Long-Term Effect of Benzene in C57BL/6N Mice

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Weanling male C57BL/6N mice received repeated subcutaneous injections of benzene in corn oil for 54 weeks.

These mice and surviving controls were killed 104 weeks after the first injection. There was no evidence of carcinogenic activity in benzene-injected mice. A high incidence of amyloidosis developed in mice of all treated and untreated groups. Butylnitrosourea, used as a positive control, induced lymphomas or leukemias (in most exposed mice) and intestinal tumors in a few.

Many reports suggest that in man industrial exposure to benzene increases the risk of leukemia.[1-4] However, attempts to induce neoplasms in laboratory animals have generally failed.[5-11] Since C57BL mice have been shown to be quite sensitive to agents like radiation or chemicals that induce leukemia. this strain of mice was used to test the potential effect of benzene and develop an animal model.[12,13] Whereas the normal route of exposure in man is by inhalation or by cutaneous absorption, we elected to develop the model by testing benzene by subcutaneous injection, mainly in order to administer as high a dosage as possible. If positive results were obtained, then other mutes simulating more closely the human route could be used.

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MATERIALS AND METHODS

Weanling male C57BL/6N mice were obtained from the Animal Production Section, Division of Research Resources, National Institutes of Health. They were housed. ten to a polycarbonate age (29 × 17 × 12.5 cm) in an air-conditioned animal room. Food was laboratory chow (Wayne Lab Blox) and hardwood chips were used for bedding. After one week of acclimatization, the mice were injected subcutaneously with benzene (80 mice) or corn oil (20 mice) or were untreated (20 mice). The benzene (99% pure) for injection was prepared as a 30% solution by measuring 30 ml of benzene and diluting it with liquid corn oil (Mazola), to a final volume of 100 ml. After several toxicity trials, the injection schedule developed for this experiment was as follows: mice received injections twice weekly for 44 weeks, and then once weekly until 54 weeks. Mice given 30% benzene were injected with 0.05, 0.1, 0.1, and 0.2 ml for the first four weeks, respectively, followed by 0.2 ml until the last injection. Oil-injected mice received 0.025, 0.05, 0.05, and 0.1 ml, respectively, for the first four weeks followed by 0.1 ml until the last injection. The lower dose given to the oil-injected mice was used when it was found that 0.2 ml of corn oil injected twice weekly did not disperse rapidly enough. However, mice receiving 30% benzene in corn oil readily absorbed the 0.2 ml injection, of which 0.14 ml consisted of corn oil.

At 104 weeks after the first injection, all surviving mice were killed (108 weeks of age) and a complete necropsy was performed. as had been done with all mice that died Tissues were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 6μ and stained with hematoxylin-eosin. Selected tissues were stained with Congo red for amyloid and by the periodic acid-Schiff method. Portions of liver from one mouse with amyloidosis were fixed in glutaraldehyde for ultrastructural examination, and processed for study in an electron microscope.

As a positive control, butylnitrosourea (melting point 82 C), synthesized according to the procedure of Werner,[14] was given to 20 male C57BL/6N mice, 4 weeks of age, in the drinking water at a concentration of 1 mg/3 ml (approximate daily dose) of water for 120 days. Surviving mice were killed at 52 weeks.

sensitive sites. Release or activation lit a leukomogenic virus also deserves consideration.

The lymphomas induced by butylnitrosourea were identical to those reported previously in mice." This is the first report of the finding of intestinal tumors in mice, although they were found previously in some rats given the chemical.[14,25]

The development of intestinal neoplasms is similar to the recorded finding of such tumors after oral dosage of methylnitrosourea." It would appear that the fairly polar chemical is not absorbed readily, migrates down into the lower intestine, and thus leads to carcinogenesis along the entire tract.

The finding of mites on the chronic skin lesions is reminiscent of previous reports on the subject.[27-29] Evidently, the scratching caused by the mites leads to acute skin lesions. In more recent experiments, with C57BL/6N mice, the scratching begins at 7 to 9 months of age in mice kept on hardwood chips only. Cedar bedding or mixtures containing cedar chips had both a preventive and curative effect. For an unknown reason, the black mice were more susceptible to thin condition than were other strains. The chronic skin lesions that developed led to amyloidosis in internal organs and eventual death.[27,28,30] The mean survival of these mice would probably have been increased if mites were not present or if cedar bedding had been used." However, benzene exposure did not appear to affect this disease process.

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References

1. Aksoy MKN, Akgun T, Erdem S, *et al*: Hematological effects of chronic benzene poisoning in 217 workers. *Br J Ind Med* 28:296-302, 1971.

2. Browning E: *Toxicity and Metabolism of Industrial Solvents*. Amsterdam, Elsevier Publishing Co, 1965, pp 3-65.

3. Goldwater LJ: Disturbances in the blood following exposure to benzene. *J Lab Clin Med* 26:957-964, 1941.

4. de Gowin RL: Benzene exposure and aplastic anemia followed by leukemia five years later. *JAMA* 185:748-751, 1963.

5. Ishimaru T, Okada A, Tomiyasu T, et al: Occupational factors in the epidemiology of leukemia in Hiroshima and Nagasaki. *Am J Epidemiol* 98:157-165, 1973.

6. Rondanelli EG, Gorini P, Genu G, *et al*: Pathology of erythroblastic mitosis in occupational benzene erythropathy and erythremia. *Bibl Haematol* 35:1-188, 1970.

7. Tareeva EM, Konchalovskaya NM, Zorina LA: Benzene leukemia. *Acta Un Int Cancer* 19:751-755, 1963.

8. Vigliani EC, Saita G: Benzene and leukemia. *N Engl J Med* 271:872-876, 1964.

9. Amid JL: Negative attempt at induction of leukemia in mice by benzene. *Rev Franc Etudes Clin Biol* 5:198-199, 1960.

10. Iversen OH, Iversen U: A study of epidermal tumorigenesis in the hairless mouse with single and repeated applications of 3-methylcholanthrene at different dosages. *Acta Pathol Microbiol Scand* 62:305-314, 1964.

11. Kirschbaum A, Strong LC: Influence of carcinogens on the age incidence of leukemia in the high leukemia strain F of mice. *Cancer Res* 2:841-845, 1942.

12. Luerum OD: Reticulum cell neoplasms in normal and benzene treated hairless mice. *Acta Pathol Microbiol Scand* (A) 81:57-63, 1973.

13. Kaplan HS, Brown YB: A quantitative dose-response study of lymphoid-tumor development in irradiated C57 Black mice. *J Natl Cancer Inst* 13:185-208, 1952.

14. Yokoro K, Imamura N, Takizawa S, et al: Leukemogenic and mammary tumorigenic effects of N-nitrosobutylurea in mice and rats. *Gann* 61:287-289, 1970.

15. Werner EA: The constitution of carbamides: Part IX. The interaction of nitrous acid and mono-substituted ureas; The preparation of diazomethane, diazoethane, diazo-n-butane, and diazocyclopentane from the respective nitrosoureas. *J Chem Soc* 115:1093-1102, 1919.

16. Dunn TB, Deringer MK: Reticulum cell neoplasm, type B, of the "Hodgkin's-like Lesion" of the mouse. *J Natl Cancer Inst* 40:771-821, 1968.

17. Dunn TB: Normal and pathologic anatomy of the reticular tissue in laboratory mice. *J Natl Cancer Inst* 14:1281-1433, 1954.

18. Matsushita T: Experimental studies on the disturbance of hematopoietic organs due to benzene intoxication. *Nagoya J Med Sci* 28:204-234, 1966.

19. Noeske K, Martens H: Histological and histometrical investigations of benzene-induced lesions of rat bone marrow. *Virchows Arch [Pathol Anat]* 355:50-65, 1972.

20. Philip P, Jensen MK: Benzene induced chromosome abnormalities in rat bone marrow cells. *Acta Pathol Microbiol Scand (A)* 78:489-490, 1970.

21. Deichmann WB, MacDonald WE, Bernal E: The hemopoietic tissue toxicity of benzene vapors. *Toxicol Appl Pharmacol* 5:201-207, 1963.

22. Yeh S: Skin cancer in chronic arsenicism. *Hum Pathol* 4:469-485, 1973.

23. Grover PL, Hewer A, Sims P: Formation of K-region epoxides as microsomal metabolites of pyrene and benzo-a-pyrene. *Biochem Pharmacol* 21:2713-2726, 1972.

24. Kaubisch H, Daly JW, Jerina DM: Arene oxides as intermediates in the oxidative metabolism of aromatic compounds: Isomerization of methyl-substituted arene oxides. *Biochemistry* 11:3080-3088, 1972.

25. Odashima S: Leukemogenesis of N-nitrosobutylurea in the rat. *Gann* 61:245-253, 1970.

26. Leaver DD, Swann PF, Magee PN: The induction of tumours in the rat by a single oral dose of N-nitrosomethylurea. *Br J Cancer* 23:177-187, 1969.

27. Dunn TB: Amyloidosis in mice, in Cotchin E, Roe FJ (eds): *Pathology of Laboratory Rats and Mice*. Philadelphia, FA Davis Co, 1967, pp 181-212

28. Galton M: Myobie mange in the mouse leading to skin ulceration and amyloidosis. *Am J Pathol* 43:855-862, 1963.

29. Baton WE, Deringer MK: Hereditary renal disease and amyloidosis in mice. *Arch Pathol* 46:49-58, 1948.

30. Tsuang PJ: Senile amyloidosis in mice. *Gerontologia* 1:269-279, 1957.

31. Storer J B Longevity and gross pathology at death in 22 inbred mouse strains. *J Gerontol* 21:404-409, 1966.

Note: has a plausicable explaination
as to why ~~[illegible]~~ animals
studies do not react the
same as man to Benzene
exposure.