[CANCER RESEARCH 36, 1779-1783, May 1976]

Tumorigenic Activity of Lead Chromate[1]

Arthur Furst, Michael Schlauder, and Daniel P. Sasmore

Institute of Chemical Biology, University of San Francisco, San Francisco, California 94117

SUMMARY

Lead chromate was investigated for its carcinogenic potential in both rats and mice. Results show that this compound is a very potent carcinogen in rats when administered i.m. Sixty-four % of the animals treated developed malignant tumors at the injection site. Three renal carcinomas were also found after i.m. treatment with lead chromate. Since lead powder is a comparatively weak carcinogen in rats, whether given p.o. or i.m., it is suggested that the combination of lead and chromium (also a weak carcinogen) accounts for the high carcinogenic activity of lead chromate in rats. Swiss albino female mice could not tolerate the same high dose level as did the rats; at the lower dose administered to the mice, no tumors were detected.

INTRODUCTION

Lead compounds are capable of inducing renal tumors in rodents. This observation was first made in 1953 by Zollinger (26), who injected lead phosphate s.c. into a series of rats and found adenomas, papillomas, and cystadenoma of the kidney cortex. These observations were confirmed by others, including Tonz (23) and Balo (1). The more soluble basic lead acetate, given to mice as 0.1% of their diet, also produced renal tumors (24), as did lead acetate in rats (4). In the latter case, adenocarcinomas of the renal cortex were observed. The route of administration seems to be of no consequence; Coogan *et al.* (5) treated over 1000 Sprague-Dawley rats with lead subacetate by the p.o., i.p., and s.c. routes and reported the same incidence of renal cortical carcinomas in each route.

One study reported a high incidence of lymphomas late in life in female Swiss mice that had received as neonates s.c. injections of a tricaprylin (trioctanoin) solution of tetraethyl lead (6). The significance of these unique results would be enhanced by repetition of the experiment by others. Lead carcinogenesis is summarized in 2 volumes of the *International Agency for Research on Cancer (IARC) Monographs* (10, 11) and by Boyland (3). After the publication of Ref. 11, Maltoni *et al.* (17) reported the induction of local rhabdomyosarcomas after the injection of either chromium orange or molybdenum orange in 65 and 85% of their experimental rats. This tumor yield was higher than that reported by Hueper (9). In our laboratories we inaugurated a study to compare lead with lead chromate as potential tumorigenic compounds in Fischer 344 rats and Swiss albino mice.

[1] This study was supported by National Cancer Institute Contract NO1-CP-33229.

Received November 10, 1975; accepted January 29, 1976.

MATERIALS AND METHODS

Weanling Fischer 344 rats of both sexes (A. R. Schmidt, Madison, Wis., or Simonsen Laboratories, Gilroy, Calif.) and female NIH-Swiss mice (Microbiological Associates, Bethesda, Md.) were first quarantined for 2 weeks upon arrival. They were then ear-punched for individual identification and placed in cages randomly, 5 rats/stainless steel cage, or 10 mice/polycarbonate cage. All animals were maintained throughout on Wayne Lab Blox and tap water *ad libitum*. Cages were changed 3 times a week. Animals were palpated and weighed weekly for the 1st 6 to 8 weeks, then monthly until the end of the experiment.

Corn oil was used as the suspending medium for the p.o. administration of lead powder in the rats; trioctanoin (tricaprylin) was used for the substances administered i.m. in the rats and mice. The latter vehicle was first purchased from Eastman Organic Chemicals (Rochester, N. Y.) but was later synthesized in our laboratories (7). Lead powder (99.9% pure) was supplied by Alpha Chemical Co. (Beverly, Mass.), and lead chromate (98% pure) and calcium chromate (99% pure) were obtained from Research Organic/Research Inorganic Co. (Sun Valley, Calif.). No attempt was made to purify these compounds further. The fine powdered agents were suspended in the vehicle ultrasonically just before administration. No sign of decomposition of suspensions was noted. Suspensions were prepared fresh each time.

Each rat experiment consisted of about 50 animals, equally divided by sex; the mouse tests utilized 25 females.

Lead powder was given p.o. to rats by stomach tube; the controls were given an equal volume of corn oil. All animals given the test compounds were given i.m. injections of 0.2 ml of the trioctanoin containing the suspension; controls received an equal volume of pure vehicle. For the mouse experiment, an additional cage control was evaluated. The data related to the animals, dosages, and weights are given in Table 1. When a nodule was found, the animal was isolated. At the time the growth reached at least 2 x 2 cm, or when an animal was moribund, it was killed with an overdose of chloroform. Complete necropsies were performed on all animals killed (Table 2). Suspicious tissues were preserved in 10% buffered formalin; histological slides were then made.

Preliminary toxicity studies were made of lead chromate injections given i.p. to the female rats and mice and the male rats. The pigment was suspended in trioctanoin, and animals were given a single injection of 0.2 ml of the suspension. Dose ranges of lead chromate for mice were from 5 to 20 mg/mouse and from 10 to 40 mg/rat. In the experiments with rats, females and males were evaluated individually. There were 5 animals/group; each was weighed daily,

Table 1
Data on administration of lead compound to rats and mice

Compound	Species and strain (supplier)	Sex	Av. wt. (g) Start	Finish	Route	Dose/animal Each	Total	Frequency of administration
Corn oil	Fischer 344 (A. R. Schmidt)	F	94	257	p.o.	0.5 ml	12 ml	0.5 ml twice/mo. for 12 injections
		M	106	392				
Lead powder	Fischer 344 (A. R. Schmidt)	F	107	232	p.o.	10 mg	240 mg	10 mg twice/mo. for 12 injections
		M	119	355				
Trioctanoin	Fischer 344 (A. R. Schmidt)	F	97	251	i.m.	0.2 ml	2.4 ml	0.2 ml/mo. for 12 injections
		M	129	387				
Lead powder	Fischer 344 (A. R. Schmidt)	F	91	255	i.m.	10 mg	95 mg	10 mg/mo. for 9 injections, then 5 mg/mo. for 3 injections
		M	111	319				
Trioctanoin	Fischer 344 (A. R. Simonsen)	F	102	269	i.m.	0.2 ml	2.4 ml	0.2 ml/mo. for 12 injections
		M	119	411				
Lead chromate	Fischer 344 (Simonsen)	F	148	285	i.m.	8 mg	72 mg	8 mg/mo. for 9 injections
		M	196	366				
Calcium chromate	Fischer 344 (Simonsen)	F	87	267	i.m.	4 mg	48 mg	4 mg/mo. for 12 injections
		M	130	407				
Cage controls	NIH-Swiss (Microbiological Associates)	F	23.2	33.2				
Trioctanoin (USF)	NIH-Swiss (Microbiological Associates)	F	26.6	34.6	i.m.	0.2 ml	1.2 ml	0.2 ml/mo. for 6 injections
Lead chromate (ROC/RIC)	NIH-Swiss (Microbiological Associates)	F	24.1	32.7	i.m.	3 mg	12 mg	3 mg/mo. for 4 injections

and the ratio of average weight of the group to their average initial weight was plotted against time daily for 20 days (Charts 1 to 3). No special toxicity studies were made for the calcium chromate, for this compound has been tested a number of times (18) and was included for comparison.

RESULTS

In the i.p. toxicity studies of lead chromate, male rats were able to tolerate the material better than the females (Charts 1 and 2). At 10 mg/rat, in less than 2 weeks, the weight gain of these males became indistinguishable from that of controls given injections of trioctanoin. The dose of 10 mg/rat was then selected for the i.m. experiments for both sexes in the long-term experiment. Characteristically, all mice lost weight when first given injections, and, as would be expected, about 5 days after the injection, weight gain became evident in the controls (Chart 3). The dose of 3 mg/mouse was selected since, at a higher dosage, the anticipated mortality might be so great that an insufficient number of mice would survive. During the long-term experiment, it was found that the mice could tolerate only 4 injections of 3 mg each before toxic symptoms appeared and weight losses were observed.

Table 2 gives the data obtained at the termination of the experiment on the yields of the various neoplasms. Listed for rats are lymphomas, lymphocytic leukemia, fibrosarcomas, and rhabdomyosarcomas at the site, and renal carcinomas. For mice are listed the lymphomas, leukemias, and pulmonary adenomas. The numbers in parentheses correspond to the mean time, in months, when the tumors appeared.

The occurrence of spontaneous fibromas, Leydig cell tumors of the testes, and the occasional skin lesions was the same statistically for both treated and control animals and are not listed. Emphasis here is on 3 tumor types: (*a*) the various sarcomas at the site of injection; (*b*) the time of appearance of the various lymphomas; and (*c*) tumors not seen in the controls. The time of appearance, the number of lymphomas of the spleen, and instances of lymphocytic leukemia did not differ between the treated and the control animals for any of the compounds under test. Lead powder given p.o. did not seem to induce any tumors in the gastrointestinal tract or in the kidneys, but when administered i.m. the tumors noted included a metastatic osteogenic sarcoma to the lungs without primary site identification, a mesothelioma of the urogenital tract, an adenoma, and an adenocarcinoma of the thyroid. (The latter tumors have been noted in controls for other experiments.) Two tumors of the pancreas were also found in this group, a fibrolipoma and a villous adenoma.

Table 2
Occurrence of neoplasms in rats and mice after treatment with lead compounds

Compound	Route	Species	No. animals autopsied	Sex	Tumor count
Corn oil	p.o.	Fischer 344	16/20	F	L[a] = 3 (23.5)[b]
			13/20	M	
Lead	p.o.	Fischer 344	23/25	F	L = 1 (22); LL = 4 (20)
			24/25	M	
Trioctanoin	i.m.	Fischer 344	16/25	F	L = 2 (23.5)
			12/25	M	LL = 1 (17); Fs = 1 (17)
Lead	i.m.	Fischer 344	18/26	F	LL = 3 (22.3); Fs = 1 (22)
			19/24	M	LL = 3 (20)
Trioctanoin	i.m.	Fischer 344	24/25	F	L = 2 (21.5); LL = 2 (20)
			20/25	M	
Lead chromate	i.m.	Fischer 344	24/25	F	L = 2 (9.5); Fs = 11 (14); Rh = 10 (13); Os = 1 (25)
			23/25	M	Fs = 3 (13); Rh = 7 (14); Re = 3 (24)
Calcium chromate	i.m.	Fischer 344	23/25	F	L = 5 (22); Fs = 2 (16)
			22/25	M	L = 2 (19); Fs = 1 (16); Rh = 2 (16)
Cage controls		NIH-Swiss	15/25	F	L = 1 (21); LL = 5 (19); Ca = 1 (25)
Trioctanoin	i.m.	NIH-Swiss	22/25	F	LL = 2 (16); Ca = 1 (24)
Lead chromate	i.m.	NIH-Swiss	17/25	F	L = 2 (16); Ca = 3 (24)

[a] The abbreviations used are: L, lymphomas; LL, lymphocytic leukemia; Fs, fibrosarcoma at injection site; Rh, rhabdomyosarcoma at injection site; Ca, lung alveologenic carcinoma; Re, renal carcinoma; Os, osteogenic sarcoma.
[b] Number in parentheses, mean time in months after start of experiment when tumor was first detected.

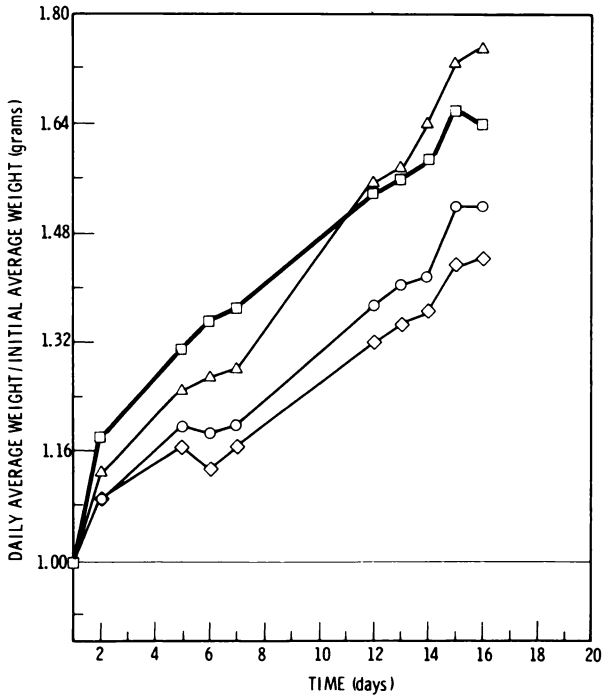

Chart 1. Lead chromate toxicity study. Male Fischer 344 rats were given i.p. injections of 0.2 ml of a suspension of lead chromate in trioctanoin. △, 10 mg/rat; ○, 20 mg/rat; ◇, 40 mg/rat. Controls (□) received 0.2 ml pure trioctanoin.

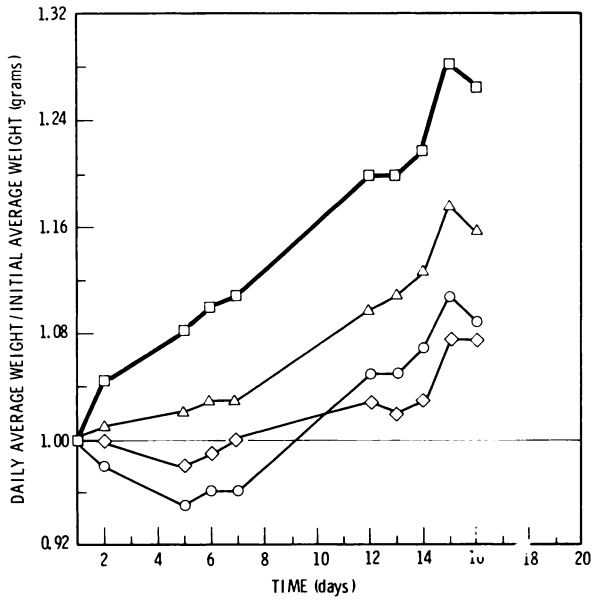

Chart 2. Lead chromate toxicity study. Female Fischer 344 rats were given injections i.p. of 0.2 ml of a suspension of lead chromate in trioctanoin. △, 10 mg/rat; ○, 20 mg/rat; ◇, 40 mg/rat. Controls (□) received 0.2 ml pure trioctanoin.

Lead chromate seems to be tumorigenic mainly to rats but not to mice under these experimental conditions. A total of 21 fibrosarcomas and rhabdomyosarcomas were found in the 25 female rats, and 10 were found in the 25 male rats. A metastatic osteogenic sarcoma was also found. In addition, 3 renal tumors were found in the males: 2 adenocarcinomas and a scirrhous carcinoma. One fibrosarcoma was observed in the treated mice, but none occurred in either of the 2 control groups. Three alveologenic carcinomas were diagnosed in the treated mice, and 1 was diagnosed in each of the 2 control groups. Only 1 fibrosarcoma was found in

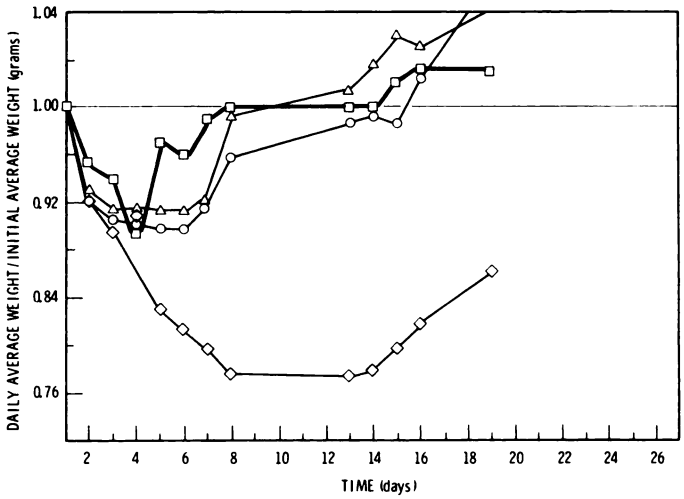

Chart 3. Lead chromate toxicity study. Female Swiss albino mice were given i.p. injections of 0.2 ml of a suspension of lead chromate in trioctanoin. △, 5 mg/mouse; ○, 10 mg/mouse; ◇, 20 mg/mouse. Controls (□) received 0.2 ml pure trioctanoin.

the rats treated i.m. with lead powder, and 5 were found in the rats given calcium chromate.

DISCUSSION

Lead chromate is tumorigenic in rats under the conditions of this experiment. Even when this insoluble compound was administered i.m., 3 of the rats developed renal tumors. This observation is similar to that of other workers who have injected the phosphate. Both compounds are very sparingly soluble in water. The chromate compound dissolves to the extent of 7×10^{-6} part by weight in 100 parts of water; the amount for the phosphate is 1.4×10^{-5}/100 parts of water. In spite of these low handbook values, sufficient lead must dissolve in the biological fluids to be transported to the kidney in order to induce tumors there.

No significance can be attached to the alveologenic carcinoma of the lung found in the mice treated with lead chromate.

Lead powder did not seem to produce any appreciable number of tumors. The few fibrosarcomas found in the rats, when compared to those in the controls, can be considered a random occurrence.

Lead is one of the few chemicals that, as an element, is not active, while one of its compounds is. Low doses of lead, 5 ppm in the drinking water, as evaluated by Kanisawa and Schroeder (13), did not induce any type of tumor in rats. The activity of lead chromate may be the result of the combination with the chromium cation. Chromates, of themselves, have been considered carcinogenic, but weakly so. Injection s.c. of sintered calcium chromate in C57BL mice did not produce tumors (18); on the other hand, when Roe and Carter (21) administered i.m. injections of calcium chromate to rats, 18 of 24 rats developed spindle-cell carcinoma. The few rats that developed fibrosarcomas after calcium chromate treatment is comparable to the results of previous workers (9). Hueper (8) implanted a variety of chromate compounds intrapleurally and found most of them active; lead chromate produced tumors at the implantation site in 3 of 34 rats, with a latent period of 16 months. Hueper (9) summarized most of his results in a review. Chromium pigments (a mixture of chromates of lead, barium, and zinc) failed to produce tumors when administered by the inhalation route. Lead chromate given i.m. induced fibrosarcomas in 9% of his rats. Laskin *et al.* (15) implanted calcium chromate by the intrabronchial pellet technique and obtained squamous cell carcinoma in 100 rats. It seems that rats are more sensitive to chromate carcinogenesis than mice, an observation that is corroborated by our experiments, although our mouse dose was less than that of the rat.

The high yield of tumors at the site of implantation relates well with the results of Maltoni *et al.* (17), who obtained 65% tumor yield in both sexes. We obtained 88% in our females and 40% in the males. Maltoni *et al.* used Sprague-Dawley rats and a single s.c. injection of 30 mg; we gave multiple i.m. injections for a total of 72 mg. Thus, for lead chromate (of varying purity), Fischer 344 rats are not more sensitive than Sprague-Dawley rats. These results are somewhat different from the sensitivity of rat strains summarized by Sunderman (22).

Although the purity of the lead chromate was 98% and one of the impurities was the calcium salt, impurities cannot be a major factor in these results, since our results compare with those of Maltoni *et al.* (17), and the mixture of chromates tested by Hueper (9) was essentially with no activity.

Little information about lead chromate carcinogenesis in man is available. A recent study (14) implies that lead chromate workers are more prone to develop bronchial carcinoma than is the general population as a whole. Thus, chromates *per se* are under suspicion. Both Machle and Gregorius (16) and Bidstrup and Case (2) believe higher tumor mortality can be found among chromate (not lead chromate) workers than can be expected among the population as a whole. Until further work is done, chromates will remain in the category of weak carcinogens. At the present time, lead is the only anion that can enhance the tumorigenic action of chromate. Lead has been only tenuously implicated as a carcinogen; a cerebral tumor reported in one lead worker (19) is not significant, although a relationship was found between lead dust and lung cancer in other workers (12).

The mechanism of the action of lead compounds as renal carcinogens is unknown, but porphyrin metabolism is disturbed, and lead may influence and enhance the synthesis of porphyrins in the renal tissue (25). Neither testosterone or xanthopterin influenced the induction of renal neoplasms in the rat after lead phosphate injection (20).

REFERENCES

1. Balo, E. J. Experimental Adenomas of the Kidney Produced by Chronic Administration of Lead Phosphate. Vopr. Onkol., *9:* 144–151, 1965.
2. Bidstrup, P. L., and Case, R. A. M. Carcinoma of the Lung in Workmen in the Bichromate-Producing Industry in Great Britain. Brit. J. Ind. Med., *13:* 260–264, 1956.
3. Boyland, E. Recent Progress in Carcinogenesis. Biochemical Aspects. Progr. Exptl. Tumor Res., *1:* 162–178, 1960.
4. Boyland, E., Dukes, C. E., Grover, P. L., and Mitchley, B. C. The Induc-

tion of Renal Tumours by Feeding Lead Acetate to Rats. Brit. J. Cancer, *16:* 283–288, 1962.
5. Coogan, P., Stein, L., Hsu, G., and Hass, G. The Tumorigenic Action of Lead in Rats. Lab. Invest., *26:* 473, 1972.
6. Epstein, S. S., and Mantel, N. Carcinogenicity of Tetraethyl Lead. Experientia, *24:* 580–581, 1967.
7. Furst, A., Davis, J. W., Jr., and Dunicz, B. L. Direct Synthesis of Trioctanoin from Glycerol and Octanoic Acid. Org. Prepn. Proc. Ing., *6:* 73–75, 1974.
8. Hueper, W. C. Environmental Carcinogenesis and Cancer. Cancer Res., *21:* 842–857, 1961.
9. Hueper, W. C. Occupational and Environmental Cancers of the Respiratory System. Recent Results Cancer Res., *3:* 77–79, 1966.
10. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 1, pp. 40–50. Lyon: World Health Organization, 1972.
11. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 2, pp. 100–125. Lyon: World Health Organization, 1973.
12. Jecklin, L. Lead Dust and Lung Cancer. Schweiz. Med. Wochschr., *86:* 891–892, 1956.
13. Kanisawa, M., and Schroeder, H. A. Life-term Studies on the Effect of Trace Elements on Spontaneous Tumors in Mice and Rats. Cancer Res., *29:* 892–895, 1969.
14. Langard, S., and Norseth, T. A Cohort Study of Bronchial Carcinomas in Workers Producing Chromate Pigments. Brit. J. Ind. Med., *32:* 62–65, 1975.
15. Laskin, S., Kuschner, M., and Drew, R. T. Studies in Pulmonary Carcinogenesis. *In:* M. G. Hanna, P. Nettesheim, and J. R. Gilbert, Jr. (eds.), Inhalation Carcinogenesis. U. S. Atomic Energy Commission Symposium Series No. 18, pp. 327–351. Oak Ridge, Tenn.: U. S. Atomic Energy Commission, 1970.
16. Machle, W., and Gregorius, F. Cancer of the Respiratory System in the United States Chromate-producing Industry. U. S. Public Health Rept., *63:* 1114–1127, 1948.
17. Maltoni, C., Sinibaldi, C., and Chieco, P. Subcutaneous Sarcomas in Rats following Local Injections of Chromium Orange and Molybdenum Orange. *In:* E. Davis and C. Maltoni (eds.), Advances in Tumor Prevention, Detection and Characterization, Vol. 1, pp. 133–134. New York: American Elsevier Publishing Co., Inc., 1974.
18. Payne, W. W. Production of Cancers in Mice and Rats by Chromium Compounds. A. M. A. Arch. Ind. Health, *21:* 530–535, 1960.
19. Portal, R. W. Cerebral Tumour in a Lead Worker. Brit. J. Ind. Med., *18:* 153–156, 1961.
20. Roe, F. J. C., Boyland, E., Dukes, C. C., and Mitchley, B. C. Failure of Testosterone or Xanthopterin to Influence the Induction of Renal Neoplasms by Lead in Rats. Brit. J. Cancer, *19:* 860–866, 1965.
21. Roe, F. J. C., and Carter, R. L. Chromium Carcinogenesis: Calcium Chromate as a Potent Carcinogen for the Subcutaneous Tissue of the Rat. Brit. J. Cancer, *23:* 172–178, 1969.
22. Sunderman, F. W., Jr. The Current Status of Nickel Carcinogenesis. Ann. Clin. Lab. Sci., *3:* 156–180, 1973.
23. Tonz, O. Renal Tumors in Rats with Lead Phosphate. Z. Ges. Exptl. Med., *128:* 361–377, 1957.
24. Van Esch, G. J., and Kroes, R. The Induction of Renal Tumours by Feeding of Basic Lead Acetate in Mice and Hamsters. Brit. J. Cancer, *23:* 765–771, 1969.
25. Zawirska, B., and Medras, K. Role of the Kidneys in Disorders of Porphyrin Metabolism during Carcinogenesis Induced by Lead Acetate. Arch. Immunol. Therap. Exptl., *20:* 257–272, 1972.
26. Zollinger, H. U. Durch Chronische Bleivergiftung erzeugte Nierenadenome und Carcinome bei Ratten und ihre Beziehungen zu des entsprechender Neubildungen des Menschen. Virchows Arch. Pathol. Anat. Physiol., *323:* 694–710, 1953.