Direct contact of tar with lung tissue induces primary lung tumors in mice which possess the tendency to develop lung tumors spontaneously. Campbell (1) exposed mice to heavy concentrations of road-dust containing 2 to 3 per cent tar and found lung tumors in 74 per cent, while similar tumors arose in 14 per cent of the control group. Mice breathing dust from which the tar had been removed with benzene had a 45 per cent incidence of lung tumors.

No experiments are recorded in which dusts or carcinogenic compounds were introduced directly into the trachea of mice, probably because of the relative difficulty and tediousness of the procedure. Yet the intratracheal route of administration would obviate certain criticisms of the dusting technic, in which the substances may gain access into the body through the skin or the digestive tract as well as through the lungs, and in which the dose actually introduced into the lungs cannot be conveniently determined.

Intratracheal injection into larger animals has been tried by several investigators, with indifferent results. Kimura (2), in 1923, claimed to have produced an adenocarcinoma of the lung in one guinea-pig six months after the intratracheal insufflation of coal tar. Schabad (3) was unable to reproduce the observation, although a pseudo-cancerous inflammatory hypertrophy of the epithelium was obtained in two guinea-pigs.

Burrows and Boyland (4) failed to obtain neoplasia in two rabbits after the intratracheal injection of 1:2:5:6-dibenzanthracene. Valade (5) injected 0.2 to 2.0 mg. methylcholanthrene in oil intratracheally into 50 rats, and produced no epithelial tumors of the lung, although in three months 14 animals had peritracheal sarcomas or esophageal rhabdomyosarcomas.

The following investigations, in which the effect of the intratracheal introduction of carcinogenic hydrocarbons into mice was studied, were started in August 1938.

**Experimental**

*Technic:* Mice of A and L strains and back-cross albinos (ABC), weighing 25 to 30 gm., were anesthetized by the intraperitoneal injection of 0.2 to 0.3 c.c. of 0.6 per cent sodium pentobarbital solution. A longitudinal incision was made over the trachea, and the salivary glands and the ribbon muscles of the neck were separated by blunt dissection. The exposed trachea was lifted gently with a probe and the injections were made rapidly through a 27-gage

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1. 1:2:5:6-dibenzanthracene will be referred to as "dibenzanthracene" throughout this paper.
needle inserted between the tracheal rings and a half centimeter down toward the lungs. The neck wound was closed with one suture.

It was found that 0.1 c.c. of water, saline, horse serum, or charcoal suspension could be injected in this manner. There was a 35 per cent immediate mortality due to drowning and suffocation, but no delayed untoward effects or deaths; the wound healed within a few days.

When trypan blue was injected, 0.1 c.c. colored about two-thirds of the lung areas. Slight regurgitation occurred through the nose or the tracheotomy opening in some animals, but in the majority neither took place. Heavier and more occlusive substances, such as olive oil or lard, killed the animals invariably, even when 0.05 c.c. was used.

Intratracheal Injections: Strain A mice, about three months old, were injected intratracheally with the following substances:

1. Methylcholanthrene, 0.1 mg. dispersed in 0.1 c.c. horse serum and cholesterol (3 males, 8 females).
2. Dibenzanthracene, 0.1 mg. dispersed in 0.1 c.c. horse serum and cholesterol (5 males, 5 females).
3. Dibenzanthracene, 0.1 mg. adsorbed on 0.2 mg. charcoal in 0.1 c.c. saline (3 males, 8 females).
4. Charcoal suspension, 0.4 mg. in 0.1 c.c. saline (3 males, 7 females).
5. Horse serum and cholesterol, 0.1 c.c. (2 males, 8 females).

Strain A mice were used because they are known to be most susceptible to induced as well as to spontaneous primary lung tumors (6). Each animal received 0.1 mg. of the carcinogenic hydrocarbon. The horse serum and charcoal dispersions were prepared by Dr. Egon Lorenz according to a technic described elsewhere (7).

Intravenous Injections: To compare the efficacy of the intratracheal and the intravenous routes of administration, strain A mice, about three months old, were injected in a lateral tail vein with the following substances:

6. Methylcholanthrene, 0.1 mg. dispersed in 0.1 c.c. horse serum and cholesterol (8 males, 8 females).
7. Dibenzanthracene, 0.1 mg. adsorbed on 0.2 mg. charcoal in 0.1 c.c. saline (5 males, 5 females).
8. Horse serum and cholesterol, 0.1 c.c. (5 males, 5 females).

Results

Of the 89 mice used, only 3 died before the experiment was terminated. Half of the animals were killed at the end of four months and the rest five months after the injections.

The results are presented in Table I. It is seen that in the control groups (charcoal or horse serum intratracheally and horse serum intravenously), the incidence of primary lung tumors was 20 per cent. The animals were about eight months old, and the finding agrees with the spontaneous incidence of the tumors in animals of that age (8). In all but one instance these tumors were single.

When methylcholanthrene or dibenzanthracene was used in the form of horse serum and cholesterol dispersion, practically all animals developed multi-
Table I: Lung Tumors in Strain A Mice Four and Five Months after Intratracheal or Intravenous Injection of Methylcholanthrene or Dibenzoanthracene

<table>
<thead>
<tr>
<th>Substance Injected</th>
<th>Mice with Lung Tumors, according to Number of Tumors per Mouse</th>
<th>Percentage of Mice with Lung Tumors</th>
<th>Average Number of Tumors per Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Mice Used</td>
<td>No. without Lung Tumors</td>
<td>1</td>
</tr>
<tr>
<td>Intratracheally</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylcholanthrene in horse serum</td>
<td>11</td>
<td>1*</td>
<td>2</td>
</tr>
<tr>
<td>Dibenzoanthracene in horse serum</td>
<td>10</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dibenzoanthracene on charcoal</td>
<td>11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Charcoal suspension</td>
<td>11</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Horse serum</td>
<td>10</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Intravenously</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylcholanthrene in horse serum</td>
<td>16</td>
<td>1*</td>
<td>0</td>
</tr>
<tr>
<td>Dibenzoanthracene on charcoal</td>
<td>10</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Horse serum</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

* Lymphoma.

ple lung tumors within four months. One mouse, injected intratracheally with dibenzaanthracene, was negative for lung tumors, but this animal was killed two and a half months after injection. The two mice which received methylcholanthrene, one intratracheally and the other intravenously, and which are recorded as negative had lymphomatous infiltration throughout the lungs. In one of these, both upper lobes formed a single firm white mass which was adherent to the pleura.

With dibenzaanthracene adsorbed on charcoal, given intratracheally or intravenously, the incidence of lung tumors was 60 per cent, and in 5 of 13 animals the tumors were multiple. The decreased carcinogenicity of dibenzaanthracene adsorbed on charcoal has been noted previously (9); it is probable that the hydrocarbon is held too firmly by the charcoal to be readily dissolved in the tissues.

Although just as many mice developed lung tumors after intratracheal as after intravenous injection, the number of tumors in individual animals was consistently and significantly greater in the latter.

Only one animal, which was injected intratracheally with dibenzaanthracene in horse serum, developed a peritracheal spindle-cell sarcoma (in two and a half months). No other tumors arose at the site of injection, although when charcoal preparations were used, the charcoal particles could be seen at the end of five months in all tracheotomy scars.

Histologic sections were made of 74 lungs. Aside from the peritracheal sarcoma and two lymphomas, only typical adenomatous tumors of the lung, as described by Tyzzer (10) and many other workers, were found. These need no further portrayal here.
It was expected that the tumors elicited by the intratracheal injection of carcinogenic hydrocarbons might differ in location or in some other features from tumors occurring spontaneously or those induced by the intravenous or subcutaneous injection of such compounds. This did not prove to be the case; the tumors were identical in morphology, development, and location. They were solid growths, composed of moderately large, round or cuboidal cells with definite boundaries and large, round or oval nuclei. Mitoses were rare. All tumors apparently arose from the alveolar epithelium, and all were directly subpleural, often in contact with the thickened pleura. In five months many of the tumors assumed the papillary structure so often described in this neoplasm.

The site of tumor origin apparently did not correspond to the location of the highest concentration of the introduced carcinogens. Thus, in mice receiving charcoal and dibenzanthracene intratracheally, some charcoal deposits were seen within phagocytes, lying free in alveoli, and within the alveolar wall; most of the charcoal was found near the bronchi, with small amounts in the peribronchial lymphatics; practically none of the particles was seen immediately beneath the pleura. Although bits of charcoal were present near or within some of the tumors, there was no correlation between the point of tumor origin and the site of heaviest charcoal deposits.

That the particles may have been transported is indicated by the observation that the charcoal injected intravenously did not vary markedly in its distribution from the charcoal introduced through the trachea. With intravenous introduction, however, the masses of charcoal seemed larger, and in several areas produced embolic occlusion of small blood vessels; again, these areas were not correlated to the site of the tumors.

**DISCUSSION**

Lung tumors can be produced by numerous means in susceptible mice, *i.e.*, mice possessing the tendency to develop them spontaneously. Some of the methods, garnered from the literature, are: repeated applications of coal-tar to the skin (11); subcutaneous injection of carcinogenic hydrocarbons (12); intravenous injection of serum and charcoal dispersions of carcinogenic hydrocarbons (9); feeding large doses of dibenzanthracene (13); introduction of carcinogenic hydrocarbons into the rectum, vagina, peritoneal cavity (3) or pleural cavity (14); insertion of string impregnated with dibenzanthracene through the lung (12).

It has been suggested that the introduction of carcinogenic compounds into the body of the mouse alters the body state so that tumors arise at points of incidental irritation. The more plausible explanation is that the lung tumors are produced because very small amounts of the carcinogenic agent leave the site of application and come in contact with the extremely susceptible alveolar epithelium (15). The essential prerequisite of a proper soil, or *Grundlage*, is obvious from the histologic studies of the tumors produced in these various ways: they are identical in structure and location.

This report demonstrates that the direct application of 0.1 mg. of dibenzanthracene or methylcholanthrene to the lungs of strain A mice is sufficient to
produce lung tumors. It is evident also that this technic is too tedious for routine use, and that the intravenous injection of test substances is easier, as well as more efficacious. In further experiments on the possible carci-
genicity of certain dusts, especially those containing chrome or arsenic, the latter method will be employed.

**Summary**

(1) Mice weighing about 30 gm. tolerate the intratracheal injection of 0.1 c.c. of water, saline, or serum suspensions; the mortality from the procedure is about 35 per cent.

(2) Primary lung tumors were produced in over 90 per cent of strain A mice within four months after the intratracheal introduction of 0.1 mg. 1:2:5:6-dibenzanthracene or methylcholanthrene dispersed in 0.1 c.c. horse serum and cholesterol.

(3) The intratracheal route of administration is not as convenient nor as efficacious as the intravenous.

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**Bibliography**

13. LORENZ, E.: Data to be published.