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Benzene: A Multipotential Carcinogen. Results of Long-Term Bioassays Performed at the Bologna Institute of Oncology

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Until recently. the incidence of benzene carcinogenicity was based only on the association between benzene occupational exposure and human leukemia. with man! limited case reports and scanty epidemiological data. Available experimental studies up to 1976 on animals were rare. fragmentary. and inadequate. and had failed to prove the carcinogenic effects of benzene. However. an integrated project of long-term carcinogenicity bioassays. begun in our laboratory in 1976 and still continuing. has shown that benzene produces a variety of tumors in rats including Zymbal gland carcinomas. carcinomas of the oral cavity. hepatocarcinonias. and possibly mammary carcinomas. lymphoreticular neoplasias. and other malignancies. Some of the tumors caused by benzene are uncommon or unusual in the breed of rats studied. Therefore benzene must be considered, under the studied experimental conditions. a strong multipotential carcinogen. The need for more experimental research is emphasized. particularly to assess the carcinogenic effects of low doses. Also recommended are more comprehensive epidemiological investigations. extended to all types of malignancies. and the application of adequate measures for primary prevention.

Key words: benzene carcinogenesis, benzene and rat tumors, carcinoma of the oral cavity, carcinoma of the nasal cavity, hepatoma, Zymbal gland carcinoma

INTRODUCTION

In 1976, a project of integrated experimental carcinogenicity bioassay was set Up at the experimental Bentivoglio Unit of the Bologna Institute of Oncology. The first experiment, studying the effects of long-term oral administration of the compound at two dose levels (250 and 50 mg/kg body weight was started that year.

In November 1977. preliminary results indicating carcinogenic effects of benzene were published, and the findings were updated on various occasions in Italy (Istituto Superiore di Sanità) and in the United States (Meeting on "Coping with chemical crisis," New Orleans. November. 1977; American Cancer Society: National Cancer Institute [NCI]; National Institute of Environmental Health Sciences

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[NIEHS]; American Petroleum Institute [API]. Those data represented the first experimental demonstration of the carcinogenic potential of benzene.

Other experiments were then started in sequence and are still in progress. The present report describes the results of those experimental studies, in an attempt to further establish the scientific basis of benzene carcinogenicity. so that preventive measures may be adopted.

The reasons for giving priority to carcinogenicity bioassays on benzene in 1976 were: (1) the great production, widespread use, and industrial importance of the compound; (2) the large number of people potentially exposed; **(3)** the already available data on the association of benzene exposure and leukemia in humans; and (4) the failure of animal experiments to show benzene carcinogenicity. Such failure had been supporting and consolidating the belief that benzene (together with arsenic) was an example of noncorrelation between animal and human data, and so caused doubt and skepticism about the predictive value of long-term carcinogenicity bioassays in animals.

BENZENE—CHEMICAL AND PHYSICAL PROPERTIES, PRODUCTION, AND USES

Benzene (C_6H_6) has the following chemical and physical properties: molecular weight. 78.1: clear. colorless. highly flammable liquid: boiling point. 80.1"*C:* melting point, 5.5°C; density. d_4^{15} 0.8787: refractive index, n_D^{29} 1.5016; volatility. the vapor pressure is 74.6 mm Hg at 20°C; and solubility, slightly soluble in water (0.8 parts by weight in 1,000 parts of water at 20°C): miscible with acetone. alcohol, carbon disulphide, carbon tetrachloride. chloroform. ether. glacial acetic acid. and oils.

Benzene has been produced industrially from coal since 1849. and from petroleum since 1941. At present the major source of benzene is petroleum. It is one of the most widely produced industrial compounds. Worldwide production is estimated at over 15 million tons per year. Its major producers are the United States. Japan. and Western Europe. Italy produces over half a million tons per year.

Its major use in the past was in blends with gasoline. Although this use has been reduced in the United States. benzene is still extensively employed in many countries for the production of commercial gasoline. The benzene content in gasoline varies from country to country. and its range is estimated to he from 1% to 15%.

Presently. benzene is also widely used as a chemical intermediate for the production of many important industrial compounds. such as ethyl benzene (used in the production of styrene). phenol. cyclohexane. maleic anhydride. aniline. dichlorobenzenes. etc. which. in turn, supply numerous sectors of the chemical industry, especially those producing plastics, resins, elastomers, dyes, and pesticides.

In the past, benzene was also used as a solvent for paints and rubber. in the production of rubber cement (widely used in the shoe and garment industries) and in the manufacture of artificial leather. It has also been used in medicine in the treatment of hemoblastomas (leukemias, polycythemia, and malignant lymphomas) and in veterinary medicine for disinfecting wounds.

Nowadays. benzene is found as an impurity in rubber cement and solvents (ie. toluene. xylene). and in nunierous other chemicals in whose synthesis it is an intermediate.

EXPOSED POPULATIONS AND ACCEPTABLE LEVELS

The following population groups may be exposed to benzene: workers engaged in its production; workers in chemical industries using benzene as an intermediate; workers in industries producing materials containing benzene as a constituent (gasoline), as a solvent (rubber cement). or as an impurity (industrial toluene): people living near factories producing or using benzene. or compounds containing it: the general population (particularly in industrialized towns). since benzene is contained in gasoline. in drinking water, and in many other goods. and is highly volatile.

It has been estimated that 2 million workers in the United States are exposed to benzene, either during its production or through contact **with** substances containing it.

The levels of benzene permitted in workplace environments vary from country to country. Until 1978, in the United States, the Occupational Safety and Health Administration (OSHA) standard for benzene prescribed an eight-hour time weighted average (TWA) of 10 ppm with an acceptable ceiling concentration of 25 ppm. In addition, this standard allowed excursions above the ceiling to a maximum peak concentration not exceeding 50 ppm, providing that such exposure occurred for no more than ten minutes in any eight-hour work period. In **1978 OSHA** stated that "1 ppm **TWA,** with 5 ppm ceiling limit for 15 minutes during the eight-hour day, is the level which most adequately assures, to the extent feasible, the protection of workers exposed to benzene." This rule was annulled by the US Supreme Court, and the previous regulation is still maintained.

The permissible levels (TWA or ceiling) in several other countries are 30 mg/m^3 (equivalent to 10 ppm) in Italy. Belgium. Holland, Poland. Sweden. Switzerland; **80** mg/m^3 in Australia and Japan: 50 mg/m^3 in Czechoslovakia. Yugoslavia and East Germany; *32* mg/m^3 in Finland: 26 mg/m^3 in West Germany: 20 mg/m^3 in Hungary; 5 mg/m^3 in the **USSR.**

MYELOTOXICITY

Prolonged exposure to benzene causes toxic effects on bone marrow, both in animals and humans. The toxic effects on the hemopoietic system in humans have been known for about **80** years and are well-documented in the literature. The classical clinical finding in benzene hematotoxicity is a decrease in the various formed elements of circulating blood (pancytopenia) as a consequence **of** the decrease in identifiable granulocyte, erythrocyte, and platelet precursors within the bone marrow.

The association between long-term exposure to benzene and the occurrence of leukemia was suggested as early as **1928** by Delore and Borgomano, who reported a lymphoblastic leukemia in a worker who had been exposed to benzene for five years.

CARCINOGENICITY

General considerations. In spite of the industrial importance and widespread use of benzene. the number of people potentially exposed. and the reports **of** occupational leukemias. after more than one century of commercial production of benzene (80 years since the first report of benzene hematotoxicity and nearly 50 Years since the first report of a case **of** a benzene-associated leukemia). there had been no adequate epidemiological investigations nor adequate experimental research until the middle of the 1970s.

The mechanism of action of benzene, in relation to its leukemic and generally carcinogenic action, has nevertheless been the subject of many hypotheses for which experimental evidence was insufficient.

History to the Mid-1970s

Human data. These data are based, almost exclusively, on reports of a series of clinical cases of leukemia (generally in individuals with a history of benzene myelotoxicity) and on one indirect epidemiological investigation on the correlation between benzene exposure and the incidence of leukemias.

Since the first report of Delore and Borgomano [1928], many leukemias in people exposed to benzene have been the subject of case reports (Table I).

Vigliani in 1976 stated that " . . . an approximate estimation of the available literature, including some unpublished North Italian cases of which we have knowledge, puts the number of known cases of leukemia attributed to benzene at, at least; 150."

Acute myelogenous leukemia has been the most frequent form of leukemia found associated with benzene exposure. Other forms of leukemia which have also been associated with benzene are erythroleukemia, thrombocytemia, acute lymphoblastic leukemia. chronic lymphocytic leukemia, and lymphomas of various types.

The majority of the cases have been found in Italy [Vigliani and Saita, 1964; Vigliani, 1976], France [Goguel et al, 1967; Girard et al, 1968, 1970, 1971a,b; Girard and Revol, 1970], and Turkey [Aksoy et al, 1972. 19741. Several cases occurred among shoemakers and garment industry workers handling rubber cement.

The variety in type and distribution of the described leukemias in the major case reports (with a more or less pronounced prevalence of acute myelogenous leukemia) may be due to several factors, among which the following must be considered: (1) difference in level of exposure; **(2)** exposure in association with other agents, with toxic effects on hemopoietic tissues; **(3)** individual responsiveness; and, last but not least, **(4)** differences in the morphological interpretation of the hemopathological pictures. which vary with time, country, and school.

All of the above-mentioned reports deal with a limited series of cases and therefore the cases are fragmented into a large number of reports (Table I). In most of these reports. the causal relation between benzene exposure and leukemia is developed more from the history of the single cases. showing characteristic sequence. benzene exposure-myelotoxocity-leukemia. than from statistical and biological evaluation.

Moreover. results of one epidemiological investigation. performed in Japan, have been reported. Ishimaru and co-workers [1971] examined 303 cases of leukemia that occurred among adult survivors of the atomic bomb explosions at Hiroshima and Nagasaki and compared them with 303 matched controls exposed to the same amount of ionizing radiation from' the bomb. They found that the risk of leukemia was approximately 2.5 times higher among those with a history of a probable exposure to benzene or its derivatives and to medical X-rays.

Benzene has been shown to be genotoxic to human blood cells. Increased rates of both stable and unstable chromosome changes have been described both in lymphocyte and bone marrow cells of patients with benzene hemopathy. and in lymphocytes of workers with past exposure to benzene but without signs of poisoning [Forni. 1966; Forni et al. 1971a.b: Khan and Khan. 1973]. Chromosome damage from benrene can persist for years in long-lived lymphocytes. and sometimes may

result in the formation of abnormal cell clones in the absence of any sign of disease [Forni et al. 1971a].

There have been a number of reports of cases of benzene pancytopenia, in which the observed bone marrow cellular atypias were progressing toward acute leukemias.

Experimental data. The available experimental data prior to 1976. summarized in Table II. are scanty and insubstantial. In IARC Monograph No. 7 [1974]. it was concluded that "Benzene has been tested only in mice by subcutaneous injection and skin application. The data reported do not permit the conclusion that carcinogenic activity has been demonstrated." It should haw added "has not been demonstrated because **of** inadequacy of the experiments."

Present Status (From the Mid-1970s to the Present)

Human data. Infante et al, [1977] reported an increased risk of leukemias among workers at two rubber hydrochloride factories who were exposed to benzene in the years 1940–1949. In that investigation. the vital status of 75% of the population was ascertained. The level of exposure was estimated to be not greater than present standards would allow.

Rinsky et al [1981] reported the findings of a more extensive investigation on the same rubber workers. The major findings of this important study may be summarized as follows: (1) the workers were exposed to only one agent that has been associated with blood dyscrasias—benzene; (2) exposure data, uncommonly complete throughout the study period (1940–1975), indicated that the exposures of the workers were, for the most part. within limits permissible at the time (these limits are not greatly higher than the current legal standards); and *(3)* the vital status (alive, dead. and cause of death) for 98% of the study population was ascertained. There were seven deaths from leukemia (all myelocytic or monocytic) among 748 workers: this rate is 5.6-fold greater than would be expected in a comparable population. For workers exposed five or more years. there was a 21-fold increased risk of death from leukemia.

From data presented by Rinsky et al [1981], extrapolation has predicted that 8.4% of leukemias in American workers are secondary to benzene exposure [Rosenstock, 1982]. Because **of** benzene's **ubiquitous** nature, nonoccupational exposure may be significant, with an estimated **75%** of the population having been exposed [U.S. Department of Health and Human Services, 19811.

Data on experimental animals. A long-term experimental carcinogenicity bioassay on benzene, administered by gavage at two dose levels (250 and 50 mg/kg bw) to Sprague-Dawley rats, was started in 1976 at the Bologna Institute of Oncology, as mentioned in the introduction. Results of this experiment [Maltoni and Scarnato, 1977, 19791 showed that benzene produced Zymbal gland carcinomas at the two dose levels, with dose-response relationships. Moreover, a dose-correlated increase in hemolymphoreticular neoplasias ("leukemias") and in mammary carcinomas was observed. In animals treated with the high dose, some rarely occurring tumors were found, ie, two skin carcinomas of the head, one subcutaneous angiosarcoma, and one hepatoma. The incidence of malignant tumors, as a whole, was clearly greater in the treated group, with a sharp correspondence between dose and response.

Benzene was reported to produce Zymbal gland carcinomas in rats exposed by inhalation [Maltoni et al, 1982a,c].

Snyder et al [1980] reported observing eight cases of hemolymphoreticular neoplasia (six thymic lymphomas, one plasmacytoma, and one hemocytoblastic leukemia), among 40 C57BL mice exposed to 300 ppm of benzene, six hours daily, five days weekly, for their entire life span. Among 40 control C57BL mice, only two developed hemolymphoreticular neoplasia (two lymphocytic lymphomas, without thymus involvements). The increase in such neoplasias among treated mice was statistically significant ($p < .005$).

Goldstein et al [1980] reported observing, in the course of a long-term carcinogenicity bioassay using benzene, a few cases of acute and chronic myelogenous leukemia, namely, in one Sprague-Dawley rat, and two in CD-I mice. The plan and the results of that experiment are shown in Table III. The authors concluded that "Although not statistically significant as compared to the respective control groups. the fact that myelogenous leukemia has not been reported in control animals of these two strains suggests a causative role for benzene." In support of their assumption. these authors refer to studies. performed on historical controls, in which examination of 1,000 CD-I mice and 1,278 Sprague-Dawley rats failed to show cases of this form of leukemia. In our opinion, the data of Goldstein et al [1980] do not allow any definitive conclusion since (1) the control groups of their experiments are too small: *(2)* the use of the results on historical controls cannot be applied when small and peculiar differences have to be evaluated. because of the experimental and methodological differences existing not only from laboratory to laboratory, but also in the same laboratory from time to time: and **(3)** in untreated Sprague-Dawley rats and Swiss mice of the Bologna Institute of Oncology. the incidence of myeloid leukemia. although infrequent. is not exceptional (Table IV).

MATERIALS AND METHODS

The Project of Long-Term Carcinogenicity Bioassays in Progress at the Bentivoglio Laboratory of the Bologna Institute of Oncology

Plan of experiments. The project includes **a** series of experiments. which were started in sequence. They were designed to study the effects of benzene through different routes (inhalation and ingestion), at different concentrations (when given by ingestion). for different periods (when given by inhalation). on animals of different species (rats, mice. hamsters). strains (Sprague-Dawley and Wistar rats). sexes. and ages (adults and embryos).

Four sets of experiments have been started:

Experiment 1 (BT 901). This experiment studies the effects of benzene by ingestion (stomach tube), at two dose levels (250 and 50 mg/kg bw). on adult Sprague-Dawley rats (Table V.)

Experiment 2 (BT 4004 and BT 4006). This experiment studies the effects of benzene administered by inhalation. for two different periods of time. **on** Sprague-Dawley rats (adults and embryos) (Table **VI).**

Experiment 3 (BT 902–906). This experiment studies the effects of benzene. administered by ingestion (stomach tube), at **a** slightly toxic dose level (500 mg/kg bw), on adult Sprague-Dawley rats. Other groups of animals were similarly treated with toluene, xylene, and ethyl benzene to provide comparative data on the effects of the test compound with other similar aromatic solvents (Table VII).

Experiment 4 (BT 907–909). This experiment studies the effects of benzene administered by ingestion (stomach tube) on adult Wistar rats. Swiss mice, and Syrian golden hamsters. This experiment started more recently and data are not yet available.

Other experiments. dealing with transplacental carcinogenesis in mice and the effect of concurrent administration of alcohol in benzene carcinogenesis have been proposed.

Materials

In all our studies. the test substances—benzene, toluene. xylene (a mixture of ortho-, meta-. and para-xylene), and ethyl benzene—were analyzed in order to determine the levels of impurities.

All test compounds had a high degree of purity. namely. benzene, **99.93%** (paraffin. 0.06%; toluene. −.01%); toluene. **98.34%:** o-xylene. over **99%:** m-xylene. over **99%**: p-xylene. over **99%:** and ethyl benzene. **99.578.**

Extra-virgin olive oil. as vehicle, was used in the gavage experiments.

Sprague-Dawley rats. Wistar rats. Swiss mice. and Syrian golden hamsters, of the breeds used at the Institute of Oncology for many years. were utilized. It should be pointed out that. whatever their use, all the animals of the Bologna Institute of Oncology undergo periodic examinations and complete autopsy. giving us extensive information concerning their current pathology, with particular regard to the background tumorigram.

The background incidence of the four tumors of major interest in the present report—namely. Zymbal gland carcinomas. carcinomas of the oral cavity, carcinomas of nasal cavities, and hepatomas—among 3,326 Sprague-Dawley rats, untreated or treated with extra-virgin olive oil (by stomach tube and by intraperitoneal and subcutaneous injection) and kept under observation until spontaneous death, is given in Table VIII.

Methods and Conduct

The animals, weaned and classified by sex, when 4–5 weeks old were numbered by ear-punch and randomly divided into groups for each experiment. After weaning, the animals were fed ad libitum an adequate commercial diet. The animals were kept in groups of five **in** makrolon (polycarbonate) cages, with **tops made** of stainless steel wire (ingestion experiments) or in groups of ten in stainless steel cages. with a solid bottom of the same metal (inhalation experiments). For ingestion exposure, the compounds were dissolved in extra-virgin olive oil and administered by stomach tube (made of stainless steel).

The chambers for inhalation exposure were stainless steel, with two glass **doors,** and measure 135 x 98 x 65 cm. The volume was 860 liters. Continuous air flow provided 12–15 air changes **per** hour. Before introduction, air was filtered and the chamber arrangement was such that air flowed from one part of the chamber to the other without recirculation. The internal pressure was about 1 mm Hg less than that of the room where the chamber was situated, to avoid any possible contamination of the outside environment. Chambers were maintained at 21 "C ± 3°C and at 50% ± 10% relative humidity. Lighting was provided by room light. The chambers were cleaned at monthly intervals. Exposure chambers were provided with **a** fixed point

matrix for checking the distribution of the test substance. During treatment, the distribution was continuously monitored by gas chromatographs.

All of the animals were kept under observation until spontaneous death.

During the experiment, the animals were examined every two weeks; they were weighed every two weeks during the period of treatment and then every eight weeks.

All detectable gross pathological changes were recorded during the examination. When moribund, the animals were isolated in order to avoid cannibalism.

A complete autopsy was performed on each animal. Histological examination was carried out routinely on the brain. Zymbal glands, interscapular brown fat, salivary glands. Harderian glands, tongue, lungs, thymus. diaphragm. liver, spleen. kidneys, adrenals, pancreas. esophagus. stomach, different segments of the intestine, bladder. uterus. gonads, and any other organ with pathological lesions, or, although apparently normal. of particular interest on the basis of preliminary results.

Specimens were trimmed in a standard way. Sections were routinely stained with hematoxylin-eosin and, when necessary, with special techniques.

In limited groups of animals, in experiments BT 4004, 4006, and BT 902–906, hematological examinations were performed on circulating blood. The count of red (RBC) and white blood cells (WBC) was made both with the Coulter and the microscope. The smears were stained with May-Grünwald-Giemsa.

All experiments were performed with the same highly standardized procedures in order to allow comparison.

RESULTS

Experiment BT 901

This study began in 1976 and ended in 1979. Preliminary results have been published [Maltoni and Scarnato, 1977, 1979. 1981: Maltoni et al. 1982a].

Data on survival and bod!; weight are shown in Table IX (A and B). Mortality was higher in the benrene-treated groups, and dose-correlated. The increase in mortality correlates to the direct effects of the treatment in the first period of the experiment. and then both to the effects of treatment and to the higher incidence of malignant tumors caused by the compound.

The incidence ot malignant tumors is presented in Table X (A and B). Bcnrrne produces Zymbal gland carcinomas at the two dose levels. with a dose-response relationship. Moreover, it increases the incidence of hemolymphoreticular neoplasias ("leukemias") and of mammary carcinomas. This increase again was dose-dependent. In animals treated with the higher dose. some rarely occurring tumors were observed. namely, tuo carcinomas of the oral cavit. one subcutaneous angiosarcoma. and one hepatoma. In the previous preliminary reports [Maltoni and Scarnato. 1977. 1979. 1981] the two carcinomas of the oral cavity. which were invading the shin overlaying the maxilla. were misdiagnosed as cutaneous carcinomas. The incidence of nialignant tuniors. as a whole. is clearly higher in the treated groups. with a demonstrable dose-response relationship.

Experiments BT 4004 and 4006

These studies began in 1979 and are still going on. Preliminary results have been published [Maltoni et al, 1982a,c,d]. The present report gives results at 118 weeks from the start of the experiments.

Data on survival, body weight. and the values of RBC. total WBC. neutrophils. eosinophils. basophils. lymphocytes. and monocytes are shown in Table XI (A and B). Mortality at 104 weeks is higher in the animals in which the treatment was started at the embryonal stage and lasted 104 weeks. The prolonged treatment (up to 104 weeks) slightly affects the body weight of male offspring at different stages. Under the conditions of the present experiment. benzene treatment causes leukopenia (the decrease in WBC is due mainly to the lymphocytopenia).

The incidence of tumors is shown in Table XII (A and B). The results confirm that benzene causes the onset of Zymbal gland carcinomas in Sprague-Dawley rats. even when given by inhalation. The incidence of these tumors parallels the length of treatment. One carcinoma of the nasai cavities was observed in an offspring male following more prolonged exposure. Moreover. benzene. under these experimental conditions. appears to cause hepatomas and other correlated dysplastic and hyperplastic lesions (Tables **XII.** XIII).

Experiments BT 902–906

These experiments were started in January, 1981. and they are still in progress. Preliminary results have been published [Maltoni et al, 1982a,b]. The present report gives results at 92 weeks from the start of the experiments.

The data on survival. bod! weight. and the values **of** RBC. total WBC. neutrophils. eosinophils. basophils. lymphocytes. and monocytes are given in Table **XIVA** (A and B). At 500 mg/kg bw benzene affects body weight and some hematological parameters. such as total RBC and total WRC (the decrease in WBC is due mainly to the lymphocytopenia).

The incidence of tumors is given in Table **XV (A** and **B).** The data further confirm that benzene causes the development of Zymbal gland carcinomas. Moreover. they show that the compound induces carcinomas of the oral cavity. a very unusual tumor in our colony of rats. since no such neoplasia has ever been observed in our historical controls. Also, one carcinoma of the nasal cavity has been observed. The onset of a liver angiosarcoma is of interest, given the extreme rarity of this type of neoplasia in the historical controls of our breed of Sprague-Dawley rats.

The finding of one Zymbal gland carcinoma, plus one carcinoma of the oral cavity, plus a slightly higher onset **of** "leukemias" among the rats treated with xylene is of interest.

Pathology of the Most important Observed Tumors

Zymbal gland carcinomas, whatever the type of treatment, show sebaceous, squamous, tubular and mixed patterns; they invade the surrounding tissues and can metastatize to regional lymph nodes, lungs and brain (Figs. 1–6).

The carcinomas of the oral cavity are squamocellular; they seem to arise mainly from the mucosa of the lip and invade the surrounding anatomical structures: other areas of the mouth, the maxilla, the mandible. and the head skin (Figs. 7,8).

The carcinomas of the nasal cavities are squamocellular and invasive (Figs. 9,10).

Hepatomas (hepatocarcinomas) show medullary, trabecular, acinar. adenoid, and mixed arrangement (Figs. 11–18).

The observed liver angiosarcoma is highly undifferentiated and of the type more frequently seen in our laboratory in rodents exposed to vinyl chloride (Fig. 19). The subcutaneous angiosarcoma invaded the surrounded tissues (Fig. 20).

The hemolymphoreticular neoplasia ("leukemias") include leukemia and hematosarcomas of different types (Figs. 21.22).

Mammary tumors include fibroadenomas and adenocarcinomas of the histotypes usually observed in the breed of Sprague-Dawley rats of our laboratory (Figs. **23.24).**

Some Considerations on Pathogenesis

The onset of carcinomas of the mouth, following oral administration of the test compound, would indicate a topical effect of benzene and would suggest that it could act as a direct carcinogen.

The onset of hepatocarcinomas mainly in experiment BT 4004, in our opinion, is to be correlated to the well-known fact that embryos are more susceptible to liver carcinogenesis, rather than to the type of the exposure.

In experiment BT **902,** the lower incidence of mammary tumors can be explained by the toxic action of benzene at the tested dose, which affects the body weight of the animals; in fact, the incidence of mammary tumors in rats is known to be highly dependent on body weight.

CONCLUSIONS

Results to date of our experiments show that (1) benzene is carcinogenic in rats: (2) benzene is carcinogenic when given by ingestion and by inhalation: (3) benzene produces different types of tumors in different organs. and therefore it must be considered a multipotential carcinogen: (4) benzene is a potent carcinogen. since it not only enhances the incidence of tumors frequently occurring in untreated animals of the tested colony. but also produces infrequent or unusual tumors in **the** animals used; *(5)* there appears to be a direct relationship between the dose of benzene (concentration or length of treatment) and tumor response [Maltoni. 1982].

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APPENDIX: Tables I-XVB and Figures 1-24

Benzene: A Multipotential Carcinogen. Results of Long-Term Bioassays Performed at the Bologna Institute of *Oncology.* Cesare Maltoni, Barbara Conti, and Giuliano Cotti

TABLE I. Case Reports of Hemolymphoreticular Neoplasia ("Leukemia") and Correlated Diseases Observed in Individuals Exposed to Benzene, Reported in Scientific Literature up to the Slid-1970s*

Type	No.	No. of reports
Acute myelogenous leukemia	58	28
Erythroleukemia	16	10
Acute monocytic leukemia	3	a
Chronic myelogenous leukemia	27	7
Myelofibrosis and myeloid metaplasia	7	5
Thrombocytheniia	1	1
Lymphoblastic leukemia	8	4
Lymphocytic leukemia	9	7
Lymphomas and correlated disorders	14	7
Total	143	

*Data from Goldstein [1977]

TABLE II. Long-term Carcinogenicity Bioassays on Benzene: Available Data up to the Mid-1970s[*]

Authors	Animals: Species	Strain	Sex	No.[a]	Treatment and other experimental details	Results	Observations
Lignac [1932]	Mice	"Albino"	M,F	33 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, once weekly, for 17–21 weeks (total dose, about 1 mg/kg bw)	8 leukemias (from 4 to 8 months from the start of treatment)	No control group
Kirschbaum and Strong [1942]	Mice	F	?	30 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of sesame oil	6 leukemias (30%) (from 200 to 300 days of age)	Leukemia increase in treated animals is not statistically significant
				212 C		29 leukemias (14%) (before 300 days of age)	
Amiel [1960]	Mice	DBA2	M	30 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, for the whole life span	0	Maximum survival of DBA2, C3H and C57BL6 mice: 730 days
		C3H		30 T		0	
		C57BL6		30 T		0	
		AKR	[illegible]	[illegible]T		16 leukemias (between the 7th and the 16th month of treatment); 8 animals died before the 9th month of treatment	Major survival of animals of control group
				35 C		30 leukemias	
Hiraki et al [1963]	Mice	Swiss	M,F	10 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, for 10 weeks	5 subcutaneous sarcomas (at autopsy, performed between the 162th and the 253th day from start of treatment)	2 animals died within the first 8 weeks of treatment No control group
Many	Mice Rats Rabbits	Various	M,F	A great many	Skin applications	No effects	Nonsystematic and non ad hoc planned experiments

[*]Goldstein, B: Hematotoxicity in humans. In: Benzene toxicity, A critical evaluation. Eds. S. Laskin and B. Goldstein. J Toxicol & Env Hlth Supplement 2:69–105, 1977.

[a]T = treated; C = control.

TABLE III. Incidence of Myelogenous Leukemia in Rodents (Rats and Mice) Exposed to Benzene by Inhalation Route, 6 Hours Daily, 5 Days Weekly, Until Death*

Animals				Myelogenous leukemias	
Species	Strain	No.	Treatment	No.	Observations
Rat	Sprague-Dawley	45	300 ppm	0	
		27	0 ppm	0	
		40	100 ppm	1	Chronic myelogenous leukemia
		40	0 ppm	0	
Mice	AKR	80	300 ppm	0	
		80	0 ppm	0	
		50	100 ppm	0	
		50	0 ppm	0	
	C57BL	40	300 ppm	0	
		40	0 ppm	0	
	CD-1	40	300 ppm	2	1 acute myelogenous leukemia 1 chronic myelogenous leukemia (+ 1 case of granulocytic hyperplasia)
		40	0 ppm	0	

*From Goldstein et al [1980].

TABLE IV. Incidence of Spontaneous "Leukemias." With Particular Regard to Myelogenous Leukemias, in Untreated Sprague-Dawley Rats and Swiss Mice at the Bologna Institute of Oncology

Animals			"Leukemias"			
			Total		Myelogenous leukemias	
Type	Sex	No.	No.	%[a]	No.	%[b]
Sprague-Dawley rats	M	1,179	41	3.5	1	2.2
	F	1,202	48	4.0	5	11.9
	Total	2.381	89	3.7	6	6.8
Swiss mice	M	445	18	4.0	2	11.1
	F	454	52	11.4	4	7.7
	Total	899	70	7.8	6	8.6

[a]Referred to total number of animals.
[b]Referred to total number of "leukemias."

TABLE V. Exwriment 1: BT901.* Plan of the Exwriment

Experiment and groups	Treatment	Animals	
		Sex	No. at start
BT901 I	Benzene	M	35
	250 mg/kg bw	F	35
		M and F	70
BT901 II	Benzene	M	30
	50 mg/kg bw	F	30
		M and F	60
BT901 III	None	M	30
	(Olive oil alone)	F	30
	(Controls)	M and F	60

*Protocol: 13-week-old Sprague-Dawley rats. exposed to benzene by ingestion (stomach tube), in olive oil. at doses of 250, 50 mg/kg body weight, and with olive oil alone, once daily. 4–5 days weekly. for 52 weeks, and then kept under observation until spontaneous death.

TABLE VI. Experiment 2: BT4004, 4006.* Plan of the Experiment

Experiments	Groups	Treatments: Agent	Treatments: Concentration and schedule	Animals (B and E) (Sprague-Dawley rats): Age	Sex	No. at start
BT4004	I	Benzene	200 ppm 4 hr/day, 5 days wk, 7 wk, and then	B	F	54
			7 hr/day, 5 days/wk, 12 wk, and then	E	M	75
	II		300 ppm 7 hr/day, 5 days/wk, 85 wk		F	65
					M and F	140
	III		200 ppm 4 hr day, 5 days/wk, 7 wk, and then	E	M	70
					F	59
			7 hr/day, 5 days wk, 8 wk		M and F	129
BT4006	I	None (in chambers)	(104 wk)	B	F	60
					M	158
	II		(104 wk)	E	F	149
					M and F	307

*Protocol: 13-week-old breeder (B) and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 weeks, and then kept under observation until spontaneous death

TABLE VII. Experiment 3: BT902–906.* Plan of the Experiments

Experiments	Treatments	Animals: Sex	Animals: No. at start
		M	40
BT902	Benzene	F	40
		M and F	80
		M	40
BT903	Toluene	F	40
		M and F	80
		M	40
BT904	Xylene	F	40
		M and F	80
		M	40
BT905	Ethyl-benzene	F	40
		M and F	80
	None	M	50
BT906	(Olive oil alone)	F	50
	(Controls)	M and F	100

*Protocol: 7-week-old Sprague-Dawley rats, exposed to benzene, toluene, xylene, and ethyl-benzene by ingestion (stomach tube), in olive oil, at the dose of 500 mg/kg body weight, and olive oil alone, once daily, 4–5 days weekly, for 104 weeks, and then kept under observation until spontaneous death.

TABLE VIII. Background Incidence of Zymbal Gland Carcinomas, Carcinomas of the Oral and Nasal Cavities, and Hepatomas Among Sprague-Dawley Rats, of the Breed Used at the Bologna Institute of Oncology (Historical Controls)

Treatments	Animals		Malingnant tumors							
			Zymbal gland carcinomas		Carcinomas of the oral cavity		Carcinomas of the nasal cavities		Hepatomas	
	Sex	No.	No.	%	No.	%	No.	%	No.	%
None	M	1,179	7	0.6	0	—	0	—	2	0.2
	F	1,202	6	0.5	0	—	0	—	0	—
	M and F	2,381	13	0.5	0	—	0	—	2	0.08
Ingestion of olive oil (repeated administration by stomach tube)	M	325	3	0.9	0	—	0	—	0	—
	F	325	3	0.9	0	—	0	—	1	0.3
	M and F	650	6	0.9	0	—	0	—	1	0.1
Intraperitoneal injections of olive oil	M	70	0	—	0	—	0	—	0	—
	F	70	0	—	0	—	0	—	0	—
	M and F	140	0	—	0	—	0	—	0	—
Subcutaneous injection of olive oil (una tantum)	M	75	1	1.3	0	—	0	—	0	—
	F	80	1	1.2	0	—	0	—	0	—
	M and F	155	2	1.3	0	—	0	—	0	—
Total	M	1,649	11	0.7	0	—	0	—	2	0.1
	F	1,677	10	0.6	0	—	0	—	1	0.06
	M and F	3,326	21	0.6	0	—	0	—	3	0.09

TABLE IXA. Experiment I: BT901.* Results After 104 Weeks—Survival and Bod! Weight

Experiments and groups	Treatment	Animals		Surviving animals 26 weeks		52 weeks	
		Sex	No. at start	No.	%	No.	%
	Benzene	M	35	32	91.4	22	h2.X
BTOOI	250 mg kg	F	35	32	91.4	26	74..3
I	bw	M and F	70	64	91.4	48	68.6
	Benzene	M	30	28	93.3	'1	70.0
BT901	50 mg/kg	F	30	30	100.0	'8	93.3
II	bw	M and F	60	58	96.7	49	81.7
	None	M	30	28	93.3	21	70.0
BT901	(Olive oil alone)	F	30	30	100.0	30	100.0
III	(Controls)	M and F	60	58	96.7	51	85.0

*Protocol: 13-week-old Sprague-Dawley rats, exposed to benzene by ingestion (stomach tube), in olive oil, at closes of 250, 50 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 52 weeks, and then kept under observation until spontaneous death.

TABLE IXB.

Experiments and groups	Surviving animals 78 weeks		104 weeks		Average body weight (in g) 26 weeks	52 weeks	78 weeks	104 weeks
	No.	%	NO.	%				
	13	37.1	0	—	524.53	564.77	610.38	—
BT901	16	45.7	6	17.1	303.28	332.88	408.61	431.00
I	19	41.4	6	8.6				
	19	63.3	8	26.7	541.60	555.95	584.21	531.42
BT901	19	63.3	10	33.3	300.66	353.39	398.40	381.00
II	38	63.3	18	30.0				
	18	60.0	4	13.3	570.00	590.23	611.67	520.00
BT901	28	93.3	10	33.3	323.33	358.33	405.71	451.87
III	46	76.7	14	23.3				

*Protocol: 13-weeks-old Sprague-Dawley rats, exposed to benzene by ingestion (stomach tube), in olive oil, at doses of 250, 50 mg/kg bod! weight, and with olive oil alone, once daily, 4–5 days weekly, for 52 weeks, and then kept under observation until spontaneous death.

TABLE XA. Experiment 1: BT901.* Results After 144 Weeks (End of the Experiment)—Incidence of Solid Malignant Tumors and of Hemolymphoreticular Neoplasias ("Leukemias")

Experiment and groups	Treatment	Sex	Animals		Malignant tumors					
					Zymbal gland carcinomas		Carcinomas of the oral cavity		Carcinomas of the nasal cavities	
			No. at start	Corrected number[a]	No.	%[b]	No.	%[b]	No.	%[b]
BT901 I	Benzene 250 mg/kg bw	M	35	33	0	—	0	—	0	—
		F	35	32	8	25.0	2	6.2	0	—
		M and F	70	65	8	12.3	2	3.1	0	—
BT901 II	Benzene 50 mg/kg bw	M	30	28	0	—	0	—	0	—
		F	30	30	2[c]	6.7	0	—	0	—
		M and F	60	58	2	3.4	0	—	0	—
BT901 III	None (Olive oil alone) (Controls)	M	30	28	0	—	0	—	0	—
		F	30	30	0	—	0	—	0	—
		M and F	60	58	0	—	0	—	0	—

*Protocol: 13-week-old Sprague-Dawley rats, exposed to benzene by ingestion (stomach tube), in olive oil, at doses of 250, 50 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 52 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 20 weeks.

[b]The percentages are referred to the corrected number.

[c]1 with sarcomatous component.

[d]1 lymphoblastic leukemia; 3 hematosarcomas.

[e]1 lymphocytic leukemia.

[f]1 myelogenous leukemia; 1 hematosarcoma.

[g]1 hematosarcoma.

[h]Subcutaneous.

TABLE XB Experiment 1: BT901.* Results After 144 Weeks (End of the Experiment)—Incidence of Solid Malignant Tumors and of Hemolymphoreticular Neoplasias ("Leukemias")

Experiment and groups	Treatment	Malignant tumors											
		Mammary carcinoma		"Leukemias"		Angiosarcomas		Hepatomas		Others		Total	
		No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]	No.	%[b]
	Benzene	0	—	4[d]	12.1	1[h]	3.0	1	3.0	2	6.1	8	24.2
BT901	250 mg/kg	7	21.9	1[e]	3.1	0	—	0	—	3	9.4	21	65.6
I	bw	7	10.8	5	7.7	1	1.5	1	1.5	5	7.7	29	44.6
	Benzene	0	—	0	—	0	—	0	—	1	3.6	1	3.6
BT901	50 mg/kg	4	13.3	2[f]	6.7	0	—	0	—	2	6.7	10	33.3
II	bw	4	6.9	2	3.4	0	—	0	—	3	5.2	11	19.0
	None	0	—	0	—	0	—	0	—	2	7.1	2	7.1
BT901	(Olive oil alone)	3	10.0	1[g]	3.3	0	—	0	—	2	6.7	6	20.0
III	(Controls)	3	5.2	1	1.7	0	—	0	—	4	6.9	8	13.8

*Protocol: 13-week-old Sprague-Dawley rats, exposed to benzene by ingestion (stomach tube), in olive oil, at doses of 250, 50 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 52 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 20 weeks.

[b]The percentages are referred to the corrected number.

[c]1 with sarcomatous component.

[d]1 lymphoblastic leukemia; 3 hematosarcomas.

[e]1 lymphocytic leukemia.

[f]1 myelogenous leukemia; 1 hematosarcoma.

[g]1 hematosarcoma.

[h]Subcutaneous.

TABLE XIA. Experiment 2: BT4004, 4006.* Results After 118 Weeks—Survival, Body Weight, and Hematological Red Blood Cells (RBC) and White Blood Cells (WBC) Values

Experiments	Groups	Treatments: Agent	Treatments: Concentration and schedule	Animals (B or E) (Sprague-Dawley rats): Age	Sex	No. at start	Surviving animals after 26 weeks: No.	26 weeks: %	52 weeks: No.	52 weeks: %
BT4004	I	Benzene	200 ppm 4 hrlday. 5 daysiwk, 7 wk. then 7 hrlday, 5 daysiwk, 12 wk. then 300 ppm 7 hrlday. 5 daysi wk. 85 wk	B	F	54	54	100.0	51	94.4
					M	75	75	100.0	74	98.7
	II			E	F	65	64	98.4	64	98.4
					M and F	140	139	99.3	138	98.6
	III		200 ppm 4 hrlday. 5 days/wk, 7 wk. then 7 hr/day. 5 days/wk, 8 wk		M	70	70	100.0	69	98.6
					F	59	59	100.0	59	100.0
					M and F	129	129	100.0	128	99.2
BT4006	I	None (in chambers)	(104 wk)	B	F	60	60	100.0	57	95.0
					M	158	156	9X.7	149	94.3
	II		(104 wk)	E	F	149	148	99.3	147	98.6
					M and F	307	304	99 0	296	96.4

*Protocol 13-week-old breeder (B), and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 \seeks, and then kept under observation until spontaneous death

Surviving animals after 78 weeks		Surviving animals after 104 weeks		Surviving animals after 118 weeks		Average body weight (in g) at 26 weeks	Average body weight (in g) at 52 weeks	Average body weight (in g) at 78 weeks	Average body weight (in g) at 104 weeks	Average body weight (in g) at 118 weeks
No	%	No	%	No	%					
44	81.5	30	55.5	5	9.3	305.84	332.55	341.25	354.55	392.00
72	96.0	21	28.0	11	14.7	477.47	532.70	524.44	526.60	491.36
54	83.0	34	52.3	23	35.4	283.83	316.17	332.59	350.29	380.43
126	90.0	55	39.3	34	24.3					
60	85.7	36	51.4	21	30.0	500.23	571 73	576.08	552.38	542.61
58	98.3	34	57.6	21	35.6	288.06	322.54	359.39	367.43	390.71
I18	91.5	70	54.3	42	32.6					
46	76.7	29	48.3	11	18.3	312.00	362.72	393.26	392.95	367.27
143	90.5	74	46.8	39	24.1	512.94	587.58	563.28	562.79	554.61
143	96.0	93	62.4	47	31.5	290.85	321.90	311.53	367.53	362.87
286	93.2	167	54.4	86	28.0					

TABLE XIB. Experiment 2: BT4004, 4006.* Results After 118 Weeks—Survival, Body Weight, and Hematological Red Blood Cells (RBC) and White Blood Cells (WBC) Values

		Average hematological values after 91 weeks[a]													
		RBC ($\times 10^6$/mm^3)		WBC ($\times 10^3$/mm^3)											
				Total		N		E		B		L		M	
Experiments	Groups	No.	SD	No.	SD	No.	SD	No.	SD	No.	SD	No.	SD	No.	SD
BT4004	I														
		8.76	0.98	8.96	2.35	3.21	0.93	0.16	0.16	0	—	5.37	1.79	0.20	0.07
	II	7.37	0.56	6.77	1.39	1.96	0.52	0.08	0.08	0	—	4.11	1.62	0.49	1.07
	III														
BT4006	I														
		8.86	0.77	12.80	3.35	4.00	2.17	0.22	0.16	0	—	8.27	1.36	0.28	0.11
	II	7.44	0.47	7.21	2.00	2.22	1.37	0.15	0.21	0	—	5.66	2.19	0.19	0.13

*Protocol: 13-week-old breeder (B), and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 weeks, and then kept under observation until spontaneous death.

[a]The averages are based on 10 animals per sex for each experiment.

TABLE XIIA. Experiment 2: BT4004, 4006.* Results After 118 Weeks—Incidence of Zymbal Gland Carcinomas, Carcinomas of the Oral and Nasal Cavities, Hepatomas, Leukemias, and Mammary Tumors

Experiments	Treatments: Groups	Agent	Concentration and schedule	Animals (B or E) (Sprague-Dawley rats): Age	M and F	No. at start	No. of survivors	Corrected No.[a]
BT4004	I		200 ppm	B	F	54	5	54
			4 hr/day, 5 days/wk,		M	75	11	75
	II		7 wk, then	E	F	65	23	64
			7 hr/day, 5 days/wk,		M and F	140	34	139
		Benzene	12 wk, then					
			300 ppm					
			7 hr/day, 5 days/wk					
			85 wk					
	III		200 ppm		M	70	21	70
			4 hr/day, 5 days/wk,	E	F	59	21	59
			7 wk, then		M and F	129	42	129
			7 hr/day, 5 days/wk,					
			3 wk					
BT4006	I	None (in chambers)	(104 wk)	B	F	60	11	60
	II		(104 wk)		M	158	39	157
				E	F	149	47	148

*Protocol: 15-week-old breeder (B) and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 22 weeks, when the first tumor (a mammary tumor) was observed.

[b]The percentages are referred to the corrected number.

TABLE XIIB. Experiment 2: BT4004, 4006.* Results After 118 Weeks—Incidence of Zymbal Gland Carcinomas, Carcinomas of the Oral and Nasal Cavities, Hepatomas, Leukemias, and Mammary Tumors

	Animals with tumors																	
	Zymbal gland carcinomas			Carcinomas of the oral cavity			Carcinomas of the nasal cavities			Hepatomas			Leukemias			Mammary tumors		
Experiments	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)
BT4004																		
[c]	3	5.6	96.3	0	—	—	0	—	—	1	1.8	67.0	0	—	—	29	53.7	87.9
	4	5.3	86.7	0	—	—	1	1.3	114.0	2	2.7	100.5	2	2.7	91.0	8	10.7	84.9
[d]	4	6.3	76.0	0	—		0	—	—	1	1.6	98.0	0	—	—	29	45.3	86.2
	8	5.8	81.4	0	—		1	0.7	114.0	3	2.2	99.7	2	1.4	91.0	37	26.6	85.9
	1	1.4	75.0	0	—	—	0	—	—	0	—	—	0	—	—	7	10.0	95.1
[e]	1	1.7	58.0	0	—		0	—		3	5.1	92.3	0	—	—	31	52.5	88.7
	2	1.6	66.5	0	—	—	0	—	—	3	2.3	92.3	0	—	—	38	29.5	89.9
BT4006																		
[c]	1	1.7	46.0	0	—	—	0	—		0	—	—	0	—	—	20	33.3	94.5
	1	0.6	94.0	0	—	—	0	—	—	0	—	—	1	0.6	25.0	16	10.2	84.5
[d]	1	0.7	59.0	0	—	—	0	—	—	0	—	—	0	—	—	75	50.7	86.0
	2	0.7	76.5	0	—		0	—	—	0	—	—	1	0.3	25.0	91	29.8	85.7

*Protocol: 13-week-old breeder (B) and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 22 weeks, when the first tumor (a mammary tumor) was observed.

[b]The percentages are referred to the corrected number.

[c]Group I.

[d]Group II.

[e]Group III.

TABLE XIII. Experiment 2: BT4004, 4006.* Results After 118 Weeks—Hepatomas and Other Correlated Hyperplastic and Dysplastic Changes

Experiments	Groups	Treatments: Agent	Treatments: Concentration and schedule	Animals (B or E) (Sprague-Dawley rats): Age	Sex	No. at start	No. of survivors	Corrected no.[a]	Animals with hepatic Hepatomas: No.	% [b]	Average latency time (wk)	ou er No	dı spl No
BT4004	I	Benzene	200 ppm 4 hr/day, 5 days/wk, 7 wk, then	B	F	54	5	48	1	2.1	67.0	5	0
	II		7 hr/day, 5 days/wk, 12 wk, then	E	M	75	11	74	2	2.7	100.5	1	0
			300 ppm 7 hr/day, 5 days/wk, 85 wk		F	65	23	59	1	1.7	98.0	5	2
					M and F	140	34	133	3	2.3	99.7	6	
	III		200 ppm 4 hr/day, 5 days/wk, 7 wk, then	E	M	70	21	64	0	—	—	2	0
			7 hr/day, 5 days/wk, 8 wk		F	59	21	59	3	5.1	92.3	7	2
					M and F	129	42	123	3	2.4	92.3	9	2
BT4006	I	None (in chambers)	(104 wk)	B	F	60	11	52	0	—	—	0	0
	II		(104 wk)		M	153	39	146	0	—	—	0	0
					F	140	47	146	0	—	—	0	0
					M and F	307	86	292	0	—	—	0	0

*Protocol: 13-week-old breeder (B), and 12-day embryo (E) Sprague-Dawley rats, exposed to benzene by inhalation, at concentrations of 200–300 ppm, 4–7 hours daily, for 15 or 104 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 67 weeks, when the first lesion of oncological interest (a hepatoma) was observed.

[b]The percentages are referred to the corrected number.

TABLE XIVA. Experiment 3: BT902–906.* Results After 92 Weeks—Survival, Body Weight. and Hematological Red Blood Cell (RBC) and White Blood Cell (WBC) Values

		Animals		Surviving animals			
				26 weeks		52 weeks	
Experiments	Treatments	Sex	No. at start	No.	%	NO.	%
BT902	Benzene	M	40	40	100.0	39	97.5
		F	40	40	100.0	40	100.0
		M and F	80	80	100.0	79	48.7
BT903	Toluene	M	40	40	100.0	39	97.5
		F	40	40	100.0	40	100.0
		M and F	80	80	100.0	79	98.7
BT904	Xylene	M	40	39	97.5	35	87.5
		F	40	40	100.0	38	45.0
		M and F	80	79	98.7	73	91.2
BT905	Ethyl-benzene	M	40	40	100.0	38	95.0
		F	40	40	100.0	37	92.5
		M and F	80	80	100.0	75	93.7
BT906	None (Olive oil alone) (Controls)	M	50	47	93.0	45	90.0
		F	50	49	98.0	49	98.0
		M and F	100	96	96.0	94	94.0

*Protocol: 7-week-old Sprague-Dawley rats, exposed to benzene. toluene. xylene. and ethyl-henrcnc by ingestion (stomach tube). in olive oil. at dose of 500 mg/kg body weight, and with olive oil alone. once daily. 4-5 days weekly, for 104 ueeks. and then hept under observation until spontaneous Jcath.

Surviving animals				Average body weight (in g)			
78 weeks		92 weeks		26 weeks	52 weeks	78 weeks	92 weeks
No.	%	NO.	%				
38	95.0	29	72.5	423.87	451.79	496.05	444.65
31	77.5	24	60.0	251.12	268.62	301.12	276.25
69	86.2	53	66.2				
34	85.0	31	77.5	516.00	553.20	599.41	611.77
36	90.0	30	75.0	290.37	333.50	395.83	429.50
70	87.5	61	76.2				
25	72.5	20	50.0	527.82	556.42	611.00	626.25
36	90.0	26	65.0	303.62	352.36	413.48	437.11
61	76.2	46	57.5				
31	11.5	28	70.0	512.75	532.76	580.64	583.57
33	82.5	29	72.5	290.75	319.45	372.57	385.68
64	80.0	57	71.2				
37	74.0	29	58.0	516.17	534.33	525.92	490.68
40	80.0	33	66.0	300.01	329.79	356.50	389.09
77	77.0	62	62.0				

TABLE XIVB. Experiment 3: BT902–906.* Results After 92 Weeks—Survival, Body Weight, and Hematological Red Blood Cell (RBC) and White Blood Cell (WBC) Values

		Average hematological values after 84 weeks[a]													
		RBC ($\times 10^6$/mm^3)		WBC ($\times 10^3$/mm^3)											
				Total		N		E		B		L		M	
Experiments	Treatments	No.	SD	No.	SD	No.	SD	No.	SD	No.	SD	No.	SD		
BT902	Benzene	7.30	0.70	7.84	1.61	3.94	1.77	0.15	0.10	0	—	3.65	0.41	0.92	0.06
		6.60	0.67	7.88	1.21	3.77	1.08	0.12	0.08	0	—	3.86	2.06	0.13	0.09
BT903	Toluene	9.04	0.23	9.00	1.34	3.38	1.04	0.16	0.08	0	—	5.27	0.80	0.18	0.09
		8.02	0.24	9.52	1.98	3.26	1.15	0.26	0.20	0	—	5.90	0.96	0.11	0.07
BT904	Xylene	9.05[b]	0.13	10.65[b]	0.66	2.97	3.06	0.26	0.18	0	—	7.21	0.89	0.21	0.10
		7.58	0.61	9.00	1.82	3.16	0.71	0.20	0.08	0	—	5.51	1.31	0.12	0.08
BT905	Ethyl-benzene	9.16	0.21	9.72	1.10	2.93	0.52	0.29	0.19	0	—	6.34	1.18	0.16	0.06
		8.00	0.35	10.48[c]	4.46	3.46	2.27	0.19	0.21	0	—	6.64	2.01	0.19	0.13
BT906	None	8.72	0.57	12.92	2.29	5.41	1.36	0.39	0.18	0	—	6.89	1.04	0.23	0.06
	(Olive oil alone) (Controls)	7.70	0.50	10.80	2.74	3.68	1.56	0.35	0.25	0	—	6.61	1.45	0.16	0.06

*Protocol: 7-week-old Sprague-Dawley rats, exposed to benzene, toluene, xylene and ethyl-benzene by ingestion (stomach tube), in olive oil, at a dose of 500 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 104 weeks, and then kept under observation until spontaneous death.

[a]The average values are based on 5 animals per sex for each experiment, unless otherwise specified.

[b]The average value is based on 4 animals, since 1 was leukemic (lymphoblastic leukemia).

[c]The WBC value referred to the animal BT905/6 was abnormally high (18.20).

TABLE XVA. Experiment 3: BT902–906.* Results After 92 Weeks—Incidence of Zymbal Gland Carcinomas, Carcinomas of the Oral and Nasal Cavities, Primary Liver Tumors, Leukemias, and Mammary Tumors

Experiments	Treatments	Sex	Animals No. at start	Corrected No.[a]	Animals with tumors: Zymbal gland carcinomas No.	% [b]	Average latency time (wk)	Carcinomas of the oral cavity No.	% [b]	Average latency time (wk)	Carcinomas of the nasal cavities No.	% [b]	Average latency time (wk)
BT902	Benzene	M	40	40	6	15.0	78.5	7	17.5	84.3	1	2.5	80.0
		F	40	40	6	15.0	76.0	4	10.0	73.0	0	—	—
		M and F	80	80	12	15.0	77.2	11	13.7	80.2	1	1.2	80.0
BT903	Toluene	M	40	40	0		—	0		—	0		
		F	40	40	0		—	0	—	—	0	—	—
		M and F	80	80	0	—	—	0	—	—	0	—	—
BT904	Xylene	M	40	39	1	2.6	66.0	0	—	—	0	—	—
		F	40	40	0	—	—	1	2.5	78.0	0	—	—
		M and F	80	79	1	1.3	66.0	1	1.3	78.0	0	—	—
BT905	Ethyl-benzene	M	40	40	0	—	—	0	—	—	0	—	—
		F	40	40	0	—	—	0	—	—	0	—	—
		M and F	80	80	0	—	—	0	—	—	0	—	—
BT906	None (Olive oil alone) (Controls)	M	50	48	1	2.1	84.0	0	—	—	0	—	—
		F	50	49	0	—	—	0	—	—	0	—	—
		M and F	100	97	1	1.0	84.0	0	—	—	0	—	—

*Protocol: 7-week-old Sprague-Dawley rats exposed to benzene, toluene, xylene, and ethyl-benzene by ingestion (stomach tube), in olive oil, at dose of 500 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 104 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 52 weeks, when the first tumor (a Zymbal gland carcinoma) was observed.

[b]The percentages are referred to the corrected number.

TABLE XVB. Experiment 3: BT902–906.* Results After 92 Weeks—Incidence of Zymbal Gland Carcinomas, Carcinomas of the Oral and Nasal Cavities, Primary Liver Tumors, Leukemias, and Mammary Tumors

	Animals with tumors											
	Primary liver tumors											
	Hepatomas			Angiosarcomas			"Leukemias"			Mammary tumors		
Experiment	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)	No.	%[b]	Average latency time (wk)
	0	—	—	0	—	—	0	—	—	2	5.0	90.0
BT902	0	—	—	1	2.5	89.0	1	2.5	64.0	3	7.5	80.3
	0	—	—	1	1.2	89.0	1	1.2	64.0	5	6.2	84.2
	1	2.5	81.0	0	—	—	0	—	—	1	2.5	97.0
BT903	0			0	—	—	1	2.5	61.0	10	25.0	73.6
	1	1.2	81.0	0	—		1	1.2	61.0	11	13.7	75.7
	0	—	—	0	—	—	3	7.7	76.0	2	5.1	94.0
BT904	0	—	—	0	—	—	0	—	—	10	25.0	81.2
	0	—	—	0	—	—	3	3.8	76.0	12	15.2	83.3
	0			0			0			0	—	—
BT905	0			0	—		0	—	—	8	20.0	82.0
	0	—		0	—	—	0	—	—	8	10.0	82.0
	0	—	—	0	—	—	0	—	—	0	—	—
BT906	0	—		0	—	—	1	2.0	60.0	12	24.5	82.0
	0			0		—	1	1.0	60.0	12	12.4	82.0

*Protocol: 7-week-old Sprague Dawley rats exposed to benzene, toluene, xylene, and ethyl-benzene by ingestion (stomach tube), in olive oil, at dose of 500 mg/kg body weight, and with olive oil alone, once daily, 4–5 days weekly, for 104 weeks, and then kept under observation until spontaneous death.

[a]Alive animals after 52 weeks, when the first tumor (a Zymbal gland carcinoma) was observed.

[b]The percentages are referred toa the corrected number.



Fig. 1. Zymbal gland carcinoma with sebaceous pattern. Hematoxylin-eosin (H & E), ×200.

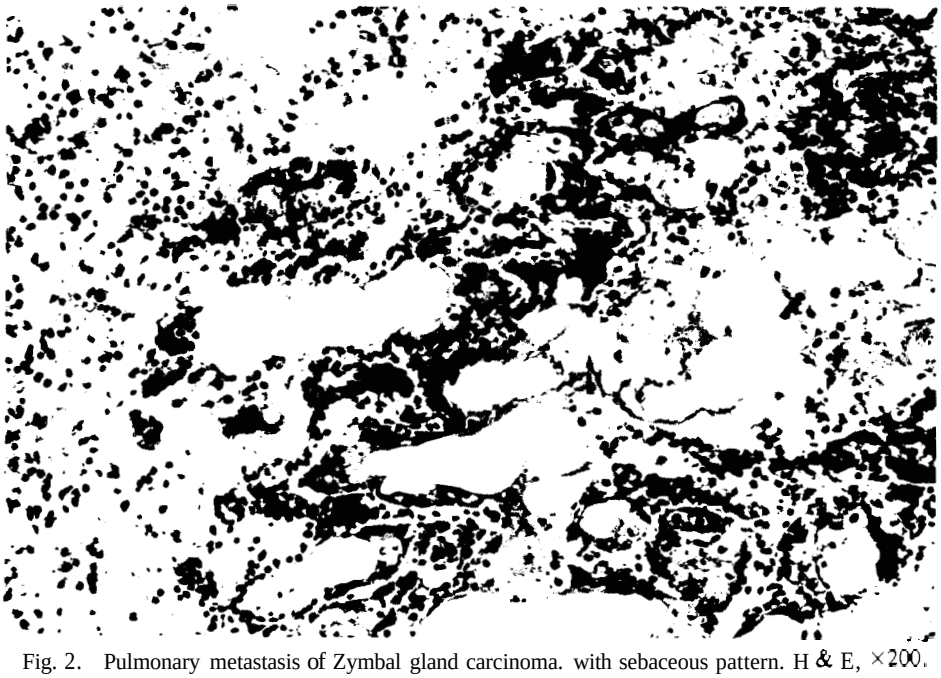

Fig. 2. Pulmonary metastasis of Zymbal gland carcinoma. with sebaceous pattern. H & E, ×200.

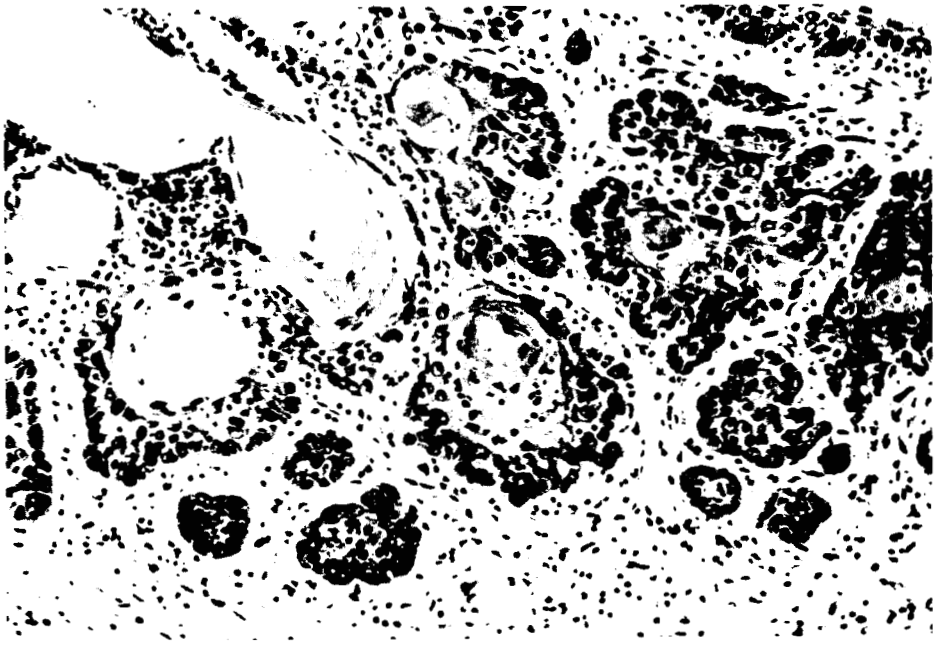

Fig. 3. Zymbal gland carcinoma with squamous pattern. H & E. ×200.



Fig. 4. Pulmonary metastasis of Zymbal gland carcinoma. with squamous pattern. H & E. x 320



Fig. 5. Zymbal gland carcinoma with tubular pattern. H & E. ×200.



Fig. 6. Metastasis to a regional lymph node of Zymbal gland carcinoma. with tubular pattern. H & E. ×200.

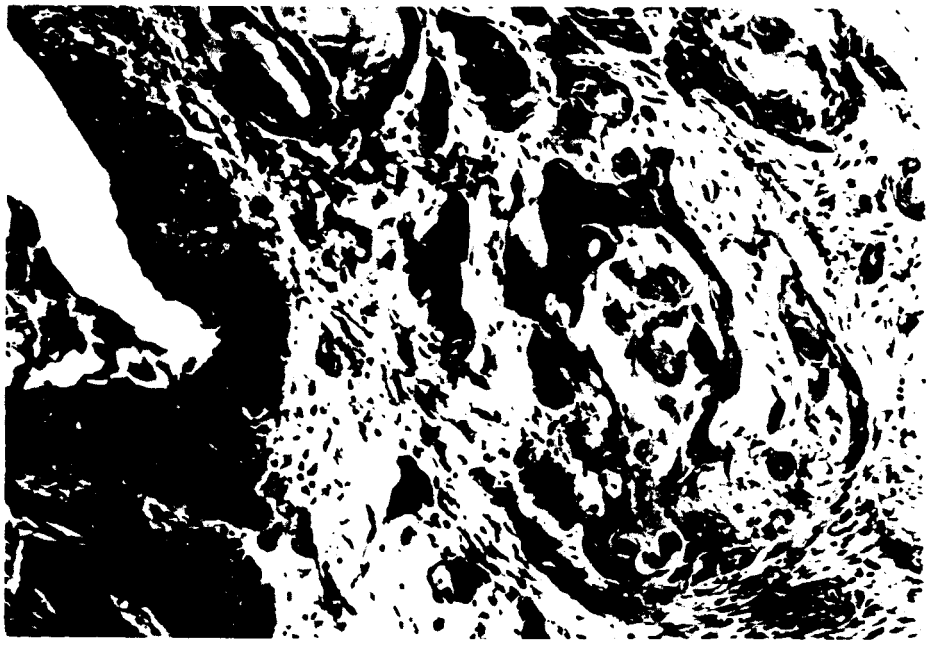

Fig. 7. Squamous cell carcinoma of the oral cavity. H & E. ×200.

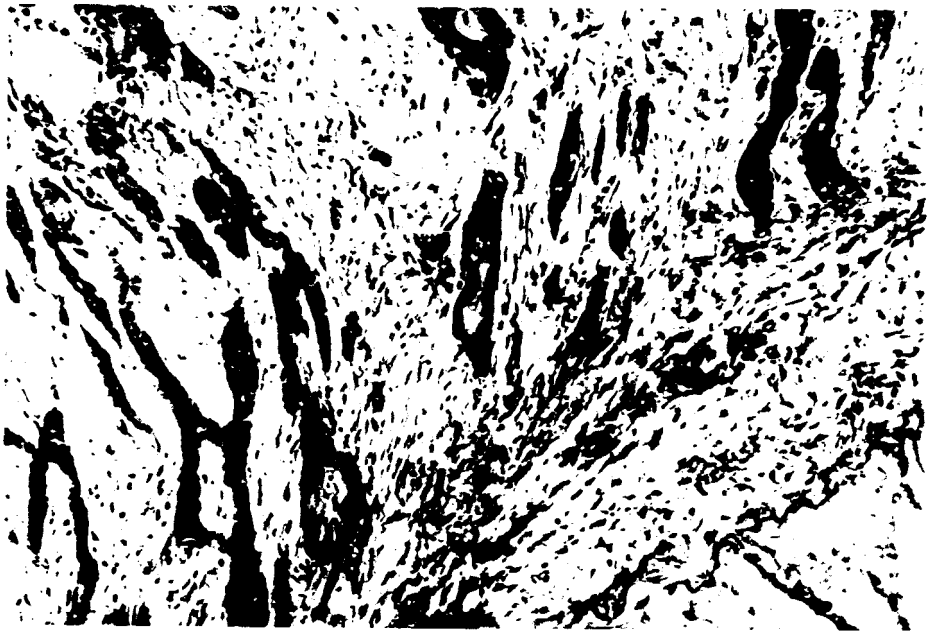

Fig. 8. Squamous cell carcinoma of the oral cavity, invading the surrounding tissues. H & E, ×200



Fig. 9. Squamous cell carcinoma of the nasal cavities. H & E, x 128.



Fig. 10. A detail of the carcinoma shown in Figure 9. H & E, ×200.

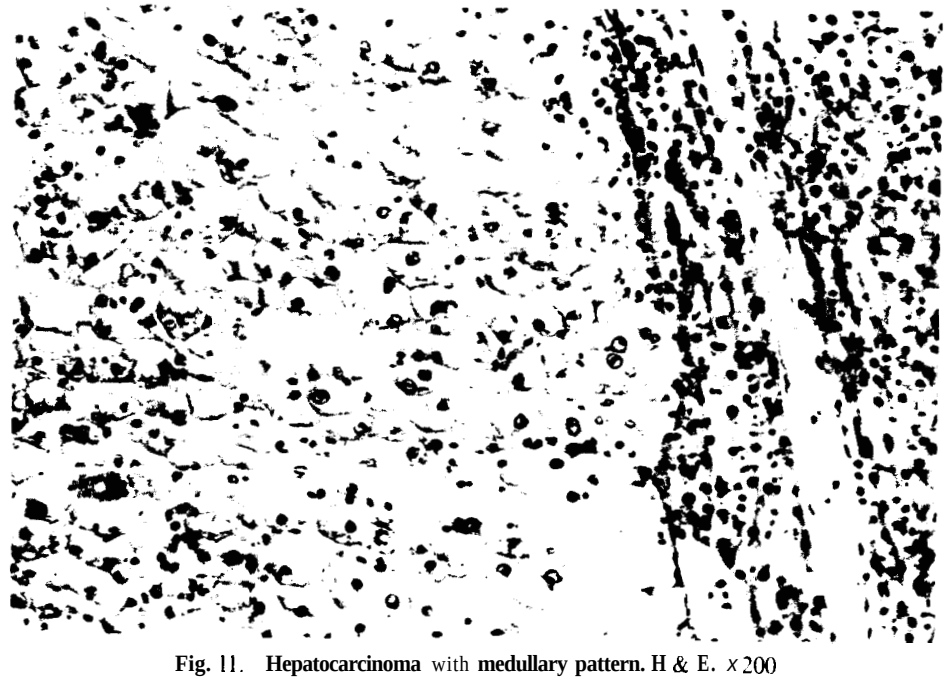

Fig. 11. **Hepatocarcinoma** with **medullary pattern.** H & E. *x* 200

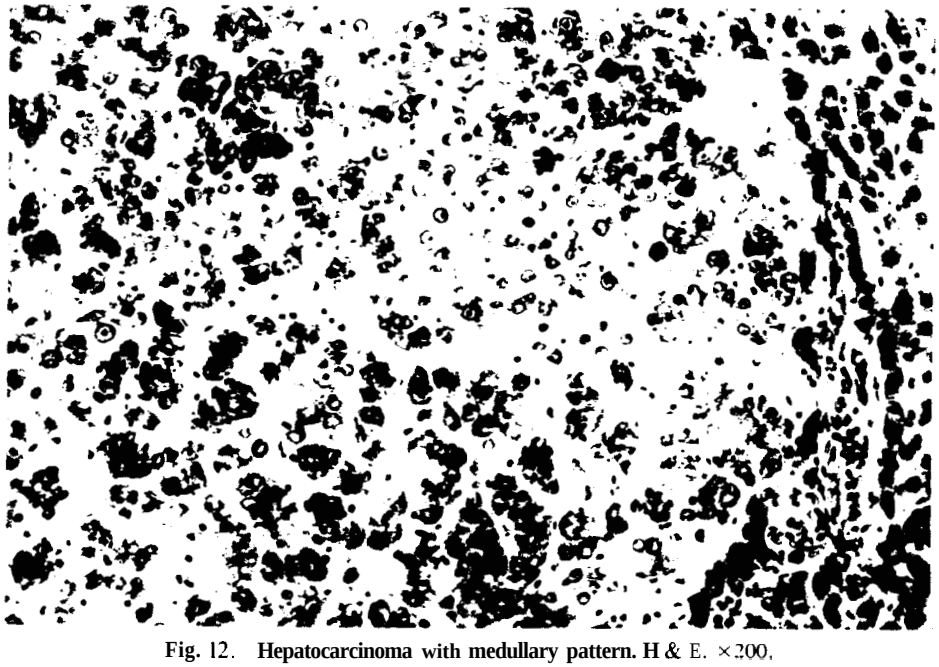

Fig. 12. **Hepatocarcinoma with medullary pattern.** H & E. ×200.

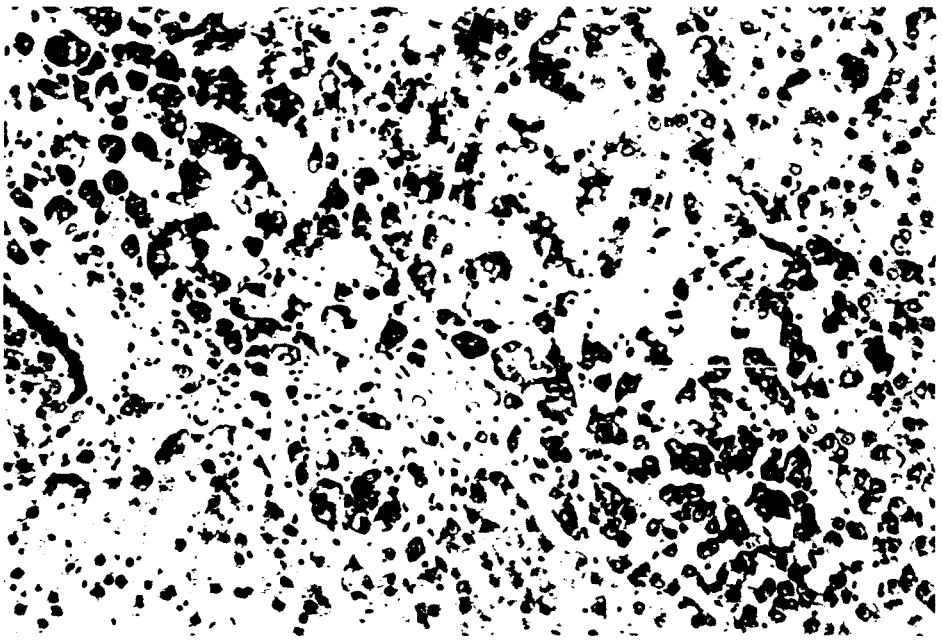

Fig 13 **Hepatocarcinoma** with **medullary—early trabecular pattern** H & E. ×200

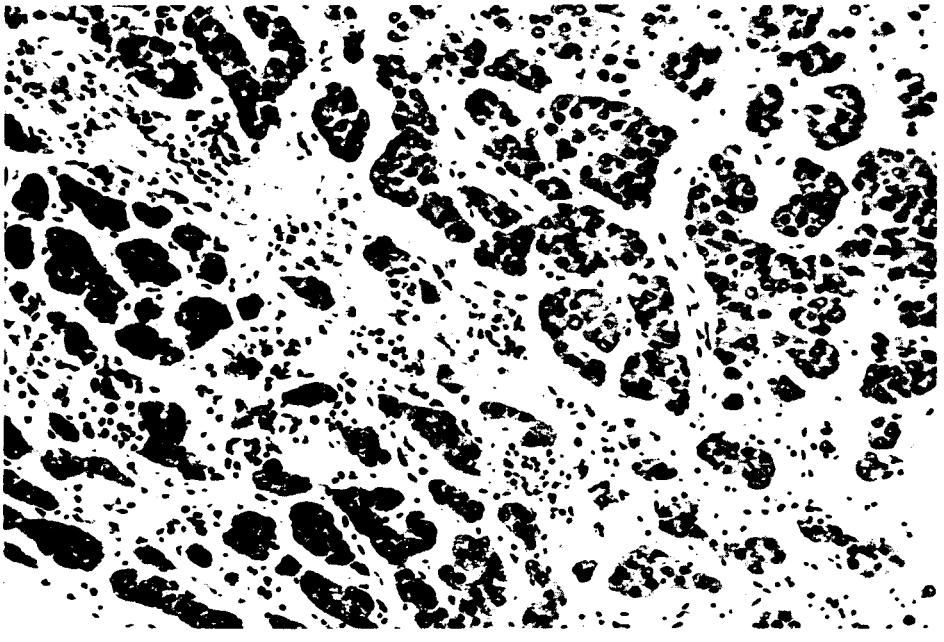

Fig. **14. Hepatocarcinoma with trabecular pattern.** H & E. ×200.

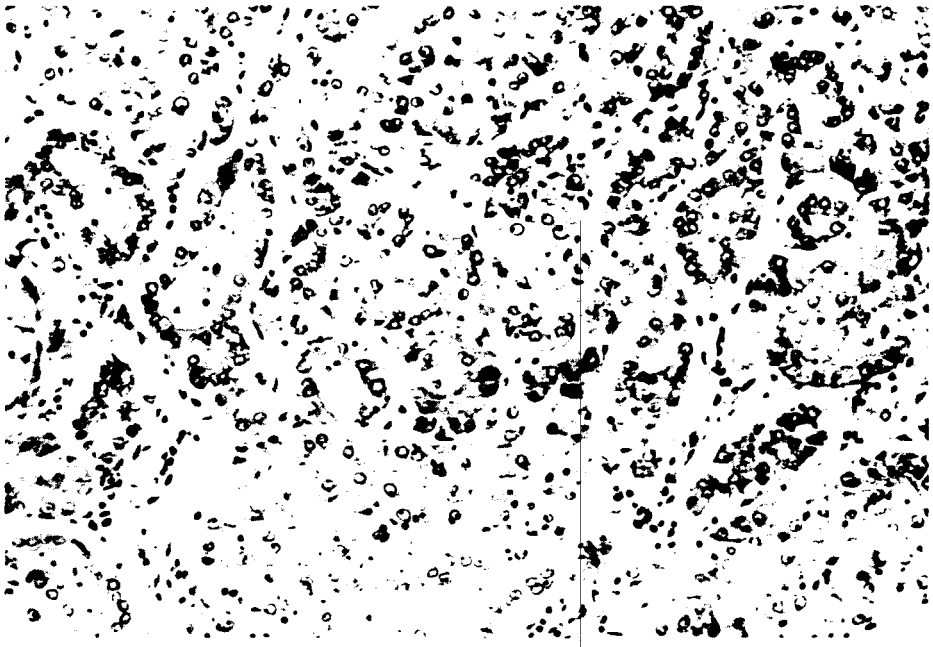

Fig. 15. Hepatocarcinoma with acinar pattern. H & E, ×200.

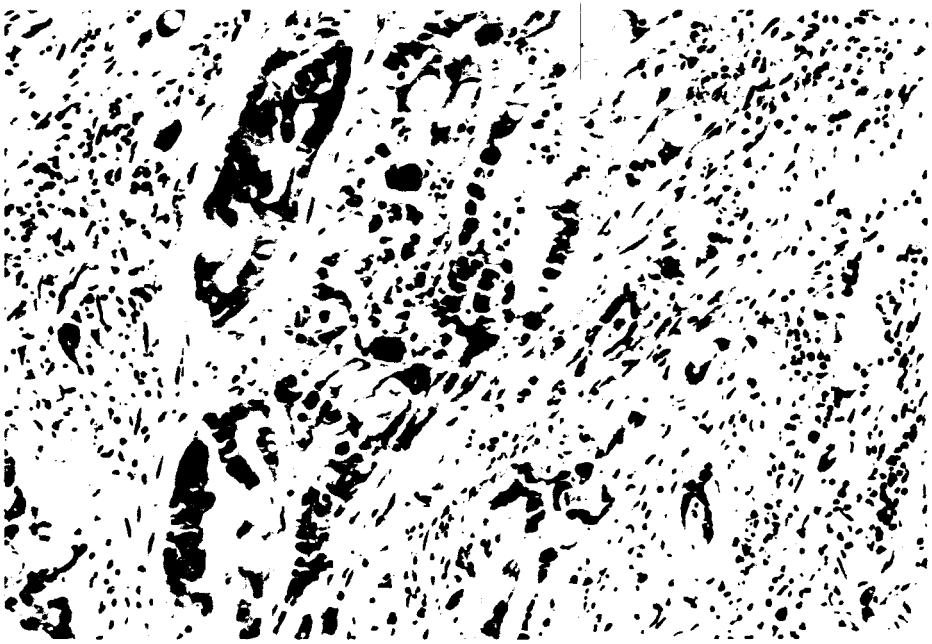

Fig. 16. Hepatocarcinoma with acinar pattern, invading the stroma. H & E. ×200.

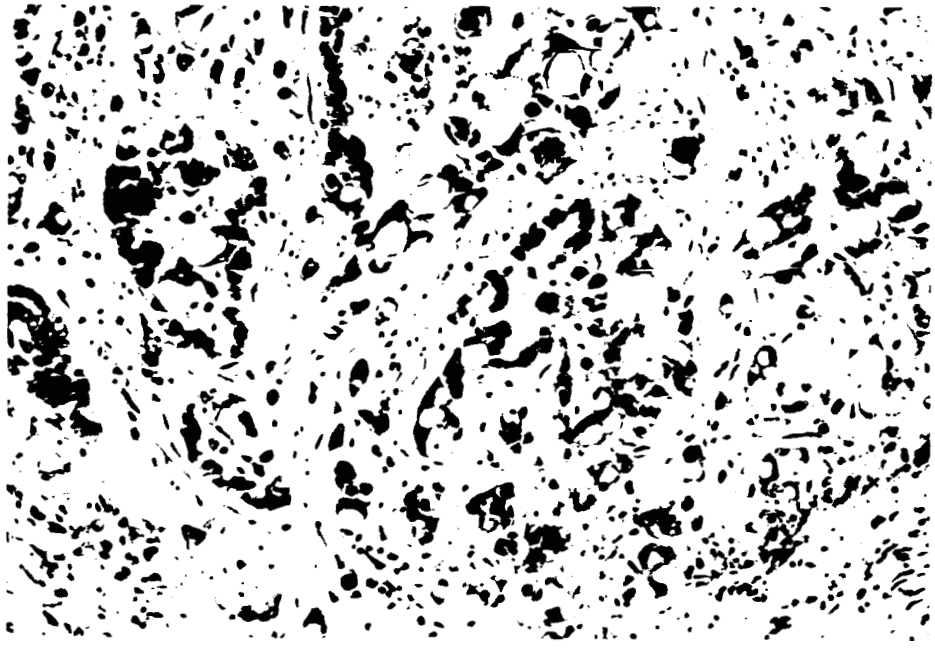

Fig. 17. Hepatocarcinoma with acinar pattern, invading the stroma. H & E, ×200

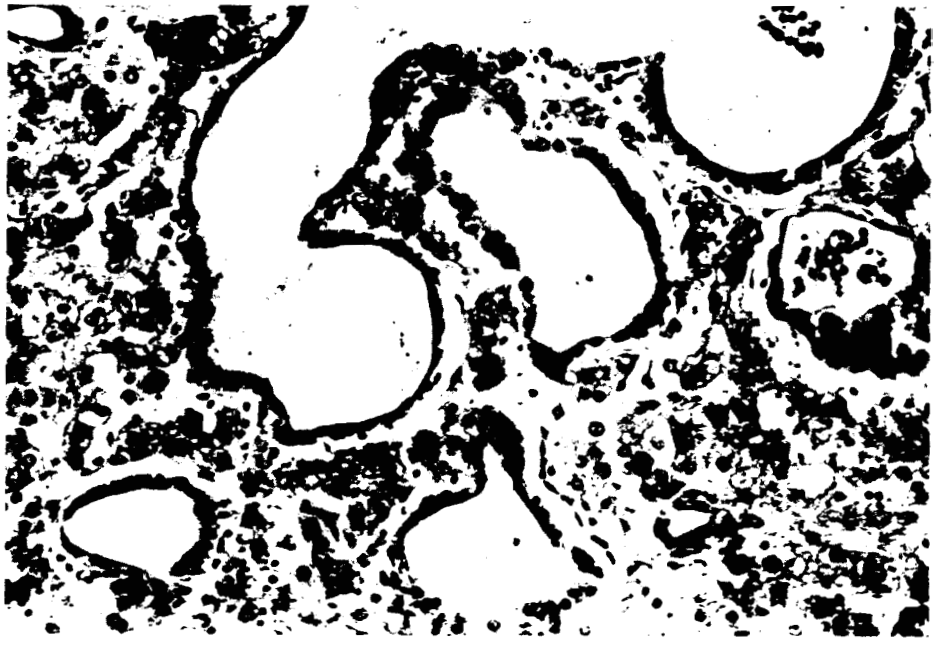

Fig. 18. Hepatocarcinoma showing areas with adenoid pattern. H & E, ×200.

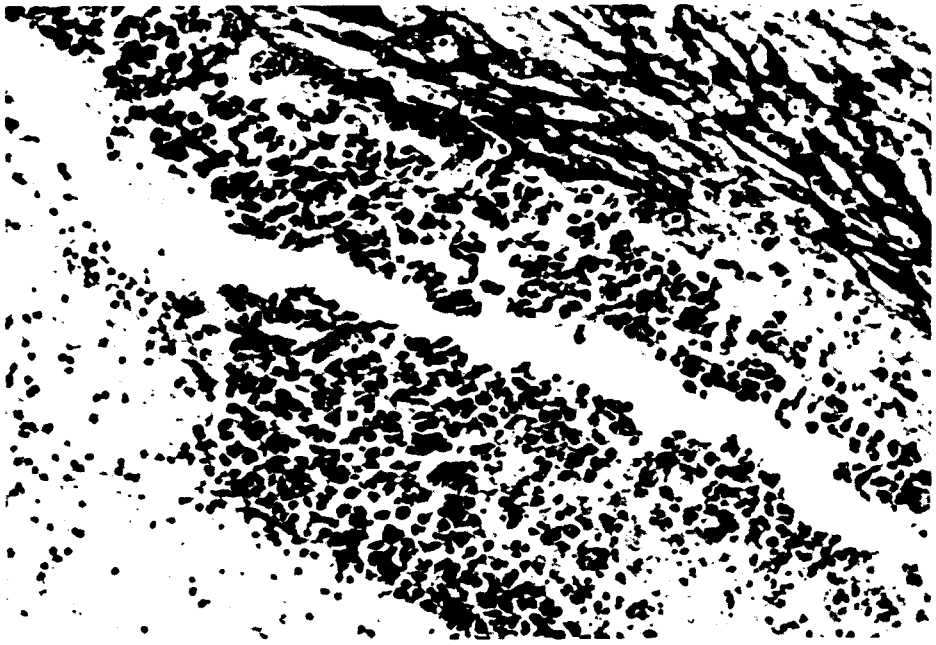

Fig. 19. Liver angiosarcoma. H & E. ×200.

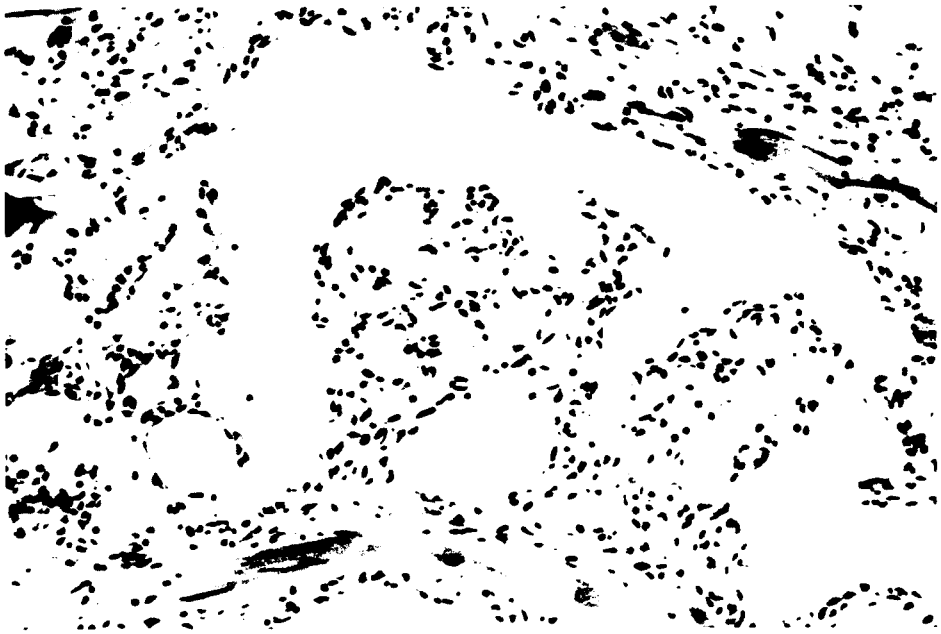

Fig. 20. Subcutaneous angiosarcoma invading the panniculus carnosus. H & E. ×200.

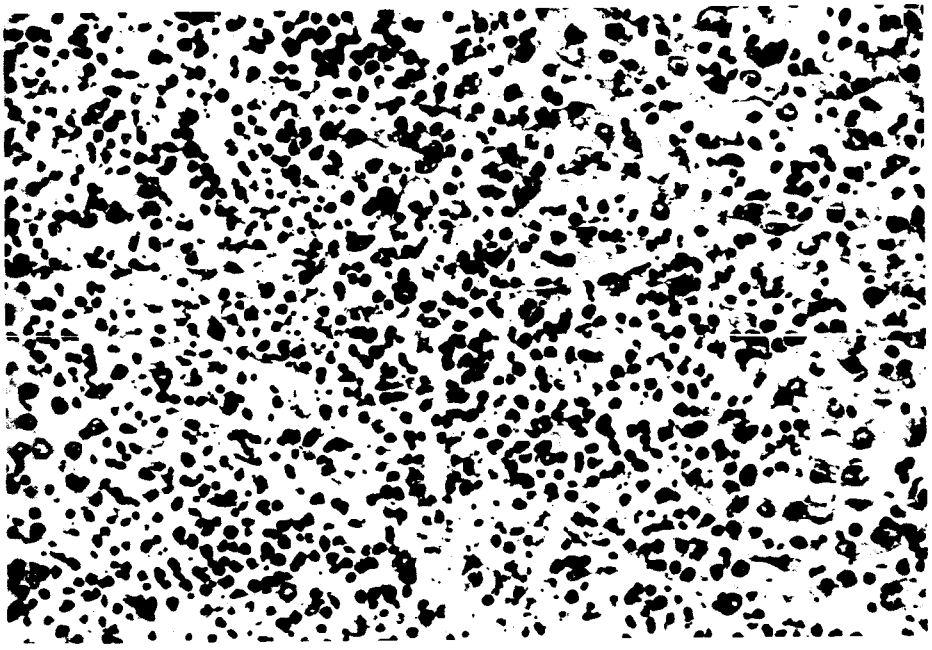

Fig. 21. Lymphoblastic leukemia (liver). H & E. ×320.

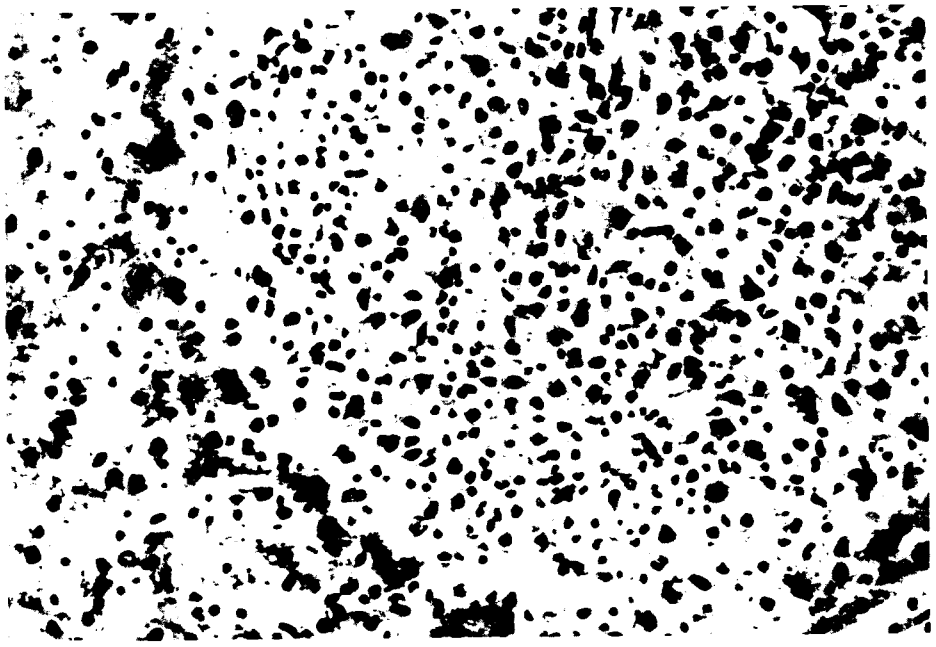

Fig. 22. Histiocytosarcoma (liver). H & E. ×320.



Fig. 23. Mammary adenocarcinoma. H & E, ×200.



Fig. 24. Pulmonary metastasis of mammary adenocarcinoma. H & E. ×200.