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# Erythropoietin-independent colonies of red blood cells and leukocytosis in a worker exposed to low levels of benzene

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**Background** — Exposure to high levels of benzene commonly results in the suppression of hemopoiesis, although cases of leukocytosis and leukocytosis with thrombocytosis have been reported. No hematologic abnormalities have generally been found with exposure to low levels of benzene.
**Methods** — A pipe fitter exposed to low levels of benzene (time-weighted average 0.9 ppm) developed leukocytosis. His blood counts and growth of erythroid burst forming units (BFU-E) was followed with and without the addition of erythropoietin.
**Results** — Erythropoietin-independent BFU-E colonies were increased to 40 per $4 \times 10^4$ cells (normal $<3$ per $4 \times 10^4$ cells). Both the leukocyte count and the number of erythropoietin-independent BFU-E colonies decreased when exposure to benzene was terminated. On reexposure the white blood count again increased. After the work was terminated, the white blood count returned to normal, as did the number of erythropoietin-independent BFU-E colonies, over a period of 12 months.
**Conclusions** — Our findings suggest that even low levels of benzene can result in perturbations of the hemopoietic system. Further studies are warranted to determine whether these findings are idiosyncratic, coincidental, or a more general phenomenon.

**Key terms** — benzene, erythroid colonies, leukocytosis, low exposures.

Although exposure to benzene generally results in the suppression of hemopoiesis, cases of leukocytosis and leukocytosis with thrombocytosis have been reported (1). Aksoy et al investigated 217 apparently healthy workers exposed to concentrations of benzene between 30 and 210 ppm for 3 months to 17 years. Five workers were found to have isolated leukocytosis, and two had leukocytosis with concomitant thrombocytosis (1). On the other hand, Townsend & Fishbeck (2) studied 282 men, most having been exposed to less than 10 ppm for less than 1 year to over 20 years, and none of them were found to have either leukocytosis or thrombocytosis. Still those exposed showed a trend towards a higher white blood cell count than the reference group did. Fishbeck et al (3) studied 10 workers exposed to varying concentrations of benzene and found one worker with intermittent leukocytosis. Collins et al (4) studied 200 persons exposed to 0.01—1.4 ppm; yet all but two workers were exposed to less than 0.2 ppm. They found no difference in the mean white blood cell values of those exposed to benzene and 268 unexposed workers at the same plant.

It has been demonstrated in vitro that the proliferation of erythroid colonies from peripheral blood and bone marrow of patients with myeloproliferative disorders occurs without the addition of exogenous erythropoietin (5—10), even in those with normal total red blood cell mass. This phenomenon has been related to hypersensitivity of the erythroid stem cell to erythropoitin (8) and interleukin-3 (9). Monocytes may promote this independence (10).

In the present case study we investigated the ability of the peripheral blood of a 43-year-old fitter with exposure to benzene and leukocytosis to produce in vitro erythropoietin-independent burst-forming unit (BFU-E) colonies, as has been found in myeloproliferative disorders.

## *Case*

A 43-year-old fitter was employed in maintenance work at a terminal for the shipping of chemicals in the Haifa harbor over a 10-year period. His job required joining, cleaning, and repairing pipelines. His chemical exposure included benzene (3% by volume), toluene, xylene, phenol, methyl chloride, white spirit, acetone, ethylacetate, amyl alcohol, and inorganic acids. His personal level of benzene exposure was found to be 0.9 ppm (time-weighted average), and that of phenol was 0.1 ppm. Monitoring tests are done in the harbor every three months and the time-weighted averages for benzene in the areas where he worked ranged from 0.3 to 1.2 ppm over the last year. During a routine annual examination, he was found to have a white blood cell

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count of 15 100 · $mm^{-3}$, with a normal differential count.

Repeated testing revealed a consistently elevated count (figure 1). The leukocyte alkaline phosphatase score was elevated on two separate occasions (230 and 300 with a control of 58, N < 100). In a urine collection the phenol level was found to be 36 mg · $l^{-1}$.

*Methods*

Peripheral blood was collected with preservative-free heparin. Low-density mononuclear cells were isolated by fractionation on Ficoll-Hypaque density gradient, washed with phosphate-buffered saline, and resuspended in Iscove medium supplemented with 10% fetal calf serum. BFU-E colonies were grown from peripheral blood according to a microwell modification of the methylcellulose technique (10). Low-density mononuclear cells ($4 \times 10^4$ cells per ml) were suspended in Iscove's modified Dulbecco's medium (Biological Industries, Kibbutz Beth Haemek, Israel) with 0.8% methylcellulose, 10% fetal calf serum, 0.01% 5-thioglycerol, and a penicillin-streptomycin solution. BFU-E colonies were scored after 14 d of incubation in a fully humidified atmosphere with 5% carbon dioxide at 37°C. The colonies with at least three clusters of 50 cells were defined as BFU-E. Selected colonies were stained with benzidine for the identification of their erythroid lineage; otherwise they were identified by their reddish color.

*Results*

The number of erythropoietin-independent BFU-E colonies was increased to 40 per $4 \times 10^4$ cells (normal < 3 per $4 \times 10^4$). When the exposure to benzene was terminated, the BFU-E (erythropoietin-independent) decreased to 20 and then to 7 and the leukocyte counts returned to normal. After reexposure, the patient's leukocyte count again increased to over 18 000. He has subsequently stopped working and stopped smoking, and his leukocyte count has remained normal over a year of follow-up. The erythropoietin-independent BFU-E colonies decreased to 2 per $4 \times 10^4$. The leukocyte alkaline phosphatase scores also returned to normal.

*Discussion*

Leukemogenesis is thought to be a multistep process. Whether the findings from our patient represent one reversible step in the carcinogenic process is uncertain, however. Benzene has been shown to activate protein kinase C both in vitro and in intact platelets (11). Protein kinase C plays a central role in signal transduction and is thought to mediate several tumor promoters (12). Introducing oncogenically activated receptor kinases has been shown to alleviate completely the requirements for epidermal growth factor for the growth of mouse keratinocytes (13). This finding may be analogous to our finding of the erythropoietin-independent growth of red cell progenitor colonies. A rapid decrease in the incidence of leukemia when exposure to benzene is terminated has been reported and is consistent with the finding of a reversible step in the carcinogenic process (14). Finally, two cases of chronic myelogenous leukemia have been reported among workers exposed to high levels of benzene (14).

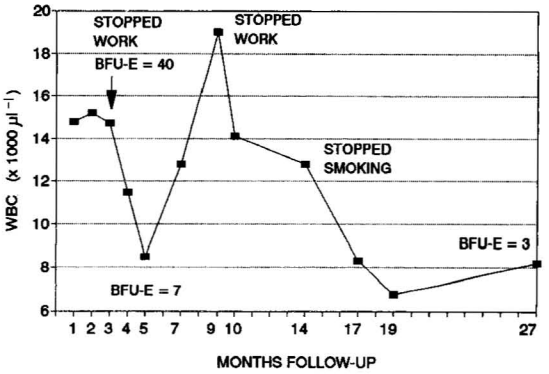


**Figure 1.** White blood count (WBC) of the patient after work was stopped on two occasions. The erythropoietin-independent erythroid burst forming units (BFU-E) decreased from 40 to 3 per $4 \times 10^4$ cells after work and smoking were stopped.

Our results should be interpreted with caution. The fitter was exposed to many other chemicals, and our findings could have been a result of exposure to one of these chemicals or merely coincidental. Still perturbations in the hemopoietic system have not been reported with the other chemicals. There are many causes of leukocytosis, such as mild chronic infections, inflammatory diseases, and allergic reactions. Our patient however was asymptomatic. The work challenge test was positive, and no leukocytosis was found during the follow-up after the exposure was terminated. Therefore we believe that benzene was likely to be responsible for the effects noted. Further studies of workers with and without leukocytosis and exposed to low levels of benzene are warranted in order to substantiate our findings. Animal studies and epidemiologic studies have suggested that the "no effect" exposure level for the hematologic effects of benzene in humans is 25 ppm, the time-weighted average suggested by the American Conference of Governmental Industrial Hygienists (4). In a study of workers exposed chronically to levels under 1 ppm with occasional levels over 100 ppm, no effects were found on hematologic or biochemical parameters, proliferative rate indices, sister chromatid exchanges, and urine mutagenicity, except for a small but significant increase in mean corpuscular volume (15). Such tests may, however, lack sensitivity.

It is uncertain whether our findings were idiosyncratic or a general phenomenon related to the devel-

opment of malignancy. We are planning a study of progenitor cells in a cohort of workers exposed to low levels of benzene. Since the abnormalities found in our patient were reversible, it is unlikely that he is at increased risk for developing leukemia. Still he is possibly more sensitive to the effects of benzene than other workers, and we have recommended that he not be returned to work in areas where there are even low exposure levels of benzene.

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