The Induction of Tumors with Extracts from Human Livers and Human Cancers*†

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The theory that there may be chemical causes for many human tumors in addition to those, internal and external, now known to be due to chemicals is comparatively new. The list of human external tumors which are chemically induced is quite large. It includes cancer of the skin following prolonged exposure to arsenic in industry (26) or in medication (17); cancer of the skin in gas works and other industries following exposure to coal tar (39) caused by the carcinogen 3,4-benzpyrene (6); cancer of the skin in cotton mule-spinners (32) due to the action of lubricating oil; and others. The list of human visceral tumors caused by specific chemicals is smaller. It includes, at the present time, aniline tumors of the urinary bladder (28) caused by β-naphthylamine (16); sarcomas of bone caused by radium salts (24); and some carcinomas of the lung caused by the inhalation of radioactive ores (13, 27), and possibly also by chrome compounds (38), asbestos (22, 15), and iron oxide (6).

Chemical agents responsible for human visceral tumors could conceivably be of exogenous or endogenous origin. If exogenous, the portals of entry would include the alimentary tract (17, 16), the respiratory tract (13, 22, 9), and the skin, and the chemical nature of the agents might show wide variation. If endogenous, they might be some pathological substance, or a physiological compound present in pathological amounts (21) or acting under abnormal circumstances.

The theory of chronic irritation as the cause of certain cancers was based upon the clinical observation that in man some cancers begin at points of prolonged, repeated, small traumas. Outstanding examples are the cancers which occur in the gall bladder and renal pelvis in relation to stones; cancers of the mouth in relation to broken teeth; malignant melanomas resulting from trauma, surgical or otherwise, to a pigmented mole; cancers of the lower lip in relation to pipestems; and cancers of the breast in relation to stasis of inspissated secretions. Although the original explanation, that such traumas led to cell proliferation for purposes of repair and replacement and that this cell reproduction finally went beyond the bounds of control and became irreversable, has not had experimental confirmation, the theory need not be discarded but may need modification. The basic observations can possibly be correlated with a chemical theory of the causation of cancers. The chronic irritation theory was based on the cell as the fundamental unit, whereas still more fundamental, chemical bases undoubtedly exist. That chemical explanations were not forthcoming, despite the old observations that certain substances such as tar, arsenic, paraffin, lubricating oil, etc., could cause cancers, seems strange until it is remembered that these examples of chemically induced cancer accounted for a comparatively small percentage of the cancers of one tissue only, the skin, and that they were thought to represent curiosities and not a general principle. Only with the isolation of 3,4-benzpyrene from tar in 1933 by Cook, Hewitt, and Hieger (6) did the relationship of the chemically induced group of tumors to a broad chemical theory for the causation of cancers finally appear. And with the principle established that some kinds of human cancer were caused by a chemical compound, related to substances occurring naturally in the body, it was reasonable to wonder if endogenous carcinogenic substances might be formed at or concentrated in sites of chronic injury and there eventually induce tumors. If this were shown to be true, the clinical observations of tumor formation at sites of chronic mechanical irritation might be correlated with work originating in the experimental laboratory. Furthermore the theory of chronic irritation as a cause of cancer would be correlated with a more fundamental chemical theory, which already has some proven basis.

Experimental attempts to find cancer-producing substances in relation to human cancers were the logical sequel to a number of converging lines of evidence and ideas of recent years. Some of these are:

(a) The demonstration by isolation and identification of the active cancer-producing principle in coal tar that this chemical, and others, are related to some naturally occurring substances in the body—the sterols, bile acids, sex hormones, etc.

(b) The synthesis of a great number of polycyclic aromatic hydrocarbons with powerful cancer-producing ability in animals, the starting point for some of which are substances occurring naturally in the body (7, 5, 11).

(c) The demonstration that certain physiological substances (e.g., the estrogens) can induce tumors in animals under specialized conditions (21). It is no longer necessary to assume that human carcinogens are all pathological compounds.

(d) The realization that cancer can be caused by a variety of agents, and is therefore not one disease but a group of diseases. In the past, the word cancer to designate a disease was more or less unconsciously employed in the sense in which the words typhoid fever or pneumonia are used instead of the broader term infectious disease. In its etiology, pathogenesis, symptomatology, pathology, and problems in diagnosis and in treatment, a carcinoma of the skin due to 3,4-benzpyrene is no more like a carcinoma of the urinary bladder due to β-naphthylamine or like a carcinoma of the stomach due to as yet unknown causes than pneumococcic pneumonia is like tuberculosis. The different cancers have in common the fundamental pathological process which is called neoplasia, just as the infectious diseases have in common the fundamental pathological processes of inflammation and immune reactions.

(e) The realization that some cancers could have more than one cause, as has been demonstrated for carcinoma of the skin and of the lung. This has its analogy in the field of infectious disease where pneumonia is caused by numerous agents.

(f) The realization that some carcinogens can cause more than one kind of cancer. The proof for this comes from experi-
ments on lower animals at the present time. In the infectious
diseases, likewise, some agents bring about more than one
disease as, for example, the pneumococcus which causes pneu-
monia, meningitis, or peritonitis.

(g) The realization that, associated with the long-known
cytopathological changes at a site of chronic tissue irritation, there
may be chemical changes, as a result of which endogenous
carcinogens may be formed.

These concepts complicate the problem of the causation of
cancers, but at the same time they break it up into units each of
which is approachable. It may prove possible and profitable to
add, one by one, additional causes for cancers to those
already known.

If such causes can be discovered and removed from the
environment it may become possible to control cancer by pre-
vention, as typhoid fever is controlled today. The immunological
processes leading to recovery from an attack of typhoid fever are
not fully understood, but the disease can be controlled in a
population. Similarly perhaps some kinds of cancer should be
controllable by prevention, even though the exact processes
within the cells which give them neoplastic properties are not
completely understood. Prevention of cancers should be possible
if the causes are exogenous chemical agents.

In the chemically induced human tumors previously men-
tioned, the conditions of exposure are so extraordinary that the
relationships of cause and effect can be traced even though many
years elapse before the tumor appears. In contrast if one of
the common visceral tumors, such as gastric carcinoma, were
due to some exogenous carcinogen, it might be a substance
which is so common in the environment in America and Europe
as to have escaped suspicion. A careful survey of the geo-
ographic and racial distribution of tumors, or chemical analysis
of many substances in the environment, might disclose valuable
clues. For example both the Bantu living under primitive conditions
in South Africa has very little gastric cancer, according to
competent observers (2); whereas in Afro-Americans, some of
whom are surely Bantus, this tumor occurs. Presumably the
genetic constitution has not changed entirely, and environ-
mental factors probably determine the tumors.

Two distinct problems crystalize from these remarks:

(a) Does chronic irritation lead to cancer because of the local
presence of carcinogenic substances?

(b) Do cancers with no obvious chemical cause have the same
different chemical causes as those which are generally recog-
nized as being due to some chemical agent?

The next step was to examine the human body itself for the
presence of carcinogenic chemicals.

In 1936 a search in man for carcinogens, exogenous or endo-
genous, was begun in this laboratory. Since 1938 this work
has been carried out vigorously, some of it in association with
Professor F. C. Koch. Many human tissues, neoplastic and non-
neoplastic, as well as body secretions and excretions, have been
studied or are now under investigation. Emphasis has been
placed on the study of potentially cancerous tissues, and on
lesions and substances found in close association with the
known occurrence of human cancer. Up to the present time
extracts from human livers have been most interesting, although
human urine has also yielded a carcinogenic extract (33).

EXPERIMENTS WITH HUMAN LIVER

There are a number of reasons why liver was se-
lected for analysis for the presence of carcinogens;
among others are the following:

(a) The chemical relationships of the liver to the
bile acids and to sterols and of these to some of the
known carcinogenic hydrocarbons (10, 31).

(b) The functions of the liver in detoxification and
excretion, which suggest that if chemical carcinogens
are present elsewhere in the body some of them may
eventually find their way to the liver.

(c) Its availability in large quantities, since it is
the largest organ in the body.

(d) The frequent occurrence of cancer of the biliary
system, extrahepatic and intrahepatic, which indicates
that exceptional influences are acting on the cells of
this tubal system. The incidence of this type of can-
cer in America is low in comparison with some other
parts of the world (2, 3). Nevertheless, even here, a
cell or group of cells in this system has a greater chance
of becoming cancerous during the life of the indivi-
dual than any other cells in the body, possibly ex-
cepting those of the uterine cervix. Even gastric
epithelial cells, considered as individuals, give rise to
cancer less commonly. The ratio of epithelial cells in
the extrahepatic bile passages to those in the stomach
is approximately 1:300, while the ratio of cancers in
these locations is about 1:60 (36).

EXPERIMENT I. NONSAPONIFIABLE LIPID EXTRACT OF
HUMAN CANCER LIVERS

The livers from persons with cancer of various
types were extracted by a method planned for recov-
ering substances of the carcinogenic hydrocarbon
group. This method involved saponification followed
by extraction with ethylene dichloride. The procedure
was:

Preservation of the liver in one volume of 95 per cent
alcohol after grinding.

Addition of water—volume equal to that of the alcohol.

Addition of 10 gm. of KOH per 100 gm. of liver tissue.

Saponification for 18 to 24 hours on a steam bath under a
reflux condenser.

Evaporation to dryness at reduced pressure.

Addition of 10 gin. of KOH per 100 gin. of liver tissue.

Extraction with ethylene dichloride; repeated 3 times.

Dehydration with anhydrous Na2SO4, followed by filtration.

Extraction 4 times with ethylene dichloride and evaporation
of the pooled extracts to dryness.

In this experiment 9,420 gin. of liver obtained from
8 persons with carcinomas was extracted. The livers
contained no gross or microscopic tumor except one
that had small scattered metastases. The primary
tumors were located in the stomach (3 cases), lung
(2 cases), esophagus, pancreas, and rectum (1 case of
each). The yield in crude extract was 65·6 gin. Ad-
ditional information regarding similar extracts has
been published (34).

This extract was tested in mice for cancer-produc-
ing ability by a single subcutaneous injection of
0.5 gin. dissolved or suspended in 0.5 cc. of sesame oil.
These mice were of our partly inbred albino stock in
Cancer Research
which spontaneous sarcoma, other than lymphosarcoma, has not been known to occur. They were of both sexes and were 55 to 83 days old at the time of injection.

This extract proved caustic in the subcutaneous tissues and many mice developed sloughs. Others had fluctuant masses at the site of injection for many months. The first tumor at the site of injection appeared in the 6th month and the mouse died in the 7th month (182 days). Thereafter other tumors developed in rapid order as indicated in Table I. The percentage yield in the effective total tested was 32.4 per cent (12 tumors in 37 mice). All of these tumors were in males.

The tumors were spindle celled or pleomorphic celled sarcomas. They grew rapidly and infiltrated the overlying skin and the underlying muscle and fascia. They appeared to arise directly in the residue from a strain inbred in this laboratory by brother to sister matings for 8 generations, were used. Each was injected subcutaneously once with 1 gm. of the liver extract in 1 cc. of sesame oil.

This amount of material caused necrosis with sloughing in some rats but not all, several showing large amounts of retained injected material at autopsy. In none did a tumor appear at the site of injection. One rat, dying in the 6th month after injection, had a mesenteric lymphosarcoma which was not considered an induced tumor. The survival time of these rats is indicated in Table I.

### EXPERIMENT

#### EXPERIMENT 1. NONSAPONIFIABLE LIPID EXTRACT OF HUMAN NONCANCER LIVERS

An extract was prepared from the pooled livers of 7 persons who died without cancer but with the following main diagnoses: hypertensive heart disease of the extract which was injected. In one mouse 2 tumors appeared to have arisen independently. In another, one tumor metastasized to the lung. Attempts at serial transplantation, carried out with 3 tumors, were successful in 2. The microscopic appearance of the tumors remained unchanged during 4 transplant generations.

### EXPERIMENT 2. NONSAPONIFIABLE LIPID EXTRACT OF HUMAN CANCER LIVERS (Rats)

In this experiment the same specimen of liver extract as that used in experiment 1 was tested in rats for carcinogenicity. Twenty-four young male rats, (2 instances), purulent meningitis, chronic glomerulonephritis, acute hemorrhagic pancreatitis, lobar pneumonia, and bronchopneumonia. This extract was prepared by the methods used in experiment 1. From 9,045 gm. of fresh liver the yield of nonsaponifiable lipid extract was 95.0 gm.

This was injected subcutaneously into mice of both sexes, of the stock used in experiment 1 and under identical conditions, except that each mouse received 2 injections, 9 weeks apart. Each injection consisted of about 250 mgm. of the extract in 0.5 cc. of the same sesame oil. Thus while each mouse received a total of 500 mgm. of the extract as in experiment 1, it also had twice as much sesame oil, a total of 1 cc. The dose of extract was divided and diluted to reduce sloughing and extrusion. This result was apparently achieved, as large amounts of extract were seen at autopsy.

The mice lived to the ages shown in Table I. Five (4 males and 1 female) developed sarcomas at the

### Table I: Carcinogenicity of Nonsaponifiable Lipid Extracts of Human Livers

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1 The mice used in all the experiments reported in this paper were from this stock. A few data on their susceptibility to tumor induction by methylcholanthrene and by crude urine extracts have been reported (33).

2 In a preliminary report of this experiment (35) 13 sarcomas were mentioned. One of these is now disqualified as possibly an undifferentiated carcinoma and not surely induced.
site of injection. In gross and microscopic appearance these tumors resembled those seen in experiment 1. The first animal died with a tumor in the 12th month. The induction time was therefore longer than that for the cancer liver extract, and the percentage yield was lower, 14.3 per cent. These indicate that the carcinogenicity of this extract was low.

**Experiment 4. Non-Saponifiable Lipid Extract of Human Non-Cancer Livers (Rats)**

Twenty-six rats were injected with the liver extract used in experiment 3. The other conditions of the experiment were the same as those of experiment 2 except that rats of both sexes were used, 12 males and 14 females. Each received 1 gm. of extract in 1 cc. of sesame oil, given once. These rats also extruded part of the extract after injection, but most of them had generous amounts at autopsy. They lived to the ages shown in Table I. None developed tumors at the site of injection.

**Experiment 5. Benzene Extract of a Human Liver**

A liver weighing 1,250 gm., from a white woman 62 years of age who died of carcinoma of the stomach, was extracted with benzene according to the method of Schabad (30), slightly modified as to the temperature at which the extractions were carried out. This liver contained a few scattered tumor metastases. It was put through a grinder 6 times, after which it was extracted with freshly distilled, thiophene-free benzene. Eight extractions were made at room temperature, each with 1 liter of benzene. Next an extraction was made with 6 liters of benzene, alternating at icebox and room temperatures. Finally an extraction was made with 4 liters of benzene over a period of 3 weeks. The total extract obtained by these 18 liters was 94.3 gm. of a brownish fat which was semiliquid at room temperature. This extract was injected subcutaneously into 32 mice (15 males and 17 females) in 0.5 cc. amounts. At 4, 8, 12, and 16 weeks after the first injection the dose was repeated for each mouse and in the same location. Thus each mouse received a total of 2.5 cc. in 5 doses during a period of 16 weeks.

This extract was well tolerated, the mice rarely showing sloughs or sinuses, but usually having soft, fluctuant masses at the site of injection. The results are shown in Table II. No sarcomas occurred in the subcutaneous tissues, although 14 mice survived for 12 months, 9 for 15 months, and 4 for 18 months.

**Experiment 6. Fractions of Benzene Extracts of Human Cancer Livers**

Although no tumors were produced at the injection site by any extract, experiments 6 A, B, C, D, E are reported because others may thus avoid un-

<table>
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<th>Experiment</th>
<th>Sex of Mice</th>
<th>Time in Months</th>
<th>Induced Sarcomas</th>
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<td>12 11  9  5  2  1  0  0</td>
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<td>4 C</td>
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% planned duplication of effort; because they show that the tumors in experiments 1 and 3 were not induced by nonspecific irritation by tissue extracts; and because of the tumors which occurred at a distance from the injection site.

**Preparation of the extracts.—12,070 gm. of liver from 11 persons with cancer was used for extraction. The cancers were primary in the following locations: stomach, 4; uterus, 2; colon, 1; ovary, 1; esophagus, 1; undetermined primary site, 1; osteogenic sarcoma, 1. Eight of these livers were free of tumor metastases on gross and microscopic examination, while 3 showed occasional small metastases not more than 1 cm. in size.

The liver tissue was dried with acetone after grinding. It was then extracted with benzene in a continuous extractor for 24 hours. This material (217 gm.)

**Table II: Carcinogenicity of Miscellaneous Human Tissue Extracts**

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was then extracted 3 times with ether. The ether-
insoluble residue (15 gm.) was used for injection in
experiments 6 B, C, and D.

The ether extract (202 gm.) was extracted with
saturated NaHCO$_3$ solution. The NaHCO$_3$ extract
was acidified and extracted with ether. This ether
extract was evaporated to dryness and yielded only
0.2 gm.

The ether extract (201.8 gm.) was treated with
water, which formed an unbreakable emulsion. The
ether was evaporated from the emulsion and the water
suspension remaining was saturated with NaCl. The
semi-solid material was filtered off and extracted with
ether. The filtrate was treated with CaCl$_2$ and this
solution was extracted with ether. The 2 ether
extracts were combined and evaporated to dryness.
The weight of the residue was 145 gm. Some of this
material was used for injection in experiment 6 A.

The above-described ether-soluble extract (135 gm.)
was saponified with KOH in alcohol-water solution
(3:1). The alcohol was evaporated and the water
solution extracted with ether. The ether extract was
washed with water and evaporated to dryness. The
nonsaponifiable residue (13.3 gm.) was used for
injection in experiment 6 E.

Experiment 6 A. Ether-soluble fraction of a benzene
extract of human cancer livers.—Each of 15 female
mice, about 5 months old, was injected subcutane-
ously with 0.5 cc. of this extract. It was well toler-
ated by the tissues, and apparently almost entirely
retained. The mice lived to the ages shown in
Table II. None had sarcomas at the site of injection,
although many showed large amounts of injected
tissue. At autopsy only a few mice showed
any residual extract and these had but traces. There-
fore this extract cannot be considered to have been
fully tested.

Experiment 6 B. Ether-insoluble fraction of a benzene
extract of human cancer livers.—This extract
was a dark brown amorphous material, brittle when
cold but plastic when warmed. It was molded into
fusiform 100 mgm. pellets. These were implanted
subcutaneously through a trochar into 15 female mice
about 6 months old.

These pellets were caustic to the tissues and were
extruded with large sloughs between the 8th and
25th days. The mice lived to the ages indicated in
Table II. No tumors occurred in the subcutaneous
tissues. At autopsy only a few mice showed
any residual extract and these had but traces. There-
fore this extract cannot be considered to have been
fully tested.

Experiment 6 C. Ether-insoluble fraction of a benzene
extract of human cancer livers.—The same ex-
tract as that used in experiment 6 B was molded into
pellets of only 25 mgm. These were implanted sub-
cutaneously through a trochar into 16 female mice
about 5 months of age.

These pellets also were not tolerated by the tissues
and were sloughed out between the 8th and the 25th
days. The mice lived to the ages indicated in Table II.
Autopsies revealed no chemical in any mouse. No
tumors developed at the site of injection. This ex-
tact also was inadequately tested.

Experiment 6 D. Ether-insoluble fraction of a benzene
extract of human cancer livers.—The same ex-
tract was used as in experiments 6 B and 6 C. Here,
however, it was pulverized at icebox temperature and
implanted under the skin through a trochar. Each
mouse received about 45 mgm., distributed along the
trochar tract which ran most of the dorsal length of
the mouse. Thirty-six mice, equally divided as to
sex and 5 to 11 weeks of age, were so injected.

This extract also was poorly tolerated and most of
the mice developed linear sloughs between the 7th
and the 30th days. No tumors occurred in the subcu-
taneous tissues. At autopsy only a few mice showed
any residual extract and these had but traces. There-
fore this extract cannot be considered to have been
fully tested.

Experiment 6 E. Nonsaponifiable fraction of a benzene
extract of human cancer livers.—Each of 17 female
mice, about 6 months of age, was injected subcutaneously with about 100 mgm. of this extract
dissolved in 0.5 cc. of sesame oil. This extract ap-
peared to be well tolerated, and to be retained with-
out loss. The mice lived to the ages indicated in
Table II. None had sarcomas at the site of injection,
although many showed large amounts of injected
extract.

EXPERIMENTS WITH HUMAN CANCERS

Experiment 7. Extract of Human Cancers

Fresh human tissue, weighing 7,970 gm., approxi-
mately 4,770 gm. of which was carcinoma tissue and
the remainder either lung or liver, was saponified
and extracted by the methods previously described.
The cancer tissue was composed of the following
types: approximately 400 gm. of carcinoma of the
rectum metastatic in the liver; about 870 gm. of car-
cinoma of the pancreas; about 500 gm. of carcinoma
of the bile ducts metastatic in the liver; about 3,000 gm.
of primary lung carcinoma and its metastases in the
mediastinum and the opposite lung.

From this tissue the nonsaponifiable lipid extract
was 33.1 gm.

Each of 45 female mice, about 3 months of age,
was given 1 subcutaneous injection of about 0.5 gm.
of this extract in 0.5 cc. of sesame oil.
The animals lived to the ages shown in Table II. The extract appeared to be almost entirely retained. Nevertheless no sarcomas arose at the site of injection.

**Experiment 8. Extract of Human Cancers**

In this experiment the human tissues used for extraction had previously been treated by the Kaiserling method. The methods were essentially those outlined by Mallory (23) except that Kaiserling II consisted of 95 per cent alcohol and thymol was omitted from Kaiserling III.

18,435 gm. of tissue composed of the following was used:

- Retroperitoneal spindle cell sarcoma: 1,585 gm.
- Lymphosarcoma of the abdomen: 2,940 gm.
- Primary leiomyosarcoma of the pleura: 1,150 gm.
- Leukosarcoma of the mesentery: 1,100 gm.
- Peritoneal mesothelioma metastatic to the liver (estimated 95 per cent tumor): 1,110 gm.
- Peritoneal mesothelioma metastatic to the liver (estimated 66 per cent tumor): 3,650 gm.
- Cancer of the breast metastatic to liver (estimated 80 per cent tumor): 5,000 gm.
- Cancer of the pancreas metastatic to liver (estimated 50 per cent tumor): 850 gm.
- Malignant melanoma metastatic to the liver (estimated 95 per cent tumor): 650 gm.
- Primary carcinoma of the stomach, plus
  - Primary carcinoma of the jejunum, plus
  - Adenomatous hyperplasia of the prostate, total of: 400 gm.

This pooled tissue was saponified and extracted by the method previously described. The yield of non-saponifiable lipids was 93.6 gm. From this, 56.4 gm. of cholesterol was removed by crystallization and the method previously described. The yield of non-saponifiable lipids was 93.6 gm. From this, 56.4 gm. of cholesterol was removed by crystallization and recrystallization from boiling 95 per cent ethyl alcohol. After evaporation a residue of 37.2 gm. of extract remained which was used for injection.

Thirty-five male mice from 4 to 6 weeks of age were injected subcutaneously with this extract. An initial injection of about 250 mgm. in 0.5 cc. of sesame oil was followed by sloughs through which apparently most of the extract was extruded. Consequently 3 additional injections were given, 8, 12, and 16 weeks after the first dose. Each consisted of approximately 100 mgm. of extract in 0.1 cc. of sesame oil. They were moderately well tolerated. At autopsy nearly every mouse showed residual injected extract, varying in amount from traces to large masses.

The mice lived to the ages indicated in Table II. None had sarcomas at the site of injection.

**Experiment 9. Sesame Oil**

In all experiments reported in this paper in which sesame oil was used as a vehicle or solvent, the specimen of sesame oil was the same. In experiments 6 E, 7, and 8, in all of which sesame oil was used, no tumors occurred at the site of injection. Unless it be assumed that the tissue extracts exerted an inhibitory action on possible tumor induction by the sesame oil, these experiments show that this sample of sesame oil was not carcinogenic. However, the oil was tested alone in this experiment.

Eighteen female mice about 8 weeks of age were injected subcutaneously with 0.5 cc. of sesame oil. One year later, no sarcomas having appeared, each mouse then living was re-injected with 0.5 cc. of sesame oil, followed 4 weeks later by another similar injection.

No tumors were induced by these injections. The mice lived to the ages shown in Table II.

**Miscellaneous Tumors**

In these experiments numerous tumors were encountered which arose at a distance from the site of the injected extracts. The problem is to decide how many if any of them should be counted as induced. If only tumors occurring directly at the point of application of a substance being tested for carcinogenicity are considered induced, true tumor-producing ability may be overlooked, because some substances fail to produce tumors at the site of first contact with the tissues, for example, o-aminophenol (49) and dimethylaminobenzene (18). Others may induce tumors both at the point of application and at a distance, for example, 1,2,5,6-dibenzanthracene (1). It is necessary, therefore, to attempt evaluation of all tumors obtained in an experiment.

Another problem in interpretation arises in connection with tumors of the mammary gland when substances are tested for carcinogenicity by subcutaneous injection. When the injection is bulky some migration, with possible direct contact with mammary gland epithelium, is inevitable regardless of the site selected. Shall mammary gland tumors be considered induced if the chemical is demonstrated to be in relation to them? Strong and Smith (37) and Bonser and Orr (4) have reported induction of mammary gland tumors by direct contact with a carcinogen.

All the tumors of experiments 1 to 9 have been tabulated as to type and time of occurrence in Tables III and IV. By referring to the corresponding experiments in Tables I and II the number of animals alive at any time can be found. In some instances the number of tumors is greater than the number of mice that died because some mice had tumors of more than one type. Multiple tumors of a single type in a mouse are tabulated as one. All of the tumors were diagnosed histologically. Ovarian cysts have been omitted.

**Lung tumors.—**These were the common white nodules, which microscopically were usually papillary adenomas or carcinomas, although some had a sar-
### Table III: Summary of All Tumors in Experiments 1 and 3

<table>
<thead>
<tr>
<th>Sex</th>
<th>Type of tumor</th>
<th>Time in months</th>
<th>Total tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous sarcoma</td>
<td>1 3 2 1 2 2</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>Lung</td>
<td>1 1 1 1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>1 1 1 1 1 1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Uterus</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
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<td>1</td>
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<td>Male</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>6</td>
</tr>
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<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous sarcoma</td>
<td>1 1 1 1 2 2</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>Lung</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>1 1 1 1 1</td>
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### Table IV: Summary of All Tumors in Experiments 5, 6, 7, 8, and 9

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</tr>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hepatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>1 1 1 1 1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Experiment 6 A</td>
<td>Female</td>
<td></td>
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</tr>
<tr>
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<td>6 7 8 9 10 11 12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hepatic</td>
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<td>1</td>
</tr>
<tr>
<td>Experiment 6 B</td>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
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<td>Mammary gland</td>
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<td>6</td>
</tr>
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<td></td>
<td>Liver</td>
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<td>1</td>
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<tr>
<td>Experiment 6 C</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>2 3</td>
<td>5</td>
</tr>
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<td>Experiment 6 D</td>
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<td></td>
</tr>
<tr>
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<td>Lung</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>Lung</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1 2 1 1 1</td>
<td>6</td>
</tr>
<tr>
<td>Experiment 6 E</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>1 1 1 2 1 1</td>
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</tr>
<tr>
<td></td>
<td>Hepatic</td>
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<td>1</td>
</tr>
<tr>
<td>Experiment 7</td>
<td>Female</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>2 1 1 2 1</td>
<td>6</td>
</tr>
<tr>
<td>Experiment 8</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Experiment 9</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>6 7 8 9 10 11 12</td>
<td>6</td>
</tr>
<tr>
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<td>Lymphatic</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>1 1 1 1</td>
<td>3</td>
</tr>
</tbody>
</table>
coma-like stroma. A few had metastasized into the mediastinal lymph nodes, and some showed invasion of bronchi, but usually they were localized parenchymal tumors.

Lymphatic tumors.—While these were not all derived from lymphocytes, they belong to the general group of tumorous diseases of the hematopoietic and lymphatic systems. Leukemias, pseudoleukemias, and lymphosarcomas of many types are included.

Mammary gland tumors.—Here are included the carcinomas and adenomas of the mammary gland. They were usually adenocarcinomas but solid carcinomas were also seen. Acantho-adenocarcinomas found in the subcutaneous tissues are included here, although their origin from mammary gland epithelium is not proved.

Uterus.—One adenocarcinoma of the uterus with metastases was seen.

Ovary.—The one tumor encountered was an adenoma.

Liver.—One adenoma of hepatic-cell type was seen.

Table V is a summary of the tumors in experiments 1 and 3 (except the induced sarcomas). Table VI summarizes the other experiments in which human tissue extracts were injected. From these two tables a comparison can be made between the miscellaneous tumors, including the mammary gland tumors, occurring after injection of tissue extracts which were locally carcinogenic and those which were not. The data in these tables were taken from Tables I to IV and only mice living more than 6 months after injection were included. Without regard to longevity of the mice the percentages of each sex that developed tumors lie within the same range. Certainly the extracts that induced sarcomas at the site of injection were associated with no more tumors at distant sites than those extracts which did not induce sarcomas.

**DISCUSSION**

In experiment 1 the induction time and the percentage yield of tumors indicate that the 0.5 gm. of cancer liver extract which was injected, expressed in terms of the potency of methylcholanthrene, probably contained a fraction of a milligram of active carcinogen. In experiment 3 the induction time was longer and the percentage yield lower, indicating that the potency of this noncancer liver extract was less. Experiment 6 E, in which the nonsaponifiable lipids from the ether-soluble fraction of a benzene extract of cancer liver were used, may have given negative results because of low dosage or because of absence of a carcinogenic factor. The ether-soluble fraction of benzene extract, tested in experiment 6 A, was not carcinogenic under the conditions which prevailed, possibly for the same reasons. The ether-insoluble fraction of this same benzene extract, reported in experiments 6 B, C, and D, was negative.

---

**Table V: Summary of Miscellaneous Tumors**

<table>
<thead>
<tr>
<th></th>
<th>Male mice</th>
<th>Female mice</th>
<th>Total mice</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Number of mice</td>
<td>Number with tumor</td>
<td>Number of mice</td>
</tr>
<tr>
<td>Expt 1</td>
<td>21</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>Expt 3</td>
<td>26</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Totals</td>
<td>47</td>
<td>10</td>
<td>47</td>
</tr>
<tr>
<td>Per cent</td>
<td>21.3</td>
<td>56.8</td>
<td>36.9</td>
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</table>

**Table VI: Summary of Miscellaneous Tumors**

<table>
<thead>
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<th></th>
<th>Male mice</th>
<th>Female mice</th>
<th>Total mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of mice</td>
<td>Number with tumor</td>
<td>Number of mice</td>
</tr>
<tr>
<td>Expt 5</td>
<td>11</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Expt 6 A</td>
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<td>..</td>
<td>15</td>
</tr>
<tr>
<td>Expt 6 B</td>
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<tr>
<td>Expt 6 C</td>
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<td>..</td>
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<tr>
<td>Expt 6 D</td>
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<td>18</td>
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<tr>
<td>Expt 6 E</td>
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<td>17</td>
</tr>
<tr>
<td>Expt 7</td>
<td>..</td>
<td>..</td>
<td>42</td>
</tr>
<tr>
<td>Expt 8</td>
<td>35</td>
<td>7</td>
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<tr>
<td>Totals</td>
<td>64</td>
<td>14</td>
<td>136</td>
</tr>
<tr>
<td>Per cent</td>
<td>21.9</td>
<td>76.5</td>
<td>59.0</td>
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</tbody>
</table>

---

Cancer Research
also. This fraction was inadequately tested since it was not retained by the animals. The experiments are reported, however, because of their information on the incidence of miscellaneous tumors in these mice.

The failure to detect carcinogenic substances in human liver by the methods of Schabad (experiment 5) is probably not significant since only a single experiment was made, and unpublished data indicate that the carcinogen is absent in many livers.
An extract similarly prepared from the livers of noncancer-bearing persons had less carcinogenic activity, having an induction time of 12 months and a percentage yield of 14.3 per cent. Five sarcomas were induced in 35 mice. Neither of these 2 extracts induced tumors in rats.

A benzene extract of cancer liver, and various fractions obtained from a benzene extract of cancer livers failed to induce tumors at the site of injection.

Extracts of cancer tissues also did not induce tumors.

A theory for the chemical causation of cancer is outlined, and its possible relationship to the theory of chronic irritation is pointed out.

I am indebted to Dr. Curtis Flory and Dr. Ralph Barris for valuable help in some phases of this work, and to Mrs. Miriam Bolyard for valuable technical assistance.—Author.

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Paul E. Steiner


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