Metal Carcinogenesis

II. A Study on the Carcinogenic Activity of Cobalt, Copper, Iron, and Nickel Compounds*

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SUMMARY

Nickel sulfide, nickel oxide, cobalt sulfide, and cobalt oxide were shown to be carcinogenic on single intramuscular injection in rats. The sulfide of each metal induced a significantly higher tumor incidence than did its oxide. Comparisons between average latent periods and between measures of tumor progression also indicated that the presence of the sulfide enhances the carcinogenic activity of these compounds. Nickel sulfate, iron and copper sulfides, and oxides failed to induce tumors in rats under these conditions.

Both C57 and Swiss mice developed tumors from single intramuscular exposure to NiS2 and to NiO; however, no tumors were induced with CoO in this species. Tumor response in mice was consistently lower than in rats, and no apparent enhancing effect of the sulfide was evidenced.

In rats, almost all tumors examined histologically were rhabdomyosarcomas, with a few fibrosarcomas, particularly among those tumors induced with NiO. Difficulty was encountered in definitely classifying the mouse tumors; whereas most appeared to be fibrosarcomas, many of these were not typical, and a few were almost certainly myomas.

It was concluded that nickel sulfide was probably the compound responsible for the carcinogenic activity of the sample of metallurgical dust originally investigated (collected from the dust flue of a nickel refinery).

The apparent specificity of nickel and cobalt compounds for striated muscle tumorigenesis is discussed.

A metallurgical powder† collected from the dust flue of a nickel refinery and milled to particle sizes of less than 5 µ has been shown to be a potent local carcinogen when introduced intramuscularly as a single injection in either rats or mice (3). In the same study, cobalt oxide (accounting for approximately 1 per cent of the dust) was also shown to be carcinogenic to rats but not to mice. A majority of the sarcomas examined in these initial experiments appeared to be of muscle-cell origin.

The present report deals with a determination of the components of this refinery dust responsible for its tumorigenicity, and our observations on the comparative tumorigenic activities of these and related compounds. The following six compounds, present in the original sample of refinery dust, were tested against rats: CuO, Fe2O3, NiO, NiS2, NiSO4·6H2O, CoO, as were also several sulfides of copper, iron, and cobalt. Subsequently, several

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† Estimated composition:

- Cupric oxide (CuO): 3.4%
- Nickel sulfate (NiSO4·6H2O): 20.0%
- Nickel sulfide (NiS2): 57.0%
- Nickel oxide (NiO): 6.8%
- Cobalt oxide (CoO): 1.0%
- Ferric oxide (Fe2O3): 1.8%
- Silicon dioxide (SiO2): 1.2%
- Miscellaneous: 2.0%
- Moisture: 7.3%

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groups of mice were exposed to single intramuscular injection with those components of the refinery dust that had proved tumorigenic to the rat.

**MATERIALS AND METHODS**

The experimental animals used were a commercial strain of Wistar rats and C57BL and Swiss mice bred in our own colony. All animals were from 3 to 3 months of age when placed on experiment. Housing and care were the same as reported previously, as also was the method of preparation and administration of suspensions of the metallurgical powders (3).

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**RESULTS**

Table 1 summarizes the findings in the initial series of tests for the carcinogenicity of the compounds listed, with the rat used as the test animal. Apparently, cupric oxide and ferric oxide, at a 20-mg. site dosage, and nickel sulfate at 5 mg/site showed no carcinogenic activity through observation periods of approximately 20 months following exposure.

Both the sulfide and the oxide of nickel induced tumors at the site of injection in a majority of the rats treated. However, marked differences in tumorigenic activity were observed between these two nickel compounds both in proportion of injection sites at which tumors developed and in average latent period. Thus, the difference between the 80 per cent tumor incidence at sites injected with NiS2 and the 41 per cent incidence with NiO is significant at the 1 per cent level ($\chi^2 = 16.622, df = 1, P < .01$). Similarly, the 302-day latent period required on the average for NiO to induce a palpable tumor is significantly longer than the 150 days required by NiS2 ($t = 7.855, df = 44, P < .01$).

Cobalt oxide had previously been shown to be carcinogenic to the rat under these conditions of experiment (3). A further test of this compound again resulted in approximately a 50 per cent tumor response (Group 6).

The fact that injection with nickel sulfide resulted in tumors developing in 89 per cent of all the exposed animals, whereas only 66 per cent of the NiO-treated rats and 50 per cent of those

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### Table 1

<table>
<thead>
<tr>
<th>Group no. and compound</th>
<th>No. on exp.</th>
<th>Effect no. rats*</th>
<th>Total inject. sites</th>
<th>Total tumors at site</th>
<th>No. tumor rats</th>
<th>Days to 1st tumor</th>
<th>Av. latent period (days)</th>
<th>Days on exp.</th>
<th>Survivors at end exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CuO</td>
<td>32</td>
<td>28</td>
<td>56†</td>
<td>0</td>
<td>0</td>
<td>595</td>
<td>10</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>2. FeO</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>601</td>
<td>14</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>3. NiSO4•6H2O</td>
<td>32</td>
<td>27</td>
<td>54‡</td>
<td>0</td>
<td>0</td>
<td>609</td>
<td>19</td>
<td>60</td>
<td>19</td>
</tr>
<tr>
<td>4. NiO</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td>36§</td>
<td>21</td>
<td>180</td>
<td>308</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>5. NiS2</td>
<td>32</td>
<td>28</td>
<td>45§</td>
<td>25</td>
<td>91</td>
<td>150</td>
<td>365</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6. CoO</td>
<td>32</td>
<td>24</td>
<td>29§</td>
<td>12</td>
<td>96</td>
<td>178</td>
<td>342</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7. CoS</td>
<td>30</td>
<td>29</td>
<td>35</td>
<td>28</td>
<td>97</td>
<td>196</td>
<td>365</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8. CuS</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>0</td>
<td>603</td>
<td>19</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>9. CuS</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>0</td>
<td>603</td>
<td>21</td>
<td>20</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>10. FeS (47% S)</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>0</td>
<td>607</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>11. FeS (88% S)</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>0</td>
<td>607</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

* Number surviving; 90 days after treatment.
† 20 mg, right leg; 6 mg, left leg.
‡ 5-mg dose/thigh.
§ Seventeen rats treated in both thighs, eleven in one thigh only.
# Five rats treated in both thighs, nineteen in one thigh only.
|| Differences significant at 1 per cent level.
** One rat (died 139 days) with large hematoma at injection site with indications of early sarcoma formation in thickened fibrous wall.

As a general rule, a single 20-mg. dose of the powder under test was injected into both the left and right thigh muscles of each rat, whereas the mice received injections of 5 mg. per thigh. Exceptions to this were made for reasons of toxicity and are noted in the text where they occurred.

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**DISCUSSION**

*Woodlyn Farms, Guelph, Ontario, Canada.*

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1. Woodlyn Farms, Guelph, Ontario, Canada.

2. 5-mg dose/thigh.

3. Seven rats treated in both thighs, eleven in one thigh only.

4. Five rats treated in both thighs, nineteen in one thigh only.

5. Differences significant at 1 per cent level.

6. One rat (died 139 days) with large hematoma at injection site with indications of early sarcoma formation in thickened fibrous wall.
receiving CoO responded to the same or heavier dosages, suggested that possibly the sulfide itself might be directly concerned in this enhancement of responsiveness. To test this hypothesis, groups of 30 rats each were exposed to single intramuscular injections of the following metal sulfides:

Cuprous sulfide
Cupric sulfide
Iron sulfide (47 per cent S)
Iron sulfide (38.3 per cent S)
Cobalt sulfide
Nickel oxide
Cobalt oxide

Results obtained in these tests are included in Table 1 (Groups 7–11). Copper and iron compounds again failed to induce tumors. However, the sulfide of cobalt proved markedly more active than the oxide in numbers of tumors induced, although no difference developed in average latent period.

The great majority, possible all, of tumors induced by nickel sulfide and cobalt oxide were pleomorphic, highly cellular sarcomas, obviously of striated muscle origin (Fig. 1), showing frequent metastasis to the lung and lymph nodes (Fig. 2). Although a majority of the nickel oxide-induced tumors could also be shown to be rhabdomyosarcomas (Fig. 3), about 20 per cent were classed as fibrosarcomas (Fig. 4). Several of the tumors examined from the CoS group were highly anaplastic and difficult to categorize definitely; however, here again the predominant histological type was a striated muscle tumor (Fig. 5).

Metastases were very frequent in animals of the nickel sulfide group, occurring in almost all animals autopsied (Table 2). Fifty-five per cent of the cobalt sulfide group also showed metastases, although these frequencies were progressively reduced when the primary tumor was induced by either nickel oxide or cobalt oxide.

In all cases the primary tumors, once established, tended to grow rather rapidly and often to a very large size around the site of injection. The rapidity with which the induced tumors resulted in the death of their hosts (tumor progress time) is compared in Table 3. Tumor-bearing animals whose death was attributable to causes

**TABLE 2**
FREQUENCY AND DISTRIBUTION OF METASTASES IN INDUCED RAT TUMORS (MOSTLY RHABDOMYOSARCOMA)

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>No. rats</th>
<th>Metastases</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>Nickel sulfide</td>
<td>21</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td>Cobalt sulfide</td>
<td>29</td>
<td>16</td>
<td>55</td>
</tr>
<tr>
<td>Nickel oxide</td>
<td>20</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>Cobalt oxide</td>
<td>12</td>
<td>3</td>
<td>25</td>
</tr>
</tbody>
</table>

**TABLE 3**
COMPARISON OF THE AVERAGE TIME FROM FIRST APPEARANCE OF TUMOR TILL DEATH IN RATS EXPOSED TO FOUR CARCINOGENIC METALS

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>No. rats</th>
<th>Av. progression time (days)</th>
<th>Difference (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel sulfide</td>
<td>20</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Nickel oxide</td>
<td>16</td>
<td>88</td>
<td>38*</td>
</tr>
<tr>
<td>Cobalt oxide</td>
<td>12</td>
<td>71</td>
<td>12</td>
</tr>
<tr>
<td>Cobalt sulfide</td>
<td>24</td>
<td>42</td>
<td>29†</td>
</tr>
</tbody>
</table>

* Significant at the 5 per cent level.
† Significant at the 1 per cent level.

Other than tumor growth and metastasis have been omitted from these calculations. The difference of 28 days in progression time that existed between animals supporting NiS₂- and NiO-induced tumors was significant ("t" = 2.219, df = 34, P < 0.05), as also was the difference of 29 days between average progression times of CoO and CoS-induced tumors ("t" = 3.41, df = 34, P < 0.01). No apparent difference existed in the rapidity with which the cobalt oxide and nickel oxide-induced tumors caused the death of their hosts.

**Tumor response of mice.**—Table 4 compares the tumor response of mice of two different strains to nickel sulfide and nickel oxide. The response of Swiss mice exposed to CoO at a heavier dose has been included for comparison.
The fact that both nickel compounds proved carcinogenic to mice is in sharp contrast to the inactivity of CoO, even when administered in double the dose and kept under observation for 2 years. No differences can be noted in the tumorigenic activity of the two nickel compounds in mice either in average latent period or numbers of tumor-bearing animals. However, in this species it would appear that the progression time of the NiO-induced tumors was appreciably shorter than that of Ni$_3$S$_2$ tumors (Table 4), an observation in direct contrast to the condition observed in rats (Table 3). There also appeared to be a strain difference in susceptibility to the nickel compounds, with the C3H being more refractory than Swiss on the basis of per cent tumor response.

**TABLE 4**
CARCINOGENICITY OF NiO, Ni$_3$S$_2$, AND CoO ON INTRAMUSCULAR INJECTION INTO C3H AND SWISS MICE

<table>
<thead>
<tr>
<th>Metal</th>
<th>Strain and no. mice</th>
<th>Total sites injected</th>
<th>Dose/s site (mg.)</th>
<th>No. tumor animals</th>
<th>Per cent affected sites</th>
<th>Av. latent period (days)</th>
<th>Av. progr’em time (days)</th>
<th>Duration of exp. (days)</th>
<th>Survivors at end of exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni$_3$S$_2$</td>
<td>Swiss-45</td>
<td>51*</td>
<td>5</td>
<td>27</td>
<td>53</td>
<td>250</td>
<td>131</td>
<td>478</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C3H-18</td>
<td>30</td>
<td>5</td>
<td>9</td>
<td>33</td>
<td>225</td>
<td>139</td>
<td>448</td>
<td>2</td>
</tr>
<tr>
<td>NiO</td>
<td>Swiss-50</td>
<td>100</td>
<td>5</td>
<td>33</td>
<td>35</td>
<td>287</td>
<td>78</td>
<td>476</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C3H-52</td>
<td>100</td>
<td>5</td>
<td>23</td>
<td>28</td>
<td>256</td>
<td>71</td>
<td>476</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Swiss-46</td>
<td>92</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Only a few Swiss mice were injected in both thighs with Ni$_3$S$_2$, owing to its toxicity.
† Average number days from appearance of first tumor to death.

DISCUSSION

Although the findings reported here demonstrate that several compounds of both nickel and cobalt are carcinogenic, they also indicate that in all probability nickel sulfide was the effective carcinogenic agent in the original crude refinery dust investigated. This view is supported by the rapidity with which Ni$_3$S$_2$ induced tumors in the rat, the failure of CoO to induce tumors in mice, and the fact that 57 per cent of the refinery dust was made up of the sulfide of nickel.

Needless to say, direct extrapolation between the induction of rhabdomyosarcomas in the rat and the occurrence of tumors of the sinus and lung in refinery workers is not justifiable—a fact that is underlined by our observation that parenterally administered cobalt oxide, while highly carcinogenic to the rat, is apparently inactive in mice. Nevertheless, the demonstrable tumorigenic capabilities of finely powdered compounds of nickel and cobalt in laboratory animals lends further support to the view that certain such dusts in the refinery industry may constitute an industrial cancer hazard (9, 11). Sinus and lung cancer occurring in plants where nickel is produced by the decomposition of gaseous nickel compounds has been included in the list of Proscribed Diseases in Britain for many years (4). During the last decade, however, evidence has been accumulating that this restriction of the industrial cancer hazard to the extremely finely divided nickel liberated from carbonyl, Ni(CO)$_4$, is probably not justifiable (1, 4).

The reports of Heath (7) on the carcinogenicity of metallic cobalt and particularly of the apparent specific affinity of this metal for muscle in the rat are corroborated by our own findings with both
the oxide and sulfide of cobalt. Of even more interest is the fact that this peculiar selectivity for muscle has, in our experience, been shown to be particularly pronounced when the sulfides of nickel or cobalt were used as the carcinogenic agent. Hueper (10) has reported a wide variation in histogenetic types of tumors resulting from intrafemoral injection of nickel and has concluded "... that no tissue specificity exists in relation to the carcinogenic action of nickel." This is in contrast to our observations on the rat which are strongly suggestive of a definite tissue preference by this carcinogen for striated muscle. A possible reason (other than the different injection site used) for this apparent divergence may have been the high incidence of a rather wide variety of "spontaneous" tumors in Hueper's strain of rats, as indicated from his report of ten neoplasms of at least five different histogenetic types in a control animal. In the light of both Heath's and our own observations and the relative rarity of occurrence of striated muscle tumors, Hueper's finding of several rhabdomyosarcomas developing along the site of intrafemoral injection is of considerable interest.

It is tempting to speculate what role the sulfides themselves may play in metal carcinogenesis, particularly in view of the significant enhancement to tumorigenic activity shown by sulfides of both nickel and cobalt over that of the oxides of these metals. As neither iron nor copper sulfides induced tumors, it seems safe to conclude that the sulfides per se have no carcinogenic activity. Thus, their function, if any, must be assumed to be one of true co-carcinogenesis, possibly involving alterations in the solubility and/or binding of the metal compounds. It has been suggested speculatively (5) that the introduction of excess metal into a system of delicately balanced metals and metal enzymes might readily interfere with normal function in such a way as to lead to a specific respiratory change and mutation. Excesses of either nickel or cobalt compounds might conceivably affect the same muscle enzyme system in the rat.

Heath (8) has reported that, in an experiment still in progress, cobalt has failed to induce tumor development in mice. This supports a similar observation by us, on the basis of which we had concluded that cobalt oxide could not be the effective carcinogen in the original refinery dust investigated (3). Furthermore, in mice, tumors classifiable as definitely of muscle cell origin rarely occurred in response to nickel compounds, and the fibrosarcomas that predominated appeared to be of a rather low order of malignancy, developing relatively slowly and rarely metastasizing. No marked enhancing effect of the sulfide of nickel occurred in this species. Such differences in response to the same carcinogens underscore both the desirability of utilizing more than one species in screening and the risk of error inherent in interpolation of findings from one species to another.

The consistently lower tumor response of mice of the C57 strain to nickel compounds, when compared with the Swiss mice tested, is probably a reflection of a general or systemic tumor resistance rather than of a specific refractoriness of local tissues to the carcinogenic agent used (13).

ACKNOWLEDGMENTS

The assistance rendered by Doctors J. D. Schroder and K. V. Jubb of the Pathology Division, Ontario Veterinary College, Guelph, in histopathology and in the taking of photomicrographs, respectively, is gratefully acknowledged. The help and cooperation received from Mrs. Agnes Anderson and Mrs. N. Gohlich in both the care and routine inspection of the experimental animals are gratefully acknowledged.

REFERENCES

8. _____, Attempts To Induce Tumors with Powdered Metallic Cobalt in Other Sites and Species. Ibid., pp. 322-23, 1959.
Fig. 1.—Nickel sulfide-induced primary rhabdomyosarcoma in rat. ×200.

Fig. 2.—Iliac lymph node, metastasis of rhabdomyosarcoma shown in Fig. 1. ×200.

Fig. 3.—Nickel oxide-induced primary rhabdomyosarcoma in rat. ×100.

Fig. 4.—Nickel oxide-induced primary fibrosarcoma in rat. ×100.

Fig. 5.—Cobalt sulfide-induced rhabdomyosarcoma in rat. ×200.

Fig. 6.—Nickel oxide-induced primary sarcoma in mouse. Note the whorled pattern suggestive of leiomyoma. ×100.

Fig. 7.—High-power view of mouse sarcoma shown in Fig. 6. Note the elongation and tendency to chromatin banding in some of the nuclei. ×600.

Fig. 8.—Nickel sulfide-induced primary sarcoma in mouse showing giant cells and syncytial arrangements suggestive of muscle cell origin. ×100.
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