

Chemical Leukemogenesis in Man

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EXHIBIT

The possibility that chemicals as well as ionizing radiations might play a role in human leukemogenesis was raised many decades ago and has been consistently discussed since then. Numerous substances have been incriminated, mostly on the basis of single observed cases. Most of these substances have in common the peculiarity of inducing bone marrow hypoplasia which may recover, proceed to aplasia, or terminate in leukemia. In a paper on this subject, Cronkite (12) stated that 'any drug or chemical capable of inducing bone marrow damage must be assumed to be potentially a leukemogen'. However, the leukemogenic effect of most chemicals in man is difficult to prove. Observation of single cases may not be significant: in some cases exposure has been to more than one substance; moreover, for most suspected substances the real number of exposed subjects is not known, so the incidence of leukemia in such subjects cannot be compared to that in the general population.

The chemical which has resisted all these criticisms is benzene, an occupational poison which has given rise in the past to epidemics of aplastic anemia and leukemia. This chapter will therefore deal mostly with benzene, and will then consider some other chemicals presently suspected to be leukemogenic in man.

Benzene, the first aromatic hydrocarbon, is a highly volatile solvent widely used in industry, in both pure and commercial forms. It evaporates at room temperature and enters the body mostly by inhalation. The vapor in very high concentration is acutely toxic to the central nervous system, while chronic exposure to benzene induces hematologic changes. Workers are exposed to benzene in the processes of extraction and purification, in the chemical industry for the production of its derivatives, and in the pharmaceutical industry for the manufacture of drugs; in most of these processes, however, cases of poisoning are very rare, due to the use of closed equipment.

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TABLE I

Cases of leukemia in subjects with chronic benzene exposure, identified in the Paris region from 1950 to 1965

Identified cases (37 males and 7 females)	44
Acute leukemia	23
Chronic myeloid leukemia	13
Chronic lymphocytic leukemia	8

From Gopuel et al. (31).

Conversely, processes involving the use of benzene as a solvent in the manufacture of rubber-containing products, in shoe manufacture, in rotary gravure printing, etc., may expose workers to chronic inhalation of benzene in high concentrations and may be responsible for the hematologic effects of chronic poisoning. Therefore the use of benzene or of mixtures containing high concentrations of benzene for these purposes has been prohibited by law in many countries and benzene has been replaced by benzene homologues (toluene, xylene) or other solvents which are non-toxic to the bone marrow. However, the risk of benzene poisoning is still present in some countries, especially in small workshops where conditions of hygiene are poor. Reduction of the alkyl-lead content of gasoline tends to raise the benzene percentage. Once it has entered the body, benzene is partly soluble in the tissues, especially those rich in lipids, and partly converted in the liver to phenol and other oxidation products such as hydroquinone and pyrocatechol, conjugated with sulphuric and glycuronic acids, and is excreted through the kidneys (see 5 and 66 for a list of references).

The depressive effect of benzene on hemopoiesis, resulting in aplastic anemia, has been known since the last century. The myelotoxic action of benzene was also exploited in the treatment of chronic myeloid leukemia in the past, but such use is now only of historical value.

Only later was benzene recognized as leukemogenic. The first report of a case of leukemia attributed to benzene was that by Delore & Borgesen (17). In a critical review of the literature on benzene hemopathy in 1955 Penati & Vigliani (55) observed that some cases diagnosed as aplastic anemia did not actually fit this diagnosis owing to myeloid proliferation, especially in the spleen and liver, and to a leukemoid reaction in the peripheral blood, which was more suggestive of leukemia than of aplastic anemia. Since then, various reports of benzene leukemia have appeared; besides the numerous cases reported by Vigliani & Saita (71), who summarized the situation in Italy up to 1964, and the large groups collected by Cavaliere

TABLE II
*Cases of benzene hemopathy in the provinces of Milan (1960-1966)
 and Pavia (1960-1964)*

44	Compensated cases	81
	Alive	49
	Anemia	49
	Dead	32
	Anemia	14
	Acute leukemia	17
	Erythroleukemia	1

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(9). Goguel et al. (31), and Girard et al. (30) in France, numerous single cases or small groups of cases have been reported, especially in Europe (1, 6, 15, 22, 23, 34, 40, 45, 46, 62, 63, 64, 67, 74). An approximate estimation of the available literature, including cases collected by Browning (5) and some unpublished North-Italian cases of which we have knowledge through the National Institute for Insurance against Accidents and Occupational Diseases, put the number of known cases of leukemia attributed to benzene at at least 150.

The most convincing cases of benzene leukemia are those occurring in factories where outbreaks of chronic benzene poisoning have been observed. This was true, for instance, for the series reported by Cavigneaux (9) and by Vigliani & Saita (71), where the incidence of leukemia in benzene hemopathy was approximately 12-15 per cent. The cases observed in the provinces of Milan and Pavia occurred in shoe factories and in rotogravure plants, where benzene was used as a solvent for glues and inks respectively. In these factories the concentrations of benzene vapor in the air of the working environment were demonstrated to be well above the threshold limit (71).

Benzene leukemias are mostly *myeloblastic* or *myeloblastic*. Most cases have very low white cell counts or show only a moderate leukocytosis with a small percentage of immature cells, except in the terminal stage. The bone marrow shows infiltration by undifferentiated cells, mostly microhemocytoblasts, or by myeloblasts which are sometimes atypical. There is generally moderate or no spleen or liver enlargement. However, in a few cases the bone marrow is poor in leukemic cells and there is leukemic infiltration in other organs such as the spleen and liver (71). Often leukemia develops in subjects with benzene-induced hyporegenerative anemia or pancytopenia of long standing; in these cases, during the course of the disease, the bone marrow changes from a hypoplastic to a leukemic pattern (65).

Generally, aplastic anemia and leukemia occur in subjects who are exposed to high concentrations of benzene; however, in a few cases there may be a long latency period (up to 15 years) between cessation of exposure to benzene and occurrence of leukemia (15, 71). In a recent unpublished case that we observed (C. A., female, 66 years old), the patient who had suffered from benzene anemia in 1957, had since had a free period of 14 years with normal blood counts before the onset of acute leukemia in 1972; she died 5 months after leukemia was diagnosed. Acute benzene-induced leukemias are invariably fatal, even though the available chemotherapy and supportive treatment may somewhat prolong survival.

Among the benzene leukemias, at least 20 cases of acute erythroleukemia have been reported (13, 23, 27, 31, 54, 64) (see 5 and 66 for additional references). Among the cases of benzene leukemia observed at the Institute of Occupational Health of the University of Milan from 1943 to 1973, 10 were acute hemocytoblastic or myeloblastic leukemias and 3 were erythroleukemias. In view of the rarity of this disease, this number of cases seems to be significant.

In the French reports (9, 30, 31), cases of chronic myeloid and chronic lymphocytic leukemia were attributed to benzene, together with the numerous cases of acute leukemia (Table I). This is neither our experience nor the experience of the Institute of Occupational Health of the University of Pavia; in both these series acute leukemia occurred at the same time as cases of aplastic anemia only in factories where high concentrations of benzene in the air were recorded (Table II). Therefore the possibility that benzene might be responsible for chronic leukemia remains an open question. For acute leukemias, Vigliani & Seita (71) calculated that in subjects exposed to benzene in the provinces of Milan and Pavia during the early nineteen-sixties, the risk of acute leukemia was at least 20 times higher than in the general population. Recently, in a retrospective statistical study of severe hemopathies in Lyon hospitals, Girard & Revol (29) found a history of exposure to benzene and/or its homologues in the preceding 10 years in a significantly higher proportion of patients with acute leukemia, chronic lymphocytic leukemia and aplastic anemia than among control subjects with diseases other than of the hemopoietic system; this was not the case, however, for chronic myeloid leukemia and other blood disorders.

It is interesting that the banning of benzene as a solvent for glues and inks, after the outbreaks of aplastic anemia and leukemia in the provinces of Milan and Pavia, led to a sharp decrease in the number of cases of chronic benzene poisoning. In the last few years only occasional cases occurred in some small workshops, where benzene was still being used despite

subjects who are still in a few cases there between cessation of exposure (71). In a recent unpublished report (71), the patient, since had a free period onset of acute leukemia diagnosed. Acute benzene in the available chemotherapy along survival. acute erythroleukemia 5 and 66 for additional observed at the Institute from 1943 to 1973, 8 cases and 3 were erythrocyte number of cases seems acute myeloid and chronic leukemia together with the more is neither our experience. Health of the University occurred at the same time as the high concentrations of benzene before the possibility that a remains an open question. calculated that in subjects in Pavia during the early 1950s, at least 20 times higher than the effective statistical study of Levol (29) found a history of leukemia in the preceding 10 years. In acute leukemia, chronic leukemia, among control subjects; this was not the case. blood disorders. benzene is a solvent for glues and used in leukemia in the province of Pavia. the number of cases of leukemia only occasional cases benzene was still being used despite

the prohibition. As previously reported, however, occasional cases of acute leukemia with a long latency period may still be observed.

All these epidemiological data are in favor of a leukemogenic action of benzene in man, either directly or through aplastic changes in bone marrow. The results of animal experiments on this subject are scanty and conflicting (5). It is also doubtful whether benzene is active *per se* or through its metabolites (phenol, hydroquinone, pyrocatechol); experimental data seem to attribute the toxic effects on hemopoiesis especially to phenol and pyrocatechol (51).

Other chemicals suspected of being leukemogenic in man are drugs such as phenylbutazone and chloramphenicol, both capable of inducing bone marrow depression as a side effect.

The first report of six cases of leukemia in subjects who had received phenylbutazone (from 10 grams to several hundred grams) for spondylitis or arthritis, was by Bean in 1960 (2). This report was followed by several others which have been reviewed by Krogh Jensen & Roll (41) and Fraumeni (25). In the latter paper, 29 cases of leukemia attributed to phenylbutazone are collected from the literature and discussed; some cases did not seem to have an established diagnosis of leukemia; of the others, only 13 had a time interval of at least 18 months between the start of drug treatment and the onset of leukemia. In a retrospective study of 55 patients with acute leukemia in Australia, 9 per cent had started receiving phenylbutazone for arthritis from 2½–6½ years before the onset of the disease and had received X-ray treatment; on the other hand, among 417 subjects with chronic leukemias and lymphomas, only 1.2 per cent had received phenylbutazone prior to the onset of their disease; in one case of acute leukemia after phenylbutazone, there was a history of bone marrow depression before the diagnosis of leukemia (72). In a short-term follow up of 24 cases registered in the United States because of bone marrow depression due to phenylbutazone, no cases of leukemia were found; 3 cases of acute myeloblastic leukemia not previously reported to the Registry of Adverse Drug Reactions were collected during the time of study and all had evidence of bone marrow depression prior to the onset of leukemia (25). In the absence of data on the actual number of people receiving phenylbutazone, it is difficult, on the evidence of sparse cases, to decide whether this drug is leukemogenic or whether the occurrence of leukemia is coincidental. It has also been suggested that elderly subjects with spondylitis or arthritis

per se be at a higher risk of leukemia, independently of treatment, but consistent data on this point are lacking. The same problems encountered with phenylbutazone arise when one considers the possible leukemogenic role of chloramphenicol, an antibiotic which since the beginning of

its use has been implicated in the causation of aplastic anemia (see references 57 and 73). According to Yunis (73), this drug can induce different types of blood dyscrasias: (1) a reversible depression of bone marrow, which may occur in many subjects during treatment with large dose over a short period of time; (2) pancytopenia, which occurs very rarely, mostly after prolonged use or repeated courses of the drug and is attributed either to a specific hypersensitivity or to an irreversible bone marrow toxicity. The first two cases of acute myeloblastic leukemia following chloramphenicol treatment were reported in 1955 and 1957 (43, 50). Fraumeni (25), in the follow up of 124 cases of bone marrow depression reported in the Registry of Adverse Drug Reactions of the American Medical Association, found three cases of leukemia, of which only one could probably be attributed to chloramphenicol; in this case aplastic anemia occurred months after the start of treatment and evolved into acute myeloblastic leukemia 2½ years later. Three more cases in subjects not registered as adverse drug reaction were reported during the study; all patients had signs of bone marrow depression prior to the onset of leukemia and developed from 2 to 15 years after the start of treatment.

In discussing the possible role of chloramphenicol and phenylbutazone in leukemogenesis, Fraumeni (25) stated that in order to suggest a causal relationship between drug use and leukemia, the following conditions should be met: relatively high dose of the administered drug; no evidence of leukemia at the time of drug use; and absence of diseases or other agents known or suspected to be leukemogenic; moreover, the cytologic picture should be chronic myeloid or acute leukemia and the interval between onset of drug exposure and development of leukemia should exceed approximately 18 months, as with radiation leukemia. Five cases reported in 1964 and 1968 actually had these characteristics (4, 11, 37). Cohen & Cline (11), while discussing their case, underlined the chemical similarity of chloramphenicol to benzene and phenylbutazone, and the common property of inducing aplastic anemia, and suggested that marrow aplasia is a condition from which leukemia can develop.

All the cases attributed to chloramphenicol were reviewed in a recent paper (26), including three more cases of acute lymphoblastic leukemia in children who had started receiving chloramphenicol 49, 49 and 8 months respectively, prior to the onset of leukemia.

Another interesting point raised in the last few years is the occurrence of leukemia in patients treated over a long period with cytotoxic drugs for cancer or for other diseases.

Long-term melphalan treatment has been implicated in the induction of acute leukemia, mostly of the myelomonocytic type, in 15 cases of multiple myeloma.

causation of aplastic anemia (see also Yunis (73)), this drug can induce two: (1) a reversible depression of bone marrow activity during treatment with large doses of chloramphenicol, which occurs very rarely and recovers after discontinuation of the drug and is attributed to an irreversible bone marrow toxicity; (2) acute myeloblastic leukemia following chloramphenicol treatment in 1955 and 1957 (43, 50). Fraumeni et al. (43) reported two cases of bone marrow depression reported in the Transactions of the American Medical Association, of which only one could probably be this case aplastic anemia occurred and evolved into acute myeloblastic leukemia in subjects not registered for the study; all patients had had no prior to the onset of leukemia and the start of treatment.

Chloramphenicol and phenylbutazone. It is stated that in order to suggest a causal relationship between the administered drug and the development of leukemia, the following conditions should be fulfilled: (1) no evidence of other causes; (2) absence of diseases or other agents which could be leukemogenic; moreover, the cytologic type of leukemia and the interval between start of treatment and the onset of leukemia should exceed approximately 1 year. Five cases reported in 1957 (4, 11, 37). Cohen & Creger (4) underlined the chemical similarity between phenylbutazone, and the common product of its metabolism suggested that marrow aplasia is induced by the development of leukemia.

Chloramphenicol were reviewed in a retrospective study of acute lymphoblastic leukemia in patients treated with chloramphenicol 49, 49 and 8 months before the onset of leukemia.

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multiple myeloma (36, 42, 52); some of these cases had been treated also with X-rays. Two more cases quoted by Kyle et al. (42) had been treated with another alkylating agent, cyclophosphamide. These authors calculated that the frequency of acute leukemia observed in 2 years (three cases among approximately 250 patients with multiple myeloma) was about 240 times the expected rate. In his interesting editorial on the subject, Holland (36) raised the questions of whether acute leukemia might be a natural evolution of multiple myeloma, formerly masked by early death; whether immune impairment in myeloma might cause failure to eliminate abnormal cell clones; whether X-rays given together with chemotherapy might play a role in the leukemogenesis in these patients; whether recurrent episodes of bone marrow depression might result in leukemia; and finally, whether the common factor of long-term chemotherapy may be responsible for the occurrence of leukemia. These questions remain open at present. Single reports of acute leukemia in subjects receiving chemotherapy for malignant diseases have appeared and are quoted by Davis et al. (14) and by Potolsky & Creger (61); the latter authors add three unpublished personal observations of acute myeloid leukemia occurring in patients with lymphoma treated only with cytotoxic drugs. The lack of untreated control cases with the same disease and the same survival times makes it impossible to decide whether acute leukemia is a consequence of treatment or whether subjects with such malignancies are at a higher risk of developing acute leukemia.

The possibility that cytotoxic drugs might be leukemogenic is of special interest, because of the increasing use of these drugs for nonmalignant diseases, with an expected long survival, and this risk should be kept in mind when deciding on their use in young people with non-fatal diseases.

It has been suggested that oxymetholone, an androgen used in the treatment of aplastic anemia, might have been responsible for the development of acute myeloid leukemia in four cases (16), but the evidence seems scanty and has been questioned (44). As is well known, a certain number of aplastic anemias evolve spontaneously into leukemia, and though it has been suggested that treatment with corticosteroids or androgens facilitates the onset of leukemia in aplastic anemia, this seems improbable since these treatments do not increase the risk of leukemia in patients treated for other diseases (44).

With regard to the mechanisms whereby chemicals can induce leukemia, at present more than one hypothesis can be suggested. The first is that the chemical might alter the genome of the hemopoietic cells by interfering directly with nucleic acid synthesis or the mitotic process, thus inducing the formation of abnormal cell clones (somatic mutation). Data supporting this hypothesis have been collected for benzene. The effect of benzene or

its metabolites on which has been known for a long time (19, 53).

In the last decade various papers have appeared on chromosome changes in lymphocytes and bone marrow cells of subjects with benzene hemopathy (20, 21, 59, 60), and in lymphocytes of subjects recovered from benzene hemopathy (21, 58). Subjects with past exposure to benzene but without history of hemopathy may show increased rates of chromosomal changes (24, 33, 69, 70). The chromosome changes observed (breaks and rearrangements) are similar to those induced by ionizing radiations, which are a recognized leukemogenic agent (see 21 for references). Such increased rate of chromosome changes may persist for many years in long-lived peripheral blood lymphocytes, thus indicating that damage has occurred; in some cases abnormal cell clones in peripheral blood could be detected in the absence of any sign of disease (21).

The significance of these findings and their possible relationship to the late occurrence of benzene leukemia are so far not clear. However, one can speculate that chromosomal damage to bone marrow cells, giving rise to stable changes, might be responsible for the formation of abnormal cell clones, one of which might induce leukemia by selective advantage. The hypothesis was put forward on the basis of the findings in a case of benzene hemopathy that evolved into acute myeloid leukemia and was followed up by multiple cytogenetic studies of peripheral blood and bone marrow. Prior to the onset of leukemia, various stable chromosome changes were found in the bone marrow, but as soon as leukemia developed a single abnormal clone was present in the cells of bone marrow and peripheral blood (22). Cell lines with abnormal karyotypes were described also in two other cases of benzene leukemia (23, 67); the karyotype abnormalities were different in the three cases, which is in agreement with the manifold cytogenetic pattern of acute leukemia.

Experimental animal studies have shown that benzene can severely disturb DNA synthesis in hemopoietic cells (49) and induce chromosome aberrations in bone marrow cells (39, 56). It is of interest that another suspected leukemogen, chloramphenicol, can induce structural chromosomal abnormalities (chromosomal vacuolization and breakage) both *in vivo* and *in vitro* in man and in experimental animals (7, 8, 48). A single case of leukemia associated with chloramphenicol treatment, reported only a few months ago, showed a marker chromosome (10). Alkylating agents are also known to induce chromosome breakage (for references, see 68).

It is still doubtful whether chromosome changes are directly responsible for malignant transformation. Some experimental studies seem to support the hypothesis that both viral and chemical carcinogenesis are mediated through changes in chromosome balance (35).

As discussed in greater detail elsewhere in this issue* individuals with Fanconi's anemia and Bloom's syndrome, who show increased rates of chromosome breaks, are at a high risk for leukemia and other neoplasias (3, 28); a high incidence of leukemia is also reported in Down's syndrome (trisomy 21). Cells from these subjects show an increased transformability by virus in culture (18, 47). It seems therefore that cells with chromosomal imbalance or instability might be more susceptible to a possible leukemogenic virus; this might be true also for cells with acquired chromosomal changes due to radiation or carcinogens. Another hypothesis is that of activation of a latent leukemogenic virus by chemical carcinogens. Results of animal experiments support this hypothesis (38), but no data are available for human leukemogenesis. Immune impairment induced by the cytotoxic chemicals might also be responsible for the development of leukemia. Alkylating agents, for instance, which can cause both chromosome damage and immunosuppression, might induce the formation of abnormal cell clones, and these clones might proliferate as a result of the depressed immune surveillance. Benzene might act in a similar way, as suggested by one of us (22).

The fact that in chemical, as well as in radiation leukemogenesis, only some of the subjects exposed develop leukemia raises the question of individual susceptibility to the leukemogenic agents. The possibility that more than one factor or mechanism might be implicated further justifies the concept of differing susceptibility, since a coincidence of various factors would be required for leukemia to occur. In chemical leukemogenesis in the animal, recent experiments have suggested that 'host responses control the proliferation of tumor cells, ... and that occurrence or non-occurrence of the disease is the end result of interactions between tumor cell and host which probably involve physiological regulatory processes, as well as immunological responses' (32). This is probably true for man also, but proof awaits the development of new experimental and statistical methods.

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