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Morphologic, Biochemical, and Cytogenetic Studies of Bone Marrow and Circulating Blood Cells in Painters Exposed to Ethylene Glycol Ethers[1]

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In a previous cross-sectional survey, up to 15% of shipyard painters were found to have mild anemia or granulocytopenia, mostly acquired since employment. Environmental studies had suggested a possible etiologic role for ethylene glycol ethers, solvents to which the men were heavily exposed and which have established myelotoxic potential. To exclude alternative hypotheses, examine possible common patterns of injury, and identify potential risk factors and markers for such an effect, the affected painters were further studied. The painters were matched with two groups of controls: exposed painters without evidence of hematologic abnormality on the previous survey and unexposed controls. Altogether 25 subjects were studied by histopathologic examination of bone marrow, cytogenetic studies of marrow cells, and peripheral lymphocytes and peripheral red cell studies of membrane and metabolic function. Except for an unexpected finding of a race-associated effect on marrow histology, insignificant differences were seen among the groups in terms of marrow morphology and cellularity, stem cell growth kinetics, and marrow or peripheral cytogenetics. Two metabolic abnormalities of peripheral red cells related to exposure or clinical status of the subjects were found. Pyruvate kinase, an established marker of acquired myelodysplasia, was significantly depressed in the subjects with previously abnormal counts. Although reduced glutathione levels and holoenzyme activities of glutathione reductase (GSHR) did not differ among groups, exposed subjects had decreased saturation of GSHR with flavin adenine dinucleotide which could be restored *in vitro,* suggesting riboflavin deficiency or impaired riboflavin metabolism. Thus, although a unique pattern of bone marrow injury by histologic or genetic assay attributable to ethylene glycol ethers was not defined, biochemical effects of possible mechanistic importance were identified. The relevance of these findings as subclinical disease markers remains to be established.

INTRODUCTION

The ethylene glycol ethers have attracted increasing attention since reports of suppressive effects on gonadal and bone marrow function at levels of exposure potentially achievable by man (Miller *et al.,* 1981; Nagano *et al.,* 1984; Dodd *et al.,* 1983). Human case reports of reversible aplastic anemia after accidental ex-

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posures have heightened this concern (Parsons and Parsons, 1938; Ohi and Wegman, 1978; Cullen *et al.*, 1983a).

In 1985 we became aware of the very extensive use of ethylene glycol ethers by the painters in a local shipyard. We conducted a cross-sectional survey of male painters and unexposed draftsmen looking at peripheral blood counts and 24-hr urine collections for the oxyacetic acid derivatives of glycol ethers, the active toxin associated with the solvents (Jonsson and Steen, 1978; Miller *et al.*, 1982). The results, previously published (Sparer *et al.*, 1988; Welch and Cullen, 1988) demonstrated insignificant mean differences in hemoglobin and granulocyte counts in exposed, compared to unexposed, men. However 14 painters, 15% of the study group, demonstrated values for one or the other parameter more than two standard deviations below populational norms, compared to none in the control group. No acute or chronic model of individual exposure to ethylene glycol ethers, or exposures to lead, ionizing radiation, or benzene, mostly trivial, could explain the findings. Further, personal data on alcohol consumption, drugs, or avocational activities failed to reveal a likely explanation for the observations. There was, however, a strong and unanticipated racial association with the effect, the observed rate of abnormalities among nonwhite painters being $10/24 = 42\%$, compared to the rate among whites of $4/70 = 6\%$, $P < 0.001$.

Given the ubiquity of etyhlene glycol ethers in the work environment (Smith, 1984), we elected to study this population further. The goals of the present study were four:

1. To exclude other causes for depressed hemoglobin and peripheral granulocyte levels among the affected painters.
2. To determine if subclinical evidence of hematologic damage is present in apparently healthy coworkers of affected painters.
3. To identify host or exogenous factors which may increase the risk of hematologic damage in exposed painters. The possibility of a genetic factor was suggested by the disproportionately high number of nonwhite painters with depressed counts. Alternatively, since the toxicologically active agent has previously been identified in animals as a metabolic product of alcohol dehydrogenase (Miller *et al.*, 1982), it was hypothesized that alcohol consumption patterns or other factors affecting biologic activity of this enzyme system might determine risk for marrow injury.
4. To identify laboratory markers of significant exposures to glycol ethers which could be used in future field investigations (Cullen, 1989). In addition to alcohol dehydrogenase itself, candidates for such markers included, a priori, glutathione reductase or its metabolite glutathione (GSH) (Lamonova and Klimova, 1977; DiSimplicio *et al.*, 1984); δ-aminolevulinic acid dehydratase (ALAD), an enzyme exquisitely sensitive to alcohol and lead exposures (Moore *et al.*, 1980; Mitchell *et al.*, 1977; Granick *et al.*, 1973); and pyruvate kinase, a well described marker of myelodysplastic change (Valentine *et al.*, 1973; Mohrenweiser *et al.*, 1981; Kahn *et al.*, 1977; Bowin *et al.*, 1975; Renoux *et al.*, 1978; Etiemble *et al.*, 1979; Arnold *et al.*, 1974; Lintula, 1986).

MATERIALS AND METHODS

The present study was begun 2 years after the completion of the original survey

and took an additional 18 months to complete. In the interim, all painters had begun the use of dermal and respiratory protection and the concentrations of glycol ethers in paint products had reportedly been diminished by about 50%.

Findings in three groups were compared: Group I, exposed painters with abnormal blood counts on the initial survey; Group II, exposed painters with normal counts on the initial survey; and Group III, unexposed men. Since the nonexposed draftsman group from the initial survey was almost entirely white and somewhat older than the painters, we chose to go outside the initial study group for the selection of group III subjects to assure racial matching.

All 14 painters with low hemoglobin (<14 g/dl) or absolute granulocyte count (<1800 cells/μl) from the 1985 survey, except for two with documented anemia on hire, were invited to participate (Group I). Among the 80 exposed painters with normal counts at the time of the survey, computer-generated "matches" for age and race were invited with a goal of one control painter for each case (Group II). Nonexposed men (Group III) were solicited independent of the initial survey by advertising in the Medical Center area. A small monetary inducement was offered for discomfort and inconvenience.

After obtaining written informed consent, a confidential interview was conducted to ascertain current occupational exposure status, health status, and detailed history of personal exposure to alcohol, drugs, medications, and household chemicals such as pesticides and solvents with potential for hematologic effects. Peripheral blood was then obtained for complete blood counts; differential, smear, and reticulocyte counts; SMA-12; thyroid function test; whole blood level by atomic absorption spectroscopy; zinc protoporphyrin; serum B12; red cell folate; serum ferritin; osmotic fragility; Hamm test; hemoglobin electrophoresis; hemoglobin A_2/F levels; and a direct Coomb's test. Sister chromatid exchange studies were done on peripheral blood lymphocytes by K.K. using previously described methods (Kelsey, 1990) and studies of red cell metabolism were done by L.S. A posterior iliac crest bone marrow aspiration and a biopsy were then performed. In addition to routine H&E, Wright's, and iron stains, routine cytogenetic and banding studies of marrow stem cells were obtained (Sandberg and Abe, 1980).

All laboratory analyses were conducted on coded specimens which did not reveal patient identification or clinical status. Bone marrow aspirate and biopsies were read independently by three investigators, including two hematologists (T.D., P.M.) and a hematopathologist (P.B.) using a preestablished coding form, with ratings for overall cellularity (as %), megaloblastic change (diffuse, focal, absent), dyserythropoiesis (diffuse, focal, absent), differential count of 200 cells (for myeloid:erythroid ratio, blasts, lymphs, plasma cells, others), fibrosis (collagen, reticulin only, absent), iron stores (increased, normal, reduced, trace, absent), ringed sideroblasts (as % of normoblasts), vacuolated pronormoblasts (present, absent), tumor cells (present, absent), and granulomas (present, absent). In addition, each reader was given the opportunity to add descriptive comments in open format.

Studies of red cell metabolism were performed (Table 1) using red cell lysates and trichloracetic acid extracts of whole blood. Specific tests were chosen for one or more of six reasons: (A) sensitive indicator of red cell age, (B) defect described

TABLE 1
DETERMINATION OF RED CELL ENZYMES AND METABOLIC INTERMEDIATES

Assay	Purpose[a]	Method	References
Glycolytic enzymes			
Hexokinase	A	Beutler, 1975;[b] Solomon, 1988	Mohrenweiser *et al.*, 1981
Aldolase	A	Beutler, 1975;[b] Solomon, 1988	Brok *et al.*, 1966
Glyceraldehyde-phosphate dehydrogenase	B	Beutler, 1975;[b] Solomon, 1988	Arnold *et al.*, 1974
Phosphoglycerate kinase	B	Beutler, 1975;[b] Solomon, 1988	Valentine *et al.*, 1973
Pyruvate kinase	A,B	Beutler, 1975;[b] Solomon, 1988	Mohrenweiser *et al.*, 1981 Kahn *et al.*, 1977 Bowin *et al.*, 1975 Valentine *et al.*, 1973 Renoux *et al.*, 1978 Etiemble *et al.*, 1979
Hexose monophosphate shunt enzymes			
Glucose-6-phosphate dehydrogenase (G6PD)	A	Beutler, 1975;[b] Solomon, 1988	Mohrenweiser *et al.*, 1981 Kahn *et al.*, 1977
6-Phosphogluconate dehydrogenase	A	Beutler, 1975;[b] Solomon, 1988	Mohrenweiser *et al.*, 1981 Kahn *et al.*, 1977
Transketolase	F	Solomon, 1988	Bamji, 1975
Glutathione reductase (GSHR)	B,F	Beutler, 1975;[b] Solomon, 1988	Arnold *et al.*, 1974 Avissar *et al.*, 1986 Powers and Thurnham, 1977 Imanishi *et al.*, 1986 Bamji, 1969 Prentice and Bates, 1981
Miscellaneous enzymes			
Adenosine deaminase	B	Beutler, 1975;[b] Solomon, 1988	Glader and Backer, 1988 Kanno *et al.*, 1988 Solomon and Crouch, 1988
Pyrimidine 5-nucleotidase	B	Solomon, 1988	Lieberman and Gordon-Smith, 1980
Acetylcholinesterase	B	Beutler, 1975;[b] Solomon, 1988	DeSandre and Ghiotto, 1960
δ-Aminolevulinic acid dehydratase (ALAD)	D	See text	Solomon and Crouch, 1990 Fujita *et al.*, 1986 Koizumi *et al.*, 1984
Aldehyde dehydrogenase	C	See text	Jonsson and Steen, 1978 Solomon and Bowman, 1986 Solomon and Hillman, 1979a Bamji, 1975
Aspartate aminotransferase	A,B,F	Solomon and Hillman, 1978	Mohrenweiser *et al.*, 1981 Miller *et al.*, 1982 Bartnik *et al.*, 1987 Carpenter *et al.*, 1956
Alanine aminotransferase	A,B,F	Solomon and Hillman, 1979b	Mohrenweiser *et al.*, 1981 Solomon and Bowman, 1986 Solomon and Hillman, 1979a
Metabolic intermediates			
Adenosine triphosphate	E	Adams, 1963	—
2,3-Diphosphoglycerate	E	Sigma, 1974	—
Glutathione	B,E	Beutler, 1975[b]	Valentine *et al.*, 1973 Arnold *et al.*, 1974

[a] Key for purpose of assay: A, measure of red cell age; B, defect described in myeloproliferative and myelodysplastic disorders or following exposure to radiation or cytotoxic drugs; C, enzyme involved in glycol ether metabolism; D, enzyme sensitive to inhibition by solvents; E, screen for functional defects in red cell metabolism not detected by direct enzyme assays; F, marker associated with deficiencies of B vitamins.

[b] Method of Beutler (1975) modified: hemolysates were prepared from heparinized blood without other additives; stabilizing solution was not used in the dilution of coupling enzymes.

in myeloproliferative or myelodysplastic disorders which may result from chemical exposures, (C) enzyme suspected to be related to glycol ether metabolism, (D) defect known to occur after exposure to aldehydes or organic solvents, (E) screen for functional defect of red cells which might not be detected by direct enzyme assay, and (F) screen for nutrient deficiency which might contribute to hematologic abnormality. Methods for the studies have generally been previously published (Adams, 1963; Sigma, 1974; Beutler, 1975; Solomon, 1988). δ-Aminolevulinic acid dehydrastase was assayed as described by Weissberg *et al.* (1971) except that red blood cell lysates were used instead of whole blood, and assays were performed in the presence and absence of 20 mM dithiothreitol. A new method was used to determine red cell aldehyde dehydrogenase. The assay mix contained 10 mM sodium pyrophosphate (pH 9.5), NADH (0.067 mg/ml), 10 mM sodium acetate, and red cell lysate to give a final hemoglobin concentration of 3–10 mg/ml. The rate of fall in optical density at a wavelength of 340 nm was then determined at a temperature of 37°C.

Statistical analyses were performed on a microcomputer using Data Desk Professional data exploration software from Odesta Corp. (Northbrook, IL). Analyses were limited to student *t* tests for comparisons of mean values of continuously distributed variables, analysis of variance for evaluation of the variability on outcome parameters contributed by categorical predictors, and Pearson correlation coefficients for evaluation of and parametric associations in the data. Simple and multiple regression analysis and diagnostics were used to explore possible causal associations in the data.

The entire protocol had been approved by the Yale University School of Medicine Human Investigations Committee.

RESULTS

Demographic Data

Ten of the 12 painters eligible for Group I based on prior data entered the study; one could not be located and one refused. Seven of the 20 eligible age-matched exposed painters with normal blood counts on the prior survey (Group II) also agreed to participate and eight previously unsurveyed controls volunteered for Group III. The groups were similar in terms of age, alcohol, and cigarette consumption. Years of painting experience were similar for Groups I and II. Racial composition was not homogeneous within all groups; Group II was mostly white, while Groups I and III were largely black. These data are summarized in Table 2. Notably, no subject admitted regular exposure to medicinal or recreational drugs or nonoccupational exposures to solvents, pesticides, or metals. None suffered from a known chronic illness.

Hematologic Data

Peripheral hematologic data for each subject obtained at the time of this study revealed recovery in two of six previously anemic subjects and in two of the four previously granulocytopenic ones at the time of this follow-up study. They are nonetheless included in their original study group in all subsequent analyses.

TABLE 2
DEMOGRAPHIC FEATURES OF STUDY SUBJECTS

Group	Age in years (Mean ± SD, range)	Racial composition (White/Black/ Hispanic)	Cigarette smoking status (Current/Ex/Non)	Years painting (Mean ± SD, range)	Alcohol consumption in drinks/day (Mean ± SD, range)
I	43.8 ± 11.3	2/7/1	4/5/1	13.9 ± 8.4	1.4 ± 2.3
(n = 10)	25–58			5–28.5	0–7
II	44.9 ± 12.6	6/1/0	5/0/2	11.1 ± 7.0	1.5 ± 2.1
(n = 7)	29–61			5.5–23	0–6
III	35.3 ± 12.2	3/5/0	3/3/2	0	0.5 ± 7.3
(n = 8)	21–61				0–2

Group II subjects have remained healthy. Group III included a clinically asymptomatic 39-year-old reporter with hematologic parameters suggestive of a myeloproliferative disease; bone marrow examination from the study confirmed Philadelphia-chromosome-positive chronic myelogenous leukemia for which he was referred for treatment. His results have been excluded from subsequent analysis. Another Group III volunteer had marginal cell counts but was included in the analysis with his recruitment group.

Hematologic tests performed to exclude established causes of anemia were unrevealing. The Coombs test and hemoglobin electrophoresis, hemoglobin A_2, and F levels were normal in all subjects, and tests of osmotic fragility showed borderline increases in two subjects from each group. Serum ferritin, serum B12, and red cell folate levels were all normal. Reticulocyte counts were also normal, suggesting that peripheral destruction was not increased.

Blood Chemistries

Tests of liver, renal, and thyroid function, performed to exclude alternative explanations for depressed blood counts, were completely normal in all subjects. Whole blood lead levels were all below 40 μg/dl, the range associated with measurable hematologic abnormalities in adults (Cullen *et al.*, 1983b), and were almost all in the range of the general population. Levels for Group I (mean, 10.6 ± 9.2 μg/dl; range, 5–36) were comparable to those for Group II (mean, 11.1 ± 3.4; range, 6–17); each was not significantly higher than that in group III (mean, 6.3 ± 4.2; range, 1–14). Zinc protoporphyrin was normal in all subjects and mean values were similar in all three groups.

Bone Marrow Histology

No abnormal features were identified in any of the marrows (excluding the CML control) except for decreased iron stores in one subject in the granulopenic group (IG) and two unexposed controls. Total marrow cellularity, averaged over the three readers, was normally distributed and ranged from 22.5 to 65%; it did not differ among the groups: Group I mean = 46.5 ± SD 9.9, Group II mean = 44.3 ± 7.0. Group III mean = 46.9 ± 17.3. Further, cellularity did not differ as a function of age, race, years of exposure to paints, or alcohol consumption.

Average myeloid/erythroid (ME) ratios varied between 1.3 (i.e., 1.3:1) and 4

(i.e., 4:1) and also showed normal distribution. ME ratio was not uniformly distributed across the study groups, with the Group I subjects having a mean ratio significantly lower than that of Group II (Group I mean = 2.25 ± 0.70 vs Group II mean = 2.96 ± 0.65, $P = 0.05$). However, the ME ratio of the unexposed controls also was lower than that of Group II, mean 2.31 ± 0.47, and did not differ from that of Group I. Analysis of variance by demographic and exposure characteristics demonstrated that race was the only significant predictor of ME ratio. Among blacks, ME ratios ranged from 1.3 to 2.5 with a mean of 2.1; among whites the range was 2.0 to 4.0 with a mean of 2.96, $P = 0.0003$. Neither group nor exposure status were significant determinants of the ME ratio.

Other than normal erythroid and myeloid precursors, the marrows did not contain any other cell lines to an appreciable degree other than lymphocytes, which ranged from less than 5% in most marrows to 25%. Mean numbers of lymphocytes did not vary among the groups, nor did the proportion with greater than 5%. Thus, no morphologic characteristic of bone marrow distinguished subjects in the three study groups.

Genetics

Routine chromosomal studies were performed reviewing five marrow precursor cells on six subjects in Group I, all seven in Group II, and four in Group III (excluding the CML case). Each subject was 46 X,Y with no deletions or translocations noted. Between 10 and 20 banded cells per subject were reviewed for chromosomal and chromatid breaks. A single chromatid break was noted in only two subjects, both in Group II.

Sister chromatid analysis of peripheral lymphocytes was also performed on 17 subjects. Values ranged from 6.96 to 11.56 with a mean of 8.64 ± 1.47. As expected from the literature (Kelsey *et al.*, 1988; Purchase *et al.*, 1980; Watanabe *et al.*, 1983), current smoking was strongly associated with SCE level, smokers having a mean of 1.80 higher levels than former and nonsmokers, $P = 0.006$. No other exposure or demographic variable significantly affected SCE levels. Uncorrected for smoking there were no significant differences in SCE among the study Groups: Group I mean = 8.22 ± 1.04, Group II mean = 8.65 ± 1.56, and Group III mean = 9.59 ± 2.17. Correcting for current smoking status using the weight derived from the data did not appreciably change the results: Group I adjusted mean = 7.71 ± 1.15; Group II adjusted mean = 7.37 ± 1.10; Group III adjusted mean = 8.39 ± 1.27.

Red Cell Enzyme and Metabolite Studies

As summarized in Table 3, metabolic studies showed few differences between the study groups. Mean G6PD levels were lower in Group I (9.48 ± 3.87 U/g Hb) than in Groups II and III (11.94 ± 1.94, $P = 0.05$). However, this was due to abnormally low levels in only two individuals, one Caucasian and one black.

Aldehyde dehydrogenase activities were similar in the three groups and did not correlate well with exposure group or history of alcohol consumption (Pearson $r = -0.09$).

ALAD also showed no relationship to exposure group in the study but did show

TABLE 3
RED CELL ENZYME AND METABOLITE LEVELS BY GROUP

Assay	Group LA (n = 6)	IG (n = 4)	II (n = 7)	III (n = 7)	Probability groups differ by ANOVA
Hexokinase	1.53 ± 0.66	1.06 ± 0.17	1.39 ± 0.52	1.27 ± 0.42	0.50
Aldolase[a]	1.85 ± 0.29	2.11 ± 0.27	1.82 ± 0.37	2.24 ± 0.62	0.41
Glyceraldehyde-phosphate dehydrogenase[a]	76.2 ± 24.1	66.8 ± 8.8	82.5 ± 19.8	91.3 ± 27.1	0.51
Phosphoglycerate kinase[a]	264.7 ± 32.9	249.3 ± 12.4	243.7 ± 14.9	237.5 ± 12.6	0.29
Pyruvate kinase	7.97 ± 2.57	6.97 ± 0.79	9.48 ± 1.05	8.89 ± 1.92	0.15
Glucose-6-phosphate dehydrogenase	8.88 ± 4.88	10.39 ± 1.84	11.87 ± 1.32	12.02 ± 2.50	0.24
6-Phosphogluconate dehydrogenase[a]	10.29 ± 1.88	8.51 ± 0.88	8.52 ± 0.93	9.01 ± 0.93	0.20
Transketolase[a]	0.73 ± 0.46	0.74 ± 0.05	0.66 ± 0.19	0.94 ± 0.38	0.52
Glutathione reductase (GSHR)	5.25 ± 2.00	6.01 ± 1.39	6.24 ± 2.04	5.26 ± 1.94	0.75
% GSHR saturation with FAD	66.7 ± 15.7	67.0 ± 6.5	68.7 ± 12.2	80.3 ± 13.5	0.26
Adenosine deaminase	1.03 ± 0.29	0.96 ± 0.22	1.23 ± 0.39	1.04 ± 0.49	0.60
Pyrimidine 5'-nucleotidase[a]	5.52 ± 1.76	7.57 ± 2.18	7.26 ± 1.59	6.71 ± 2.50	0.56
Acetylcholinesterase[a]	50.5 ± 10.0	59.7 ± 9.1	60.9 ± 6.7	54.4 ± 17.9	0.54
δ-Aminolevulinic acid dehydratase	65.8 ± 21.1	55.8 ± 39.5	56.1 ± 22.1	66.5 ± 18.7	0.78
Aldehyde dehydrogenase	0.78 ± 0.21	1.39 ± 0.53	0.90 ± 0.37	1.24 ± 0.82	0.23
Aspartate aminotransferase	4.41 ± 1.51	3.40 ± 0.96	3.69 ± 0.81	4.43 ± 1.34	0.53
Alanine aminotransferase	0.33 ± 0.18	0.27 ± 0.14	0.21 ± 0.10	0.31 ± 0.19	0.55
Adenosine triphosphate	4.05 ± 0.45	3.99 ± 0.65	4.46 ± 0.81	4.42 ± 0.81	0.47
2,3-Diphosphoglycerate	15.6 ± 2.4	14.6 ± 0.9	14.2 ± 1.4	15.5 ± 1.4	0.49
Glutathione	4.10 ± 0.69	4.62 ± 0.74	5.37 ± 2.30	4.86 ± 1.41	0.57

[a] Only the first 17 subjects studied (IA = 3, IG = 3, II = 7, III = 4).

the anticipated relationship to whole blood lead, Pearson $r = -0.5$, $P < 0.02$, despite the very low levels of lead in the population. Although the relationship was not significant, there was also a trend among all subjects together toward lower ALAD with increased alcohol consumption, $r = -0.25$. Further, the increase in ALAD activity resulting from addition to the assay of dithiothreitol was strongly correlated to the initial level of ALAD depression ($r = -0.56$, $P = 0.01$), suggesting that much of the variability in ALAD activity *in vivo* is reversible, as one would expect from lead inhibition.

There was no significant difference among the study groups for reduced GSH or activity of glutathione reductase (GSHR) assayed in the absence of flavin adenine dinucleotide (FAD) (Table 3). However, the percentage saturation of GSHR with FAD was low in both of the exposed painter groups, with half of the subjects falling below 70% saturation (mean 68.3 ± 11.9%), compared to the unexposed subjects, only one of whom had a saturation below 70% (mean, 80.3 ± 13.5%, $P = 0.05$). This is depicted in Fig. 1.

A difference among groups is suggested for pyruvate kinase (Table 3). As can seen in Fig. 2, subjects in Group I have lower PK levels than those in the two control groups, whose levels are comparable ($P = 0.05$). Regression analysis failed to reveal any demographic or exposure factor other than study group which might explain this relationship. Further, no correlation with other measured enzymes was found to suggest a red cell age effect.

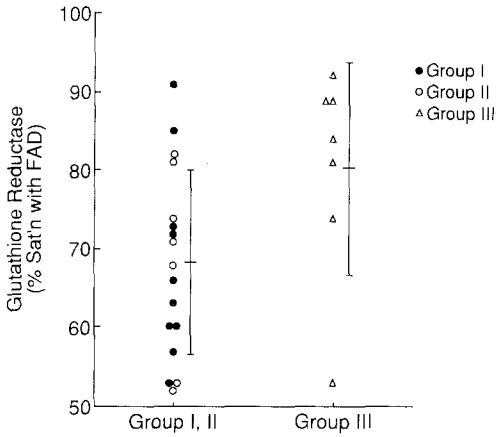

FIG. 1. Percentage saturation with FAD of glutathione reductase in exposed subjects (Groups I and II, mean 68.3 ± 11.9%) compared with unexposed controls (Group III, mean 80.3% ± 13.5, $P = 0.05$). (●) Group I, (○) Group II, (△) Group III.

DISCUSSION

The results of these investigations must be interpreted in the context of our previous survey of painters (Sparer *et al.*, 1988; Welch and Cullen, 1988) and existing knowledge about the hematologic effects of ethylene glycol ethers. As noted in the Introduction, the initial painter survey was conducted to determine if there were effects on blood counts which could be related to relatively heavy and well-documented exposure of painters to ethylene glycol ethers. The results demonstrated that a small portion of the population had marginally low hemoglobins and total granulocyte counts which were subsequently shown to have been acquired since employment in most cases. Unexpectedly, black painters were

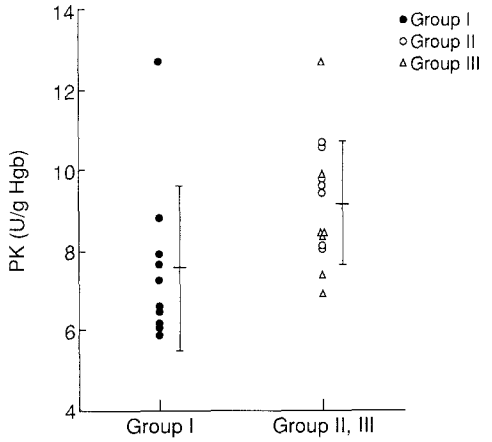

FIG. 2. Pyruvate kinase (PK) levels in the painters with originally abnormal peripheral counts (Group I, mean 7.6 ± 2.0 U/g Hb) and the exposed and unexposed controls (Groups II and III, mean 9.2 ± 1.5, $P = 0.05$). (●) Group I, (○) Group II, (△) Group III.

strongly overrepresented in the affected group. No clear dose–response relationship to glycol ethers could be shown, while exposure to other potential hazards in the painting environment, such as lead, benzene, or ionizing radiation, were reasonably well excluded. There remained three tenable hypotheses to explain the finding: (1) Some painters may have acquired mild hematologic disorders during the course of employment totally unrelated to any exposure factor at work. (2) Since blacks are known to have generally lower hemoglobins and granulocyte counts than whites (Orfanakis *et al.*, 1970; Bain *et al.*, 1984; National Center for Health Statistics, 1983), results could represent normal variability in counts within the painter population unrelated to any pathologic process, due to exposure or otherwise. (3) Some acquired or genetic host factor could predispose a minority of painters to subclinical effects from solvent exposures. This follow-up study was conducted in order to exclude the first two hypotheses and explore the third. As such, the study was designed in such a way that it might provide further indirect support for a causal relationship between ethylene glycol ether exposure and hematologic effects but could not directly test a causal hypothesis because subjects were not randomly drawn from exposed and unexposed populations.

Review of the now extensive data base on each of the subjects previously found to have mild abnormalities on complete blood count again failed to reveal a specific diagnosis unrelated to work exposures. Given no alternative diagnostic finding, we had fully anticipated some pattern histologically or on marrow cultures or cytogenetic studies to suggest a common injury effect for the Group I subjects. Further, we had supposed at the outset that similar, less prominent findings might also be demonstrable among Group II exposed subjects with still normal counts. Based on our prior experience with histologic studies of printers exposed to glycol ethers (Cullen *et al.*, 1983a), we had predicted a histologic pattern of depressed total cellularity, low myeloid–erythroid ratios, and possibly sideroblastic or infiltrative changes. As can be seen from the results, Group I subjects had average cellularity. While there was a significant depression in ME ratio as expected this was more strongly related to race than exposure. Indeed, the race effect on ME ratio, significant at the 0.0003 level, represents a strong and serendipitous finding in a study of this size. No other histologic finding was seen which differentiated the marrows of Group I subjects from the others. Marrow cytogenetics also failed to provide the expected differential features which might have allowed characterization of a glycol ether or other exposure effect. Further, previous studies of solvent-exposed workers (Kelsey *et al.*, 1988; Watanabe *et al.*, 1983) and glycol ether-exposed animals (McGregor, 1984) had suggested the possibility of an effect on sister chromatid exchange levels but no differences among our groups were found in SCE levels.

The depression of red cell pyruvate kinase among the Group I subjects provides the single finding in this study to exclude the hypothesis that the abnormalities noted on the survey represent normal variation in a largely nonwhite subgroup of painters. This enzyme marker did not vary with race or with any exposure parameter, but was depressed more than one standard deviation below the control mean in almost every subject in Group I. It is of note that low pyruvate kinase activity is the most consistent red cell enzyme defect noted in acquired hemato-

logic disorders (Valentine *et al.*, 1973; Mohrenweiser *et al.*, 1981; Kahn *et al.*, 1977; Bowin *et al.*, 1975; Renoux *et al.*, 1978, Etiemble *et al.*, 1979; Arnold *et al.*, 1974; Lintula, 1986). While we could not establish that the result is indeed an effect of marrow injury rather than a predisposing risk factor, nor exclude the possibility that the result represents a coincidental finding since many enzymes were compared, it is certainly an observation worthy of further investigation.

It has previously been suggested that glutathione and glutathione reductase may be affected by glycol ether exposure (Lamonova and Klimova, 1977). In this study, the level of saturation of GSHR and FAD was depressed among both the painter groups compared to unexposed controls. As compared with animal data (Lamonova and Klimova, 1977) our data point to a defect in riboflavin metabolism rather than a reduction in red cell GSH level. That this could be related to the observed hematologic effects is suggested by well-established animal models of riboflavin deficiency causing hypoplastic anemia (Wintrobe *et al.*, 1944; Foy *et al.*, 1968; Lane and Alfrey, 1963). That riboflavin deficiency could also be related to the defect in pyruvate kinase activity has been suggested by previous investigators (Staal *et al.*, 1975) and could provide a unifying interpretation of our study results.

Assuming that some biologic effect on marrow has indeed occurred, the investigation of possible host susceptibility factors to explain it also proved inconclusive beyond the racial association already apparent. The leading contender had been aldehyde dehydrogenase variability, genetic and/or acquired, in view of the role of hepatic alcohol and aldehyde dehydrogenase in metabolizing ethylene glycol ethers (Miller *et al.*, 1982; Nelson *et al.*, 1984). It had been hypothesized that chronic enzyme induction, by regular alcohol consumption, drug ingestion, or solvent exposure, might enhance susceptibility while exposure to enzyme inhibitors such as alcohol during exposure to glycol ethers might be protective as they are in ethylene glycol (anti-freeze) exposure. As noted, neither questionnaire nor biologic data support such a relationship. However, it must be pointed out that red cell, not hepatic, enzyme levels were measured. Further, the possibility of competitive inhibition in the actual work environment, as opposed to the conditions on the day of the study, might have confounded a real relationship which deserves further testing, perhaps in an animal model.

A possible missing link in the host response for ethylene glycol ethers was suggested by the racial clustering of cases in the initial survey. In this study, an unexpected and previously unreported difference in bone marrow histology between whites and blacks was noted. Although based on very small numbers of subjects, the difference was large and the association very strong. This finding precluding the possibility of observing any racially related risk factor for a putative glycol ether effect.

Finally there is the issue of the possible clinical relevance of the mild abnormalities of blood counts noted in the 1985 survey. None of the counts was depressed into the clinically significant range, nor was anything demonstrated on histology, marrow cultures, or cytogenetics to suggest lesions of concern. Indeed, based on all available data we have tended to be very reassuring with the study participants. However, the results of the analysis of pyruvate kinase do provide

the basis at least for pause. While we have noted that we could not prove that depressed levels among affected painters were acquired, nor establish that they are related to marrow injury as has been proposed for the myeloproliferative disorders and myelodysplastic syndromes, the consistent PK depression in the subjects raises the possibility of underlying marrow injury more serious than is obvious by other criteria. As with other speculations from the data, this consideration merits further evaluation in animal and human studies of ethylene glycol ether injury. In the meantime, the interpretation aside, pyruvate kinase may serve as a useful marker for depressed cell counts in ethylene glycol ether-exposed individuals.

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