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VINYL CHLORIDE EXPOSURE AND HUMAN CHROMOSOME ABERRATIONS*

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SUMMARY

Examination of lymphocyte cultures from 11 vinyl chloride polymerization workers and 10 controls revealed a significantly higher incidence of aberrations in the exposed population. Most of the excess damage was of the "unstable" variety and involved the grossest kinds of changes such as fragments or rearrangements. When these complex changes were regarded as the product of two breaks, the incidence of all breaking events was also significantly increased in the workers. The results indicate the presence of chromosome damage in vinyl chloride exposed workers.

INTRODUCTION

Since the pioneering radiation studies of the early 1960's it has been clear that chromosome morphology examination could uncover evidence of genetic damage in man[1–3]. The earliest of these studies discovered the now well-known link between carcinogenesis and chromosome changes (for recent review, see ref. 9). These were soon followed by the discovery that a number of chemicals are clastogenic (chromosome breaking), notably alkylating agents and cytostatic drugs, and DNA base analogues[4]. In recent years, occupational exposure to various chemicals has been recognized as another potential source of genetic change in man. Chromosome examination following human exposures had revealed the probable clastogenicity of some pesticides and herbicides[10,17]. Industrial exposure to benzene has also been implicated by a number of investigators in both leukemogenesis and clastogenesis (refs. 7, 8, 16).

Within the last year, a new industrial carcinogen has been identified[14]. Vinyl chloride, the monomer used for production of the common polymer polyvinyl chloride, has been held responsible for at least 22 angiosarcomas of the liver in exposed wor-

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kers[10]. There is additional suggestion from both animal and human data of an increased risk for cancers of the central nervous system, the respiratory system, and of the blood forming tissues[10,13,15]. With an association between clastogenicity and carcinogenesis established for other agents, it appeared that this new carcinogen might also cause chromosome damage. We therefore undertook a blind study of lymphocyte chromosomes from vinyl chloride-exposed workers and nonexposed controls.

EXPOSURE TO VINYL CHLORINE

The 11 subjects studied were male workers who had received repeated exposure to vinyl chloride in an upstate New York polyvinyl chloride polymerization plant. Major exposures came from leaks of unreacted vinyl chloride gas, fumes from polyvinyl chloride slurry, and from polymerization reactor cleaning operations. Reactor cleaning involved skin contact to polyvinyl chloride and inhalation of vinyl chloride gas residues; this operation accounted for the most intense exposures. Duration of recurrent occupational exposure in the 11 men ranged from 4–28 years with an average of 15 years (Table I). There is no record of ambient gas levels in the factory, but it is assumed that these must have exceeded 500 ppm at times, based on reports of odor detection, dizziness, and headaches.

TABLE I

AGE AND DEGREE OF VINYL CHLORIDE EXPOSURE OF SELECTED CASES AND CONTROLS

Case No.	*Age*	*Years exposed*	*Cells examined*	*Control No.*	*Age*	*Years exposed*	*Cells examined*
1	61	23	50	1	43	0	50
2	50	28	50	2	30	0	50
3	47	19	50	3	29	0	50
4	44	11	50	4	29	0	50
5	40	13	50	5	28	0	50
6	39	17	50	6	28	0	50
7	36	17	50	7	27	0	50
8	36	12	50	8	20	0	50
9	33	11	50	9	19	0	50
10	32	10	50	10	18	0	50
11	25	4	50				
Total	443	165	550		271	0	500
Average	40	15	50		27	0	50

Of the 10 healthy male controls, 4 were from within the same factory population, without known vinyl chloride exposure. Nevertheless, as long-term employment implied the possibility of some vinyl chloride exposure, albeit at low levels, we also selected 6 older controls from outside the factory environment. The average age of the controls was 27, of the subjects 40.

METHODS

The chromosome studies were performed on cultures of peripheral blood lymphocytes incubated at 37° for 65–68 h with phytohemagglutinin (Wellcome). Harvests were performed according to micromethod modifications of procedures first described by MOORHEAD *et al.*[14]. Media used in this study came from a single batch (Gibco) in order to insure uniform pH and other culture conditions.

In each individual studied, 50 metaphases were counted directly under the microscope at 1600×. The A, B, D, E, F, G, and Y chromosomes were evaluated separately so far as possible. Suspected aberrations and some normal metaphases were photographed; karyotypes were performed where helpful. From the total of 1050 cells examined there were 281 photographs taken and 140 karyotypes made from the photographs.

Confirmed aberrations were classified according to two systems in order to discern if a pattern of breakage might exist. The system of BUCKTON *et al.*[3] and COURT BROWN[5] has three categories of aberrations: B, cells with simple aberrations such as breaks and gaps; C_u, cells with "unstable" chromosome changes such as fragments, dicentrics, and rings; C_s, cells with "stable" chromosome changes such as monosomies, trisomies, deletions, and exchanges. The distinction between cells with stable and unstable aberrations is related to their tendency to either remain in or disappear from the circulation.

The system of HIRSCHHORN AND COHEN[11] differs in two respects. It considers all breakage, including the total number of breaks in cells with more than one aber-

TABLE II

VINYL CHLORIDE EXPOSURE AND CHROMOSOME ABERRATIONS (CLASSIFICATION SYSTEM OF COURT BROWN[5] AND BUCKTON *et al.*[3])

Case No.	B[a]	C_u[a]	C_s[a]
1	7	2	3
2	8	2	3
3	6	0	3
4	3	1	3
5	5	1	3
6	3	0	0
7	5	2	2
8	8	2	6
9	4	0	4
10	8	4	5
11	5	3	5
Total	62.0	17.0	37.0
Mean(±S.D.)	5.64(±1.91)	1.55(±1.29)	3.36(±1.63)
Comparison	$t = 1.84$ $0.1 > p > 0.05$	$t = 2.863$ $p = 0.01$	$t = 0.56$ $0.6 > p > 0.5$
Control No.			
1	5	1	3
2	4	0	7
3	4	0	1
4	5	0	3
5	2	1	6
6	5	0	3
7	5	0	3
8	5	1	2
9	4	0	0
10	5	0	1
Total	44.0	3.0	29.0
Mean(±S.D.)	4.40(±0.97)	0.30(±0.48)	2.90(±2.18)

[a] B, Breaks and gaps, C_u, "unstable" changes (fragments, dicentrics, rings), C_s, "stable" changes (monosomy, trisomy, deletions, exchanges).

ration, and it gives weighted consideration to those aberrations which are the apparent result of two "hits". Therefore, complex breakage (*C*) such as rings, dicentrics, and exchanges is counted twice in the total, whereas simple breaks and deletions (*S*) are counted once.

RESULTS

Gaps and breaks were the predominant aberrations among the 1050 cells examined, as seen in the *B* column of Table II. Subjects have nonsignificant increases of such aberrations when compared to controls by a *t*-test for comparison of the means ($0.1 > P > 0.05$), and marginally significant increases by an *F*-ratio for comparison of the variances ($0.05 > P > 0.01$). The difference in cells with stable aberrations, or those that BUCKTON *et al.* and COURT BROWN found to persist in circulating lymphocytes[3,5], is also nonsignificant. In our study, stable aberrations were usually cells with random chromosome loss. However, cells with unstable aberrations were observed significantly more frequently in the cultures from exposed workers: *t*, $P = 0.01$; *F*, $0.01 > P > 0.001$.

Table III focuses on breaking events only. The total of simple breaks, including

TABLE III

CHROMOSOME BREAK EVENTS (IN 50 CELLS/INDIVIDUAL) AND VINYL CHLORIDE EXPOSURE (SYSTEM OF HIRSCHHORN AND COHEN)

Case No.	*S*[a]	*C*[a]	*Total break events (S + 2C)*
1	4	3	10
2	2	3	8
3	2	0	2
4	1	2	5
5	0	1	2
6	0	0	0
7	3	2	7
8	1	2	5
9	4	0	4
10	1	4	9
11	3	5	13
Total	21	22	65
Mean(± S.D.)	1.91(± 1.44)	2.00(± 1.67)	5.91(± 3.91)
Comparison	$t = 1.12$ $0.3 > p > 0.2$	$t = 2.80$ $0.02 > p > 0.01$	$t = 2.75$ $0.02 > p > 0.01$
Control No.			
1	3	1	5
2	1	0	1
3	1	0	1
4	0	0	0
5	2	2	6
6	2	0	2
7	0	0	0
8	1	1	3
9	2	0	2
10	1	0	1
Total	13	4	21
Mean(± S.D.)	1.30(± 0.95)	0.40(± 0.70)	2.10(± 2.02)

[a] S, Single hit events (breaks, deletions); C, complex events (rings, dicentrics, exchanges).

those from multiaberrant cells, is increased but not significantly in the subjects. This is shown in the *S* column. Complex breaking events in *C* are significantly more frequent in those exposed, with: *t*, $0.02 > P > 0.01$; and *F*, $0.01 > P > 0.001$. The total of breaking events ($S + 2C$) shows a similarly significant increase in the subjects (*t*, $0.02 > P > 0.01$; *F*, $0.01 > P > 0.001$). Not included in the charts is a combined total of all breaks, gaps, and deletions. There was a marginally significant difference in these with *t*, $0.05 > P > 0.02$; *F* was not significant.

Exaggerated secondary constrictions of the No. 9 chromosome were easily noted because of their high degree of visibility. The average subject was observed to have 3.91 (± 2.47 S.D.) and the average control had 2.00 (± 1.63) in the 50 cells examined per individual. Significance was marginal at most, $P < 0.05$ by a *t*-test; *F* not significant.

DISCUSSION

There are obvious perils in drawing strong conclusions from small samples, and it would clearly be preferable to have an age-matched control group despite experimental evidence that age is generally unrelated to chromosome changes other than chromosome loss[5]. Also, the relatively high degree of breaks and gaps in controls as well as subjects is somewhat disconcerting, although subjects do have more. Within these limitations, it is clearly indicated that chronic high level exposure to vinyl chloride is clastogenic. Much of the increased damage was of the unstable variety as defined by BUCKTON *et al.*[3]. The difference between cases and controls is most evident for unstable aberrations in general and fragments in particular. HIRSCHHORN AND COHEN's system, which is a better overall index of chromosome damage, shows that long-term exposure is associated with an evidently significant increase in all breaking events.

In this small sample it has not been possible to correlate the degree of damage with either the degree of exposure or with vinyl chloride disease symptoms. The former may never be possible, as the best estimate of total exposure for any individual is only a crude guess. In general, the majority of subjects selected from this factory exhibited increased breakage rates, and those with the shortest duration of exposure showed damage similar to those with the longest duration.

Stating that chronic vinyl chloride exposure almost certainly damages chromosomes leaves us with two questions. First, can chromosome damage studies be at all predictive of environmentally induced carcinogenesis? Ionizing radiation and now vinyl chloride exposures have been studied for genetic properties *after* association with induced neoplasms. If we can reverse the order of events, perhaps in animal studies, we will know with more certainty if chromosome examination has an important role to play in predicting environmental carcinogenesis.

The second question is: what kind of genetic studies should be done with vinyl chloride? We feel strongly that chromosome study of individual concerned fathers is unwarranted. The degree of damage discovered here is unlikely to yield meaningful findings in any single individual. Women are not employed in polymerization work. However, they may work in polyvinyl chloride processing industries, in which some exposure to unreacted vinyl chloride may occur. This could be important in light of experimental findings of transplacental carcinogenesis[13].

Carefully controlled examination of larger groups with vinyl chloride and polyvinyl chloride exposure are obviously needed, first to provide the larger data base necessary to confirm clastogenicity for vinyl chloride exposure, and also to evaluate dose-response relationships. Mutagenicity is being assessed in other test systems, including bacterial studies, insect and animal studies, along with *in vitro* chromosome studies.

The case for studying other genetically suspect chemicals is now stronger than ever.

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AN EVALUATION OF THE MUTAGENIC POTENTIAL OF AN AEROSOL SPRAY ADHESIVE IN THE RAT

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SUMMARY

A commercially available aerosol adhesive formulation was examined for its mutagenic potential in adult rat bone marrow cells. Groups of rats were placed in a inhalation chamber and were exposed to 10, 15, or 20 g of the aerosol once daily. This took place 21 times during 33 days. Treated and control animals were injected with colchicine intraperitoneally 2 h prior to sacrifice. Slides of bone marrow cells were examined for the presence of chromosome and chromatid aberrations. Although no statistically significant increases in grossly visible chromosomal damage were produced, significant reductions in the mitotic index were obtained which were indicative of a cytotoxic effect. Acute toxicity was manifested by the occurrence of ataxia, eye and nasal irritation, hyperexcitability, and clonic convulsions; these overt symptoms were dose dependent.

INTRODUCTION

In 1973, a preliminary report on an aerosol adhesive was forwarded to the Consumer Product Safety Commission by Dr. J. Rodman Seely, Professor of Pediatrics and Molecular Biology, University of Oklahoma Medical Center. This report indicated a causal connection between exposure to the aerosol adhesive and chromosome damage that might lead to genetic birth defects. The chromosomal analysis of peripheral blood drawn from exposed human subjects indicated a statistically increased incidence of visible chromosome damage as evidenced by breaks and gaps. No other aberrations were reported. Upon review of these data by academic, industrial, and governmental experts, the Consumer Product Safety Commission alerted the general public to this potential hazard. Although the association between the aerosol adhesive and chromosome damage had not been positively established, the strength of the association based upon the available information and the potential for serious personal injury to exposed humans led the Commission to conclude that the distribution of these products for household use presented an imminent hazard