the increased SCE frequency in the alcoholics, the age, sex, smoking, drinking and nutritional history between the 3 groups were analyzed with the Mann—Whitney *U*, Spearman rank correlation and chi-square tests. No significant difference between sexes, or age classes of the 3 groups was found by the use of these statistical tests.

Statistical studies were also undertaken to determine the relationship, if any, between the years of alcohol consumption, the type of beverage, smoking history and nutritional status with respect to the SCE frequency. The Spearman rank correlation coefficient of -0.111 is evidence that the number of years of alcohol abuse was not considered significant in increasing the frequency of SCEs and SCE frequency does not continually increase with the years of alcohol consumption by the alcoholics.

Obe et al. (1977) has theorized that the agent responsible for the increased chromosomal aberrations in alcoholics may be the ingredients found in alcoholic beverages other than alcohol. To examine the possible relationship between the number of SCEs and the beverage most commonly consumed by the alcoholics in this study, the Mann—Whitney *U* test was used. The test showed no significance between the type of beverage (wine, beer, vodka, whiskey) consumed and the number of SCEs; therefore, no particular beverage was found to induce the SCE frequency.

Armendares et al. (1971) and Khouri and McLaren (1973) found in protein-calorie malnutrition studies that chromosome aberrations were above normal. The etiology was thought to be due to a distorted internal environment or inefficient cellular repair mechanism of the chromosome (Hillman, 1974). Protein malnutrition is often observed in alcoholics; therefore, the nutritional status of the individual must be considered in examining chromosome damage and SCEs.

The nutritional status of each alcoholic was judged as adequate or inadequate based on history and appearance with 5 of the 11 alcoholics having adequate nutrition. The chi-square method was used to examine the relationship, if any, between the nutritional status of the individual and the number of SCEs. The values calculated were not significant. Therefore, the induction of SCEs is not apparently related to nutrition in the alcoholics studied.

Cigarette smoking has been reported to increase SCEs in smokers when compared to non-smokers (Lambert et al., 1978). The induction of SCEs in smokers has not been supported by others (Hollander et al., 1978; Crossen and Morgan, 1980; Butler, 1980). The Spearman rank correlation test was used to compare the smoking history and the SCE frequency in the alcoholics and recovering alcoholic subjects. The coefficient for these variables in the alcoholic subjects was calculated to be 0.093 which showed little relationship between cigarette smoking and the number of SCEs. The coefficient for the recovering alcoholic subjects was 0.17. Thus, the number of cigarettes smoked per day had little relationship with the number of SCEs found in the alcoholic or recovering alcoholic subjects and should not be considered the cause of the higher number of SCEs in the alcoholic subjects.

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TABLE 1

NUTRITION, SMOKING AND DRINKING HISTORY OF THE CONTROL SUBJECTS

Initial	Age	Sex	Nutritional status	Smoking history	Alcohol abuse	Mean of SCEs
PD	28	male	adequate	none	none	8.3
RIB	27	female	adequate	none	none	8.2
GD	58	male	adequate	none	none	8.2
JP	41	female	adequate	none	none	7.7
MAS	28	male	adequate	none	none	8.1
RD	25	female	adequate	none	none	9.0
MGB	27	male	adequate	none	none	8.1
AV	28	male	adequate	20 cigarettes/day	none	8.5
KKS	28	female	adequate	none	none	8.3
CW	34	female	adequate	10 cigarettes/day	none	9.5

TABLE 2

NUTRITION, SMOKING AND DRINKING HISTORY OF THE ALCOHOLIC SUBJECTS

Initial	Age	Sex	Mean of SCEs	Nutritional status	Smoking history	Alcohol consumption		
						Duration (years)	Kind	Quantity/day
BH	27	male	11.3	inadequate	10 cigarettes/day	13	wine	1 quart
JW	50	female	10.7	inadequate	10 cigarettes/day	25	wine	1 quart
AB	53	male	10.4	inadequate	10 cigarettes/day	30	no pref.	not known
GS	44	male	10.1	inadequate	10 cigarettes/day	25	no pref.	not known
JG	39	male	10.3	adequate	none	23	beer	>1 gallon
AZ	37	male	10.1	adequate	10 cigarettes/day	10	no pref.	not known
JM	25	male	10.2	adequate	45 cigarettes/day	6	vodka	1 quart
HY	46	male	9.6	inadequate	40 cigarettes/day	15	whiskey	1.25 quarts
LB	40	male	10.8	adequate	15 cigarettes/day	20	beer	>1 gallon
GB	19	female	11.5	adequate	30 cigarettes/day	6	beer	>1 gallon
AL	25	female	11.7	inadequate	20 cigarettes/day	15	wine	1 quart

TABLE 3**NUTRITION, SMOKING AND DRINKING HISTORY OF THE RECOVERING ALCOHOLIC SUBJECTS**

Initial	Age	Sex	Nutritional status	Smoking history	Years recovering	Years of alcohol abuse	Mean of SCEs
AB	24	female	adequate	7 cigarettes/day	3	15	8.2
LB	31	male	adequate	60 cigarettes/day	1	10	8.7
EW	41	male	adequate	10 cigarettes/day	4	10	8.8
AM	20	female	adequate	40 cigarettes/day	1.25	7	8.2
SM	31	male	adequate	none	6	10	8.5
JV	40	male	adequate	10 cigarettes/day	3.5	20	7.8
LES	58	male	adequate	none	10	35	8.6
LZ	57	male	adequate	10 cigarettes/day	8	20	8.2
GP	29	female	adequate	30 cigarettes/day	3	4	8.6

TABLE 4

FREQUENCY OF SCEs FROM CONTROLS, ALCOHOLICS AND RECOVERING ALCOHOLICS

Subjects	Number	Age		Sex M/F	Average number of SCEs (group mean $\pm$ SD range)		Total number of cells analyzed
		Mean	Range		per cell	per individual	
Controls	10	32.4	25—58	5/5	8.4 $\pm$ 0.51	7.7—9.5	252
Alcoholics	11	36.8	19—53	8/3	10.6 $\pm$ 0.66	9.6—11.7	258
Recovering alcoholics	9	36.8	20—58	6/3	8.4 $\pm$ 0.32	7.8—8.8	188