Metal Carcinogenesis

I. Observations on the Carcinogenicity of a Refinery Dust, Cobalt Oxide, and Colloidal Thorium Dioxide

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SUMMARY

A metallurgical dust obtained from the dust flue of a nickel refinery was shown to be a locally acting carcinogen in both rats and mice. Sarcomas were induced in two strains of rats at about 45 per cent of the sites of intramuscular injection, with most of the tumors being demonstrably of striated muscle cell origin. Average latent periods approximated 6 months, and metastases occurred frequently. Mice proved less responsive than rats, showing significantly longer latent periods and an absence of metastases. A majority of the mouse tumors appeared to be fibrosarcomas.

Similar experiments utilizing single intramuscular injections of cobalt oxide powder gave a 50 per cent local incidence of rhabdomyosarcomas in rats but proved completely negative in the mice tested.

Single intramuscular injections of “Thorotrast” failed to induce a local tumor response in either rats or mice. Four out of 50 “Thorotrast”-treated mice developed malignant lymphomas in an average of 457 days. No sarcomas or malignant lymphomas were observed in control animals given injections of Procaine Penicillin G.

MATERIALS AND METHODS

The refinery dust sample investigated was calculated to be of the following composition:

An increase in the incidence of cancer involving the nasal sinuses and lungs over that in the general population has been shown to exist among refinery workers in the nickel industry in Great Britain for the period 1933–48 (1) and for the period 1948–56 (3). Another recent report concerned with the incidence of respiratory cancer in nickel workers refers to a total cancer mortality excess of over 3 times that in the population as a whole during the 9-year period 1939–48, this excess occurring entirely in the two sites referred to above (11). The latter report also points out that in the Clydach Works, “No deaths from nasal cancer have been recorded in workers engaged after 1924, at which date increased precautions were taken against dust.” The drying and powdering of copper sulfate and the arsenic content of the sulfuric acid used before 1921 are suggested as being related to the respiratory cancer excess. A somewhat similar study of cancer incidence has been published for a German nickel refinery at which workers were exposed to ores containing bismuth, nickel, cobalt, and very large amounts of arsenic (17). It was suggested in this investigation that the dust and vapors might be responsible for a primary irritation bronchitis, from which the bronchial carcinoma developed as a result of the influence of As, Ni, and Co dusts and gases.

The present investigation was undertaken as a part of an over-all inquiry into the possible carcinogenicity of dusts collected from the dust flues of a Canadian nickel refinery. The effects of single intramuscular deposits of (a) the refinery dust, (b) cobalt oxide (a component of the refinery dust), and (c) “Thorotrast” (a colloidal thorium dioxide, and not a component of the dust) are reported here.
Either one or both thigh muscles by means of a trast medium made up of @4—@6 per cent stabilized dextrin and 0.15 per cent methyl parasept as colloidal thorium dioxide in @5 per cent aqueous silver, tellurium, chromium, gold, and boron.

Spectographic traces (less than 0.01) of bismuth, less than 0.1 per cent. This powder also contains by air elutration. Exposure to the test substances in all cases consisted of a single intramuscular injection into either one or both thigh muscles by means of a laboratory pebble mill followed by saline water. The test substances, with the exception of thorium dioxide, were administered by means of a laboratory pebble mill followed by air elutration.

The “Thorotrast” employed was a sterile contrast medium made up of 24—26 per cent stabilized colloidal thorium dioxide in 25 per cent aqueous dextrin and 0.15 per cent methyl parasept as preservative. Exposure to the test substances in all cases consisted of a single intramuscular injection into either one or both thigh muscles by means of a 23-gauge needle. The test substances, with the exception of thorium dioxide, were administered as 10 per cent suspensions in an aqueous suspension of penicillin G procaine.

The experimental animals used were hooded and Wistar rats of both sexes and female Swiss mice. All animals were bred and raised in our colonies and were between 2 and 3 months of age when put on experiment. Mice were housed four or five to a glass cookie jar on pine shavings, while the rats were kept three or four to a galvanized metal cage with wire screen floor. All animals and bedding were periodically dusted with a rotenone powder. Purina Laboratory Chow and water were provided ad libitum.

Treatment groups and toxicity.—In a preliminary trial it was found that a 10 mg. per thigh dose of the refinery dust was lethal to mice within 24 hours, whereas cobalt oxide at the same dosage caused the death of 50 per cent of the mice between the 2d and 6th day after exposure. These substances apparently contained highly toxic water-soluble constituents or impurities, since repeated washing in distilled water (until the supernatant was colorless in the case of refinery dust) completely eliminated the mortality. All dusts referred to in these experiments as “washed” were treated in this manner. Rats were able to tolerate the unwashed dusts in amounts up to 50 mg. per site and to total doses of 40 mg. per animal. However, the supernatant from 10 gm. of refinery dust washed with 100 cc. of distilled water proved consistently lethal if administered in single or simultaneous doses totaling much over 0.1 cc. per rat.

The series of experiments reported here was designed to determine whether dust from the flue of a nickel refinery would prove tumorigenic when administered parenterally to either rats or mice at more or less maximum tolerable dose levels. Concurrently, screening tests utilizing cobalt oxide and a colloidal thorium dioxide were set up to parallel the metallurgical dust series. This was done because cobalt is a known, locally acting carcinogen in the rat (6), whereas Thorotrast has been reported as a remotely acting carcinogen in both species (18). Further control groups were given injections of aqueous Penicillin G procaine, the suspensory agent used throughout.

RESULTS

Refinery dust.—Table 1 lists the tumor response obtained in the several groups of animals exposed to refinery dust, to its water-soluble components (supernatant), and to single intramuscular injections of Penicillin G procaine.

From this table it is apparent that injection with refinery dust, at the dosage levels and site used, was highly carcinogenic to both mice and rats. However, repeated injections of a solution of the water-soluble components of this dust into rats of Group 5 failed to induce tumors. The latter result is in accord with the observation that no difference in tumorigenic effect existed between the unwashed and washed refinery dusts (Groups 3 and 4). Over 70 per cent of hooded rats of Groups 3 and 4 developed tumors, whereas only 40 per cent of the Wistar strain (Group 2) were affected. However, it should be noted that the former strain received two injections per rat (one in each thigh), whereas the latter received only one injection per animal. A comparison of the tumor response in these groups (2 versus 3 and 4), based on the percentage of injection sites affected, shows no significant differences to exist between them.

In general the incidence of tumors was higher in the rat groups than in the mice, both on the basis of the number of tumor-bearing animals and of per cent injection sites affected; however, none of these differences is statistically significant. There was a much longer time to the appearance of the first tumor among the mice (197 days).

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than in the rat groups (106–112 days). The mean tumor latent period for rats treated with the washed refinery dust (Group 4) was 176 days, in contrast to the 325-day mean for the mice of Group 1, given injections of dust from the same sample. This difference of 149 days between the mean tumor latent periods of rats and mice proved to be significant at the 1 per cent level ("t" = 7.96, \( df = 48, P < .01 \)).

All the primary tumors developed at and around the sites of injection and deposits of the original dusts injected could invariably be shown within these tumors (Fig. 3). Tumors induced by refinery dust in the rat were almost all of striated muscle cell origin, showing a characteristic cellularity in no instance has there been any indication of primary tumor induction by such dusts involving the tissues of the lymph nodes, and the tumors seen were clearly metastases from a primary striated muscle cell tumor. No metastases were observed among the twenty tumor-bearing mice of Group 1.

Cobalt oxide and thorium dioxide (Thorotrast).—Table 2 summarizes the tumor responses obtained in the groups of rats and mice subjected to single intramuscular injections of cobalt oxide, Thorotrast, and Penicillin.

Cobalt oxide failed to induce tumors at any of the 92 effectively exposed sites in the mice of Group 8. This was in sharp contrast to the 50 per

### TABLE 1

**Tumor Response of Mice and Rats to Intramuscular Injection with Metallurgical Refinery Dust, Its Aqueous Supernatant, and Penicillin G Procaine**

<table>
<thead>
<tr>
<th>GROUP AND STRAIN</th>
<th>NO. ANIMALS*</th>
<th>TREATMENT</th>
<th>TUMOR RESPONSE</th>
<th>DURATION OF EXP. (DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On exp.</td>
<td>Effective no.*</td>
<td>Subst.</td>
<td>Dose/site</td>
</tr>
<tr>
<td>1. Swiss mice</td>
<td>40</td>
<td>88</td>
<td>Metal dust †</td>
<td>10 mg.</td>
</tr>
<tr>
<td>2. Wistar rats</td>
<td>20</td>
<td>30</td>
<td>Metal dust</td>
<td>30 mg.</td>
</tr>
<tr>
<td>3. Hooded rats</td>
<td>35</td>
<td>27</td>
<td>Metal dust†</td>
<td>20 mg.</td>
</tr>
<tr>
<td>4. &quot; &quot;</td>
<td>31</td>
<td>28</td>
<td>Metal dust</td>
<td>20 mg.</td>
</tr>
<tr>
<td>5. &quot; &quot;</td>
<td>30</td>
<td>19</td>
<td>Supernat.</td>
<td>.6 and†</td>
</tr>
<tr>
<td>6. &quot; &quot;</td>
<td>30</td>
<td>30</td>
<td>Pen. G</td>
<td>120,000 i.u.</td>
</tr>
<tr>
<td>7. Swiss mice</td>
<td>51</td>
<td>48</td>
<td>Pen. G</td>
<td>60,000 i.u.</td>
</tr>
</tbody>
</table>

* Survivors at 90 days—approx. 2 weeks prior to appearance of first tumor in any group.
† Washed samples of refinery dust.
‡ Repeat injections of .05 in each thigh.
§ Exclusive of one pulmonary adenocarcinoma and three mammary tumors.
# Differences significant, \( P < .01 \).
DISCUSSION

Pure metallic cobalt has been reported to be carcinogenic to the rat when introduced by intramuscular injection (6, 7) with some of the observed sarcomas being classified as rhabdomyosarcomas. The present study, in demonstrating the carcinogenicity of the compound cobalt oxide, lends support to these reports by Heath on the tumorigenic activity of this metal and also emphasizes the surprisingly frequent occurrence of a type of sarcoma that is clearly of striated muscle origin.

It is of interest to note that we were unable to induce any tumors at all in mice with cobalt, whereas the refinery dust (containing 1 per cent Co) induced a high incidence of tumors in both rats and mice. In consequence, it seems safe to assume that some component compound(s) of the refinery dust, other than cobalt oxide, is actively carcinogenic. Among these metals, nickel has been reported by Hueper to be carcinogenic to both the rat and the rabbit (8, 9). However, this author failed to observe any nickel-induced tumors in C57BL mice exposed to a single injection of 0.1 mg. powdered nickel. This finding was contrasted with an observed incidence of over 25 per cent tumor-bearers among his experimental rats, receiving 50 mg. metallic nickel in one femur (repeated in the other femur after 18 months in twenty survivors). The 27 tumors described by Hueper were all sarcomas, several being myogenic and apparently arising from seepage of the nickel-gelatin suspension at the site of injection. It would seem probable that tumors might also have developed in the mice from intramuscular injection if a considerably higher dose had been used.

Recently iron, in the form of iron dextran, has been reported to be carcinogenic to rats (16) and to both rats and mice (5). These findings have stimulated considerable interest in the mechanism involved in metal carcinogenesis (4). While many of the iron-dextran-induced tumors arose locally as sarcomas at the sites of injection, other primary tumors arose at remote sites. Many of the latter tumors and some of the former involved cells of the reticuloendothelial system. No myomas were reported.

Sarcomas have been induced in rats by the subcutaneous implantation of plastic discs, glass,
although not yet completed, have to date provided roscopically visible throughout life, even though these lymph nodes almost always have metal deposits of metal particles. H. & E., X140.

It is noteworthy that rather extensive inhalation experiments have been undertaken utilizing similar samples of refinery dust to those reported here.3 These experiments have been conducted at the School of Hygiene, University of Toronto and, although not yet completed, have to date provided no evidence of the induction of pulmonary tumors in the laboratory animals used (including rats and mice). The results reported here, while throwing no direct light on the problem of respiratory cancer among refinery workers, demonstrate that more than one of the component compounds of such dusts can induce malignancy in certain specific tissues with which they are in direct and protracted contact. As Kuschner et al. (10) have demonstrated, there is considerable difficulty in inducing experimental cancer of the lung by inhalation (at least in rats and mice), even with such highly carcinogenic hydrocarbons as methylcholanthrene. However the same workers were successful in inducing lung carcinoma by the more artificial technic of introducing pellets into the bronchi, thus permitting more massive, direct, and protracted contact between epithelium and carcinogen.

The consistent involvement of striated muscle and the strict localization of the primary tumors, along with the high tumor incidence and surprisingly short latent periods, suggest that further study of these metallurgical tumors may be of particular interest in regard to the induction mechanism involved (4), as well as in the study of progression and tumor dependency (2).

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1.—Primary rhabdomyosarcoma in thigh region of Wistar rat treated with a single 50-mg. injection of metallurgical dust. Note the cellular pleomorphism with numerous giant and muscle strap cells. H. & E., X140.

Fig. 2.—Higher magnification of mid-right hand region of Fig. 1. Note vacuolated and fibrillar cytoplasm of the multinucleated myocytes, the typical tapered shape of the uninnucleated myoblasts, and their tendency to syncytial formation. H. & E., X600.

Fig. 3.—Tumor from mouse injected with 10 mg. washed metallurgical dust per thigh. Note spindle-cell type of fibrosarcoma and the distended lymphatics associated with the deposits of metal particles. H. & E., X140.

Fig. 4.—Hooded rat exposed to 20 mg. metallurgical dust per thigh muscle. Note primary tumor involving left hind leg and left flank lymph nodes with metastasis through the superficial lymphatic chain along the left side to the axillary lymph nodes. Several metastatic lesions in lungs are also visible.
Fig. 5.—Lung metastasis of rhabdomyosarcoma in a rat treated with 20 mg. metallurgical dust per thigh. H. & E., X 140.

Fig. 6.—Lung metastasis in rat exposed to single 30-mg. injection of metallurgical dust, showing granular cytoplasm of muscle giant cells with formation of cross striations. Silver impregnation, X 600.

Fig. 7.—Iliac lymph node of rat exposed to single 30-mg. injection of cobalt oxide, showing metastasis of tumor as well as extensive deposits of metal powder. Iron hematoxylin, X 45.

Fig. 8.—Higher magnification of region from cortex of lymph node in Fig. 7, showing double banding of a tumor myocyte in an advanced stage of differentiation. Note also cobalt oxide particles. Iron hematoxylin, X 600.


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