

The Role of Roasted Chromite Ore in the Production of Cancer

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Introduction

The observation of individual cases, principally in Europe, and later epidemiological studies in the United States¹⁻⁵ and England⁶ leaves little doubt that workers in chromate-producing plants experienced a higher risk to cancer of the lung than would be expected among such a population. Attempts to identify the specific causative agent by epidemiologic methods have not been successful because, particularly in the older plants, the general atmosphere has been contaminated by dusts and mists from several processes and because a worker has seldom remained in a single job for the duration of his employment. Consequently, laboratory studies were undertaken by several investigators including Hueper⁷ and Baetjer⁸ to determine whether materials from a plant would produce cancers in animals and to identify the responsible agent.

In the early animal experiments complex materials from the plants rather than pure compounds were generally used in efforts to produce cancers in animals. Using roasted chromite ore mixed with sheep fat implanted intrapleurally and intramuscularly, Hueper⁹ produced squamous-cell carcinomas coexisting with sarcomas of the lung in 2 of 25 rats and fibrosarcomas of the thigh in 3 of 31. No tumors occurred at the site of implant in 30 control rats. He interpreted these results as highly suggestive that the roasted ore contains a carcinogenic agent, but the experiment still did not identify the agent. Little evidence that some

form of chromium is the carcinogen had been presented until Hueper and Payne¹⁰ produced cancers in rats using pure chromium compounds. Additional evidence was furnished by the similar production of cancers in mice by Payne.¹¹

At the time the present experiment was started cancers had not been produced by pure chromium compounds and doubt still existed among some investigators that chromium in any form was responsible for the disease. Some had suggested that an organic carcinogen arising from the roasting kilns, the fuel for which is an industrial type fuel oil, may be the causative agent, while others believed chromium may be acting as a promoting agent rather than as a primary carcinogen.

The research described in this paper was undertaken with the approach that a further analysis of the complex materials contributing to the atmospheric contamination of the plants might yield information that would be useful in selecting materials for their cancer producing properties. Because the roasted chromite ore, commonly referred to as the "roast," produces more acute toxic effects than the residue from the first leaching of the roast, the residue was selected for fractionation, analysis, and animal testing. This material contains all of the elements present in the roast but less of the acutely toxic sodium chromate, making it possible to administer a larger dose of nontoxic materials to the animals. The residue was the material suggested by the Public Health Service study⁵ as most likely to contain the carcinogenic agent or agents.

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Procedure

1. *Particle Size Fractionation.*—The first step in the analysis of the residue dust was fractionation into four particle size fractions: (1) greater than 10 μ ; (2) 5 μ to 10 μ ; (3) 2 μ to 5 μ , and (4) less than 2 μ . The separations were made first by sedimentation in butanol according to the procedure described by Cummings²⁹ and later by elutriation with air using the Roller Particle Size Analyzer as manufactured by the American Instrument Company.

2. *Chemical Analysis.*—The four fractions of the dust as well as the whole dust were analyzed for water soluble hexavalent, acid soluble, and total chromium. The colorimetric chromite method described by Sandell³⁰ was used utilizing Beckman Model DU and Model B spectrophotometers.

Vanadium determinations were made by first separating this element from interfering metals using cupferron and then the peroxide colorimetric method described by Sandell.

X-ray diffraction was employed in an effort to determine some of the specific compounds found in the residue and to determine whether there is a variation in the amounts of these compounds in the various size fractions. The x-ray diffraction analysis was performed in another laboratory using the Debye-Scherrer x-ray diffraction technique.

3. *Animal Testing.*—The animal testing was in two parts. In the first, the materials were injected subcutaneously in mice with and without a known carcinogen, and in the second the residue was implanted surgically in mice and rats.

(a) Subcutaneous Injection in Mice: The following materials were selected for administration to mice: (1) residue less than 10 μ in size from which practically all water soluble hexavalent

chromium had been removed; (2) 5 μ to 10 μ fraction; (3) less than 2 μ fraction, and (4) chromic phosphate with no particles larger than 10 μ . These fractions not only give a comparison of the biological effect of the different size fractions but also of varying hexavalent chromium content from practically zero to about 4 mg. per administered dose. The chromic phosphate was selected for its insolubility. To investigate the effect of the residue on the action of a known carcinogen, each fraction was administered with and without 3,4-benzpyrene.

The materials were administered to C57 Black mice from the N.I.H. colony as a suspension of the dust in tricaprillin (Eastman Trioctanoin). The treatment consisted of a single subcutaneous injection in the nape of the neck of 0.2 ml. of the vehicle containing 10 mg. of dust and, in the case of the groups receiving the known carcinogen, 0.05 mg. of benzpyrene. There were 10 groups of mice with 26 males and 26 females in each group. They were housed in stainless steel suspended cages with 13 mice to a cage and had access to drinking water and food pellets at all times. The experimental scheme is shown in Table 1.

This scheme was selected so that it would be possible to evaluate not only the differences in the results produced by the chromium compounds but also the effect, either promotion or inhibition, of the chromium compounds on the production of tumors by the known carcinogen.

(b) Implantation in Mice and Rats: As a further test of the carcinogenicity of the residue, the whole dust was mixed with sheep fat and implanted as described by Hueper and Payne¹⁹ in the thigh muscle of 26 male and 26 female C57 Black mice and 35 (15 male and 20 female) Bethesda Black rats. A group of 35 rats of the same strain received implants in the right pleural cavity. As controls, an equal number of mice and

TABLE 1.—Experimental Scheme

Bio-Assay of Dust from a Chromate-Producing Plant

		Group Number				
		I	III	V	VII	IX
Material administered	Vehicle only		Water extracted residue less than 10 μ	Chromic phosphate less than 10 μ	Residue 5 μ -10 μ	Residue less than 2 μ
		Group Number				
		II	IV	VI	VIII	X
Material administered	Benzpyrene		Water extracted residue and benzpyrene	Chromic phosphate and benzpyrene	Residue 5 μ -10 μ microns & benzpyrene	Residue less than 2 μ & benzpyrene

Twenty six male and twenty six female C57 Black mice in each group of animals. Tricaprylein (Eastman Trioctanoin) was used as a vehicle. Dose: 10 mg. of dust and 0.05 mg. benzpyrene in 0.2 ml. of vehicle.

TABLE 2.—Percent Chromium Content of Particle Size Fractions of Residue Dust

	Whole	Above 10 μ	5 μ -10 μ	2 μ -5 μ	Below 2 μ
Water soluble hexavalent Cr	1.63	1.30	2.12	3.03	4.48
Total Cr	7.0	8.1	6.9	6.6	6.8

rats received sheep fat only. For mice the dose was 10 mg. of dust and 20 mg. of fat and for rats 25 mg. of dust and 50 mg. of fat.

Animals were examined weekly and killed whenever a firm mass a centimeter or more in diameter appeared at the site of implantation. A post-mortem examination was performed on each animal. Organs and tissues with gross pathological lesions were studied by histological examination.

Observations

1. Fractionation and Chemical Analysis.

The analysis of the fractions of the residue dust gave the results listed in Table 2.

The water soluble hexavalent chromium (Cr^{+6}) content of the residue increases with decrease in particle size, while the total chromium does not change appreciably. The Cr^{+6} content of the fraction under 2 μ is approximately 3.5 times that of the fraction larger than 10 μ . There was little variation in the acid soluble chromium.

None of the fractions contained amounts of vanadium large enough to measure quantitatively by the method employed.

The x-ray diffraction analysis shows that sodium chromate is present in the crystalline form, and that its concentration increases with decrease in particle size. In a less than 2 μ fraction a small amount of kaolinite is present.

2. *Animal Testing of Residue by Injection in Mice.*—With the exception of the groups receiving the less than 2 μ fraction of the residue, which contained the highest percentage of hexavalent chromium and was the most toxic, there were very few deaths from causes other than tumors during the first 15 months of the experiment. Only 17 deaths were from other causes in the 8 groups and not more than 4 in any one group. It was during the first 15 months

that a majority of the tumors were observed, only three occurring after that time. Among the groups that did not receive benzpyrene the total deaths from other causes at the end of 22 months of observation were quite uniform except for the group that received the less than a 2 μ fraction of the residue. It appears, therefore, that risks other than the Occurrence of tumors at the site of injection did not adversely affect the incidence of tumors among these groups. This was confirmed by a life table analysis.

After 22 months of observation three tumors at the site of injection had been observed among those mice receiving the water extracted residue with no benzpyrene [Group III (Table 3)]. No tumors occurred at the site of injection among the animals that received tricapyllin vehicle only, the chronic phosphate or the other fractions of the residue, although at the site of injection there was a continuing exposure of the surrounding tissue to the insoluble dust. Proof of this is the fact that as long as 21 months after injection a deposit of green material was found in the nape of the neck of the mice receiving chromic phosphate, and in some of the animals into which residue and benzpyrene were injected, brown powder was observed in the tumor sections.

From Table 4 it will be seen that the tumor incidence in the groups receiving benzpyrene and residue dust decreases with the increasing amount of hexavalent chromium (Cr^{+6}) in the dust (Figure). The reduction in the percentage of tumors from 37% to 21% with an increase of only 0.037 mg. of Cr^{+6} does not appear to be due only to the increase in Cr^{+6} . The benzpyrene

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TABLE 3.—*Tumors at Site of Administration of Residue Dust and Chromic Phosphate Without Benzpyrene*

Group	Animal and Route	NO. Animals	Material	Vehicle	Weight of Chromium (as Cr) per Dose		No. Tumors
					Hexavalent	Total	
I	Mice (Subcut.)	52	(Controls)	Tricapryllin	0	0	0
III	" "	52	Residue, extracted	"	0.037 mg.	0.50 mg.	3
V	" "	52	Chromic phosphate	"	0.003	2.64	0
VII	" "	52	Residue 5 μ -10 μ	"	0.17	3.60	0
IX	" "	52	Residue <2 μ	"	0.45	0.68	0
	Rats (Pleural)	35	Residue (whole)	Sheep fat	0.4	2.0	2
	Hats (Thigh)	35	" "	"	0.4	2.0	1
	Rota (Pleural)	35	(Controls)	"	2	0	0
	Rats (Thigh)	35	(Controls)	"	0	0	0
	Mice (Thigh)	52	Residue (whole)	"	0.16	0.79	0
	Mice (Thigh)	52	(Controls)	"	0	0	0

control animals received no dust while all the others did. The inert dust may reduce to some extent the effectiveness of the benzpyrene in producing tumors, since the chromic phosphate, which contains practically no hexavalent chromium, when injected with benzpyrene produced the same percentage of tumors as the residue containing 0.037 mg. of Cr⁺⁶ and benzpyrene. The reduction of the potency of the benzpyrene is shown further by a small increase in the latent period from the time of injection to the appearance of tumors. This reduction in the potency of the benzpyrene may not be due entirely to the larger quantity of hexavalent chromium in the smaller size fractions, but it may be caused in part by the larger total surface area of the small particles.

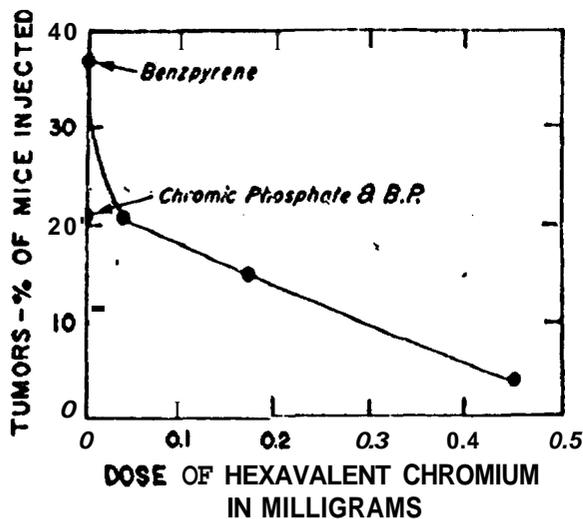
Histological examination of the tumor sections revealed that all were sarcomas. Those produced by benzpyrene were mostly of the spindle-cell type, while two of those produced by the residue were round-cell sarcomas and one was a fibrosarcoma.

3. *Animal Testing of Residue by Implantation of Mice and Rats.*—No tumors occurred at the site of administration in the mice which received implants of residue mixed with sheep fat or sheep fat alone. In the 35 rats which received intrapleural implants of the whole residue dust in sheep fat, at the end of 17 months after implantation three died with tumors in the pleural cavities, while in the intramuscular group one was killed with a tumor in the thigh. In the control groups that received the fat intrapleurally or intramuscularly no tumors occurred.

TABLE 4.—*Subcutaneous Injection of Residue and Chromic Phosphate with Benzpyrene*
Number and Percentage of Tumors at Site of Injection, Latent Period, and Dose of Hexavalent Chromium (Cr⁺⁶)

Group No.	Material	Tumors		Interval *	Cr ⁺⁶ Mg.
		No.	% †		
I	Benzpyrene	19	37	6.5	0
IV	Extracted Residue & benzpyrene	11	21	8.4	0.037
VI	Chromic Phosphate & benzpyrene	11	21	6.5	0.001
VIII	Residue, 5 μ -10 μ , & benzpyrene	8	15	7.6	0.17
X	Residue, less than 2 μ , & benzpyrene	2	4	9.0	0.45

* Interval in months between administration of material and death from tumor.
† Percentage of total number of mice injected.



Effect of varying amounts of hexavalent chromium on production of tumors in mice by 3,4-benzpyrene.

Comment

The fact that the small particles of the residue contain more hexavalent chromium than the larger particles appears to be significant in evaluating previous animal experiments. The higher hexavalent chromium content of the smaller particle size fractions increases the acute toxicity of the dust which reaches the lung.

In inhalation experiments using roasted ore, such as those conducted by Baetjer,⁸ the amount of the material that could be administered was limited by the acute toxic effects attributable to the hexavalent chromium content. In reducing the concentration of the mixed dust in the exposure to avoid acute effects of the hexavalent sodium chromate, the amount of slowly soluble material was reduced also. The addition of a dichromate to the roasted ore increased the acute toxicity.

Prior to the production of cancers in rats and mice by chromium compounds, it had been suggested that chromium may act as a promoting agent rather than as a primary carcinogen. The administration of 3,4-benzpyrene with residue dusts containing chromium gives no evidence of a promoting effect but, instead, an inhibiting effect. It is known that quinones can be formed by oxidizing 3,4-benzpyrene under various conditions.¹⁶ One of these, the 5,8-quinone, was prepared

and tested for carcinogenicity by Berenblum.¹⁰ It did not produce cancers when injected subcutaneously. The oxidation of benzpyrene by the chromate ion could account for the reduction in the tumor incidence among mice treated with both benzpyrene and residue containing varying amounts of hexavalent chromium. The reduction in the tumor incidence appears to be the result of a chemical reaction rather than tumor inhibition resulting from competition between carcinogens and anticarcinogens, as suggested by Kotin¹⁷ in explaining the inhibition of some aromatic carcinogens by their partially hydrogenated derivatives. Hill,¹⁸ in a discussion of the inhibition of 9,10-dimethyl-1,2-benzanthracene skin carcinogenesis by polycyclic hydrocarbons, proposes that the action may be a manifestation of a relatively nonspecific cellular injury by the inhibitors. Such an action by chromates seems possible, but the chemical reaction mechanism appears to be more likely.

The production of tumors in rats and mice by pure chromium compounds^{10,11} and the negative results of Cahnmann and Grogan¹⁴ in the extraction of fluorescing aromatic compounds from the roasted ore indicate that aromatic hydrocarbons from the roasting process are not responsible for the high incidence of lung cancer among the chromate workers.

In the mice to which the residue was administered without benzpyrene the tumors occurred only in the group that received the less than 10 μ fraction from which most of the water soluble material had been removed. It appears that if a means could be found to remove some of the acutely toxic sodium chromate from the residue without removing other constituents or changing the chemical or physical state of the mixture, a larger dose could be administered to the animals with a possible higher yield of tumors.

In comparing the results of the implantation of the residue in rats and mice with those obtained with pure chromium com-

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pounds by Hueper and Payne¹⁰ in rats and by Payne¹¹ in mice, it should be noted that much larger amounts of chromium were administered in the pure compounds than in the residue. The dose of the chromium compounds was 10 mg. for mice and 25 mg. for rats while less than 0.5 mg. of Cr⁺⁶ were present in the dose administered to rats. The amount of chromium in the residue injected into the mice was less than 1 mg. (Table 3). Even with the small doses used, four sarcomas were observed in the rats receiving the residue and three sarcomas in the mice of Group III.

From these observations and from the chemical analysis of the residue, it can be concluded that the residue does contain material that is carcinogenic, at least to rats and mice. The results of this experiment and the production of tumors in rats by Hueper with roasted ore indicate that the intermediate products in the production of chromium chemicals and the discarded residue should be considered hazardous.

Conclusions

Fractionation by particle size and analysis of the residue from leaching roasted chromite ore reveal that the smaller particles contain a higher percentage of hexavalent chromium than the larger particles. This increases the acute toxicity of the smaller particle size fractions which reach the lung by inhalation.

The subcutaneous injection in mice of 3,4-benzpyrene with the residue resulted in a lower incidence of tumors than when the benzpyrene was injected alone or with very insoluble chromic phosphate. The incidence of tumors decreased and the latent period increased with increasing amounts of hexavalent chromium. No promoting effect was observed.

When administered by implantation in sheep fat the residue produced sarcomas in 4 of 70 rats. A fraction of the residue from which most of the water soluble compounds had been removed produced similar tumors in 3 of 52 mice when injected sub-

cutaneously. No tumors occurred in the control groups of the same size and in groups receiving two other fractions of the residue or a group receiving chromic phosphate.

The results of this experiment give additional evidence that the intermediate products in the production of chromium chemicals and the discarded residue may be hazardous.

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