STUDIES OF NICKEL CARCINOGENESIS METASTASIZING PULMONARY TUMORS IN RATS INDUCED BY THE INHALATION OF NICKEL CARBONYL

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The high incidence of pulmonary cancer in nickel workers was first reported by Baader in 1937.[1] Since that time, the relation of nickel to pulmonary carcinogenesis has been the subject of a number of investigations.[2–21] In 1958, Doll[4] reported that 35.5 per cent of nickel workers in Wales died of cancer of the lung or upper respiratory tissues whereas the incidence among colliery workers was only 1.5 per cent. Within the past 2 years, Passey[15] tabulated 144 deaths from cancer of the lung in nickel workers and computed the average age at death in these workers to be 57.6 years. The average length of time that the affected workers were employed in nickel refineries was 27 years; the average time between the first exposure and death from lung cancer was 30.5 years. In Great Britain, cancer in the respiratory tract is a compensable disease for nickel workers.

The relationship between the inhalation of nickel and pulmonary cancer has been the subject of a number of investigations from our laboratory.[17–19] These studies have established that pulmonary cancer may be induced in rats exposed to a single heavy concentration of nickel carbonyl as well as in rats exposed to repeated inhalations of sublethal concentrations for a period of a year. It is noteworthy that cancers were not observed in these experimental animals until 2 or more years after the initial exposure.

In recent studies on pulmonary carcinogenesis,[16,20,22] nickel was found to be present in purified preparations of ribonucleic acid obtained from the livers and lungs of normal rats. In similar preparations from the livers and lungs of rats exposed to inhalations of nickel carbonyl, the amounts of nickel bound to RNA were found to be greater than those in

This study was supported in part by contracts of Jefferson Medical College with the Atomic Energy Commission and with the Rohm and Haas Company.

Accepted for publication, February 29, 1965.

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This is the seventeenth paper in a series of studies on nickel poisoning.

normal rats. Furthermore, specific changes in the physico-chemical properties of RNA were observed in purified preparations from the exposed animals.

Other studies from our laboratory have demonstrated the presence of nickel in tobacco smoke.[19] Computations of the amount of nickel inhaled annually by a heavy cigaret smoker indicate that this value is several times the amount that is carcinogenic for the rat;—an animal that is known to be unusually resistant to the development of pulmonary cancer.

The present studies covering a period of 3 years are a continuation of those previously undertaken on nickel carcinogenesis. Since "Dithiocarb" (N,N′ sodium diethyldithiocarbamate-trihydrate) [23,24] has proved to be remarkably effective in the treatment of persons acutely poisoned by inhalations of nickel carbonyl, investigations were undertaken to ascertain whether or not the administration of "Dithiocarb" to animals subjected to lethal concentrations of nickel carbonyl would be protective against the induction or development of cancer in the respiratory tract. In addition, mortality data and weights of rats exposed to nickel carbonyl have been compared to those of unexposed animals under controlled conditions.

Material and Methods

Wistar strain, male white rats weighing between 200 and 250 gm were used in our experiments. The animals were kept under preliminary observation in cages adjoining the exposure chamber for a period of a month before being exposed to nickel carbonyl. Throughout the period of study, the rats were maintained on a diet of Purina® Rat Chow. They were observed daily and weighed weekly.

The design of the exposure chamber and the method of exposing rats to nickel carbonyl have been described in a previous publication from our laboratory.[25] In brief, nickel carbonyl is dissolved in a mixture of equal parts of absolute alcohol and ethyl ether and dispersed as a vapor into an air-stream flowing at a rate of 539 l per minute for a period of 30 minutes. During each period of exposure the concentration of nickel carbonyl in the chamber is determined by chemical analysis. The results of these analyses are compared to the calculated concentrations. In general, the chemical analyses yield values which are approximately 80 per cent of the calculated nominal concentrations.

In the present investigations, the rats were divided into 6 groups: 3 groups of control animals (III, IV and VI) and 3 groups of exposed animals (I, II and V). The disposition of the 6 groups was as follows:

Group I. Two hundred and eighty-five rats in this group inhaled nickel carbonyl in a concentration of 80 parts per million (0.6 mg per l) for a period of 30 minutes. Three weeks after exposure, 214 had died and only 71 of the exposed rats had survived and appeared healthy.

Group II. Sixty rats inhaled a single dose of nickel carbonyl for 30 minutes, in precisely the same manner as group I. In addition, however, each rat received "Dithiocarb" subcutaneously in a dosage of 50 mg per kg of body weight within 15 minutes after exposure. Three weeks after exposure all rats in this group had survived and appeared healthy.

Group III. Nineteen rats in this control group inhaled 5 ml of an alcohol-ether mixture (without nickel carbonyl) vaporized into the air-stream of the exposure chamber for 30 minutes. No fatalities occurred within 3 weeks after exposure and all animals appeared healthy.

Group IV. Nineteen rats in this control group inhaled 5 ml of an alcohol-ether mixture the same as group III, but in addition they received "Dithiocarb" subcutaneously in a dosage of 50 mg per kg of body weight immediately after removal from the exposure chamber. No fatalities occurred within 3 weeks after exposure and all animals were healthy.

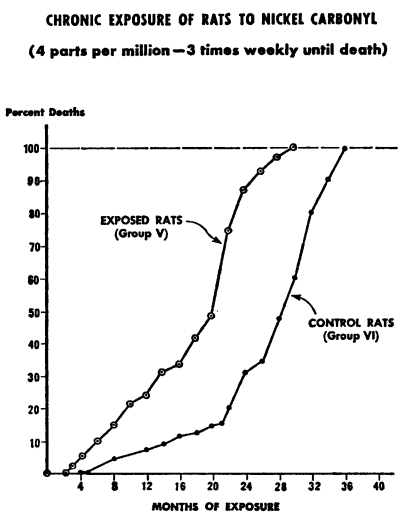
Group V. Sixty-four rats were exposed to inhalations of nickel carbonyl in a concentration of 4 parts per million (0.03 mg per l) for 30 minutes 3 times weekly for the remainder of their lives. All animals were alive after 3 weeks of exposures.

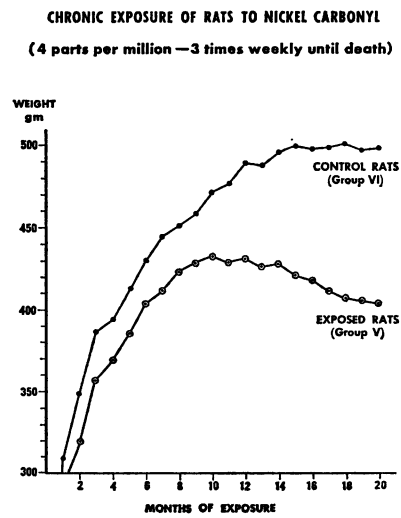
Group VI. This group of 32 rats served as controls for group V. These rats inhaled only alcohol-ether vapor (without nickel carbonyl) 3 times weekly for the remainder of their lives. No deaths occurred within the first 3 weeks after the initial exposure and all animals appeared healthy.

Necropsies were made on all animals that were sacrificed or found dead during the 3 year period of this study and representative tissues were removed and fixed for histologic studies. An attempt was made to avoid autolytic changes in the tissues by sacrificing animals that appeared moribund.

Results

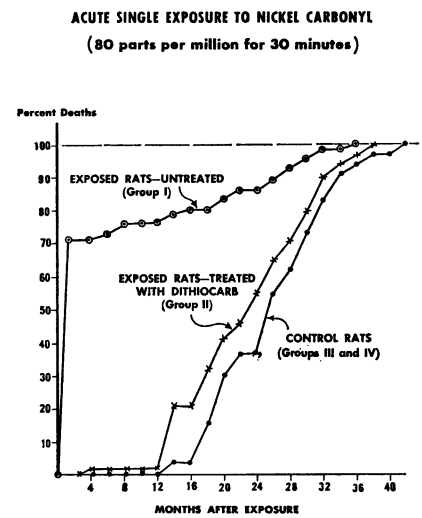
Mortality and Weight Curves. In Text-figure 1 the mortality curve of rats (Group V) exposed chronically to the vapors of nickel carbonyl in a concentration of 4 parts per million (0.03 mg per l) for 30 minutes 3 times weekly until death is compared to the mortality curve of control rats (Group VI). At the end of 1 year, 25 per cent of the rats exposed to nickel carbonyl had died compared to 7 per cent of the controls. By the end of the second year, 88 per cent of the exposed rats had died compared to 30 per cent of the controls. The last rat of the exposed groups





died 29 months after the initial exposure. The last control animal died 36 months after the beginning of the experiment.

In Text-figure 2 the mean weight curve of the rats chronically exposed to nickel carbonyl (Group V) is compared to the mean weight curve of the control animals (Group VI). It will be seen that the mean weight of the exposed rats was less than that of the control rats throughout the entire period of the study. At the end of 1 year of exposures to nickel carbonyl, the mean weight of the exposed rats was 430 gm. By comparison, the mean weight of the control animals was 490 gm. After 20 months of repeated exposures to nickel carbonyl, the mean weight of the surviving rats in this group was 405 gm as compared to 499 gm in the control animals (Group VI).



In Text-figure 3 is portrayed the mortality curve of rats in groups I and II exposed to a single heavy dose of nickel carbonyl in a concentration of 80 parts per million for 30 minutes. This curve is compared to the mortality curve of control rats in groups III and IV. Within 3 weeks after exposure, 72 per cent of the rats in group I had died, whereas none of the rats in group II (similarly exposed but treated with "Dithiocarb") had died during this same period. The rat living the longest in group I died 34 months after exposure. One rat in the control group lived for 40 months after the experiment had been started. The mortality curve for the exposed rats in group II treated with "Dithiocarb" is similar to that of the combined curve of control groups III and IV.

Histologic Features of the Pulmonary Carcinomas Found in the Ex-

posed Rats. Of the rats in group V that were exposed chronically to nickel carbonyl, rat V-82 was sacrificed after 26 months of tri-weekly exposures. At death, the animal was emaciated, having lost 100 gm during the final week of life. At necropsy the left lung was found to be emphysematous and the right lung, though smaller, contained a neoplastic lesion. Both kidneys were enlarged and contained lesions considered to be tumors. Microscopically the main pulmonary tumor was an adenocarcinoma (Fig. 1). The glandular pattern in various areas of the tumor resembled bronchiolar structures, peribronchial glands or even hyperchromatic alveolar elements with some papillary irregularities. The neoplastic change, although characterized by a major tumor, includes multicentric lesions ranging from early neoplastic transitions in alveolar epithelium (Fig. 2) to distinct masses that could be either satellite metastases from the main tumor or additional primary tumors developing from some of the multicentric foci. Metastatic adenocarcinoma was found in the regional mediastinal lymph nodes, and in the myocardium (Fig. 3). The metastases were differentiated enough to be readily recognizable as compatible with origin in the primary pulmonary lesion.

Rat I-462 died 24 months after a single exposure to nickel carbonyl. The lungs were grossly congested and in the right lung, there was a tumor nodule, approximately 1.0 cm in its greatest diameter. The liver was the site of nodules ranging from a few mm to slightly less than a cm in diameter. Unfortunately, the tissues were generally affected by autolysis. The lung tumor, however, could be readily recognized histologically as a papillary adenocarcinoma with necrosis (Fig. 4). The renal and hepatic metastases (Fig. 5) were clearly recognizable as secondary to the papillary adenocarcinoma of the lung.

The third carcinoma of the lung encountered in our investigation occurred in rat II-479, an animal exposed only once to nickel carbonyl and subsequently treated with "Diothiocarb." Death occurred 26 months after exposure. The animal was emaciated and had gross evidence of tumor in the lungs and liver with definite enlargement of the spleen. Histologically, the lungs were the site of anaplastic carcinoma of an expansile type (Fig. 6) with multicentric sites or metastases. Similarly, anaplastic metastases were encountered around the hilum of the enlarged hyperplastic spleen (Fig. 7) as well as in the liver (Fig. 8). Extensive necrosis was present in the carcinoma.

The last rat to survive in control group VI died 33 months after the initial exposure. The histologic study in this rat VI-1 revealed a single well-circumscribed, but not encapsulated, nodule approximately 1 mm in diameter (Fig. 9). The lesion was a papillary adenomatous tumor and

had some degree of nuclear hyperchromatism. In character this tumor was similar to 2 small papillary adenomas discovered in the lung of a test rat in an earlier study.[17] In that study it was pointed out that the lesions were similar to one reported by Horn and Stewart,[26] who classified it simply as a spontaneous pulmonary tumor in the rat, fundamentally similar to the papillary glandular pulmonary tumor of the mouse. We consider such tumors to be akin to those classified by Mostofi and Larsen[27] as "adenomatoid alveolar cell tumors" found in the lungs of certain strains of mice. Most investigators avoid any designation of them as malignant and we, too, accept the more conservative view.

Additional Pathologic Findings. Previous experience[17,18] indicated that protracted follow-up observation for at least 2 years was necessary for the discovery of pulmonary tumors resulting from exposure of rats to nickel carbonyl. It was also discovered that chronic inhalation of a toxic agent frequently resulted in a relatively high mortality owing to inflammatory disease and other conditions which might not be related to neoplasia.

Pulmonary lesions other than neoplastic types were practically all inflammatory and consisted mainly of pneumonitis, pneumonia, bronchitis, bronchiectasis, bronchial abcesses and reactive fibrosis. These lesions occurred commonly and were frequently lethal. Such changes were encountered in the lungs of older rats and could be extensive.

In the present studies the animals in the control groups as well as those exposed to the inhalations of nickel carbonyl were beset with inflammatory lung lesions. Therefore, there was no attempt to assign an etiologic role to nickel carbonyl in explaining the inflammatory reactions or reparative fibrosis, though the possibility of such a role is recognized and has been suggested by earlier studies on acute nickel carbonyl poisoning.[28,29]

In Table I are listed the rats that developed malignant tumors or related conditions. It will be seen that a variety of types of tumors was encountered among our experimental animals. Most of these lesions were not considered related to nickel carbonyl exposure since rats in the control groups developed similar lesions.

In the 3 control groups that were never exposed to nickel carbonyl, 44 rats were alive at the end of the 2 year period of observation and of these, approximately one-third (15) developed malignant tumors. In the 3 test groups chronically or acutely exposed to nickel carbonyl, 80 rats were alive at the end of the 2 year period of observation and of these approximately one-half (39) developed malignant tumors. Malignant lymphoma was the most common neoplasm found in both control

TABLE I

NEOPLASMS FOUND IN CONTROL RATS AND RATS EXPOSED TO NICKEL CARBONYL

Group	Type of exposure	No. of rats with specific types of tumors
I	$Ni(CO)_4$, single exposure of 80 ppm for 30 minutes (of 285 rats exposed, 71 survived)	Malignant lymphomas * ..... 9 Myelogenous leukemia ..... 1 Fibrosarcoma (subcutaneous) ..... 1 Osteosarcoma and metastases ..... 1 Leydig cell carcinoma of the testis..... 1 Squamous carcinoma of skin ..... 1 Papillary adenocarcinoma of lung with metastases ..... 1 Subcutaneous fibrosarcoma, malignant pheochromoblastoma (included in the lymphoma group because of its dominant lesion) 1
II	$Ni(CO)_4$, single exposure of 80 ppm for 30 minutes followed by "Dithiocarb" intraperitoneally (of 60 rats exposed, all survived)	Malignant lymphomas * ..... 8 Fibrosarcoma (subcutaneous and widespread metastases) ..... 4 Carcinoma of the skin ..... 2 Anaplastic carcinoma of the lung with metastases ..... 1 Anaplastic malignant tumors of uncertain origin, probably sarcoma 2
III (control)	Alcohol-ether, single exposure to concentrations similar to group I (of 19 rats exposed, all survived)	Malignant lymphomas * ..... 3 Angiosarcoma with metastases ..... 1 Squamous carcinoma of skin 1
IV (control)	Alcohol-ether, single exposure in concentrations similar to group I and then followed by "Dithiocarb" intraperitoneally (of 19 rats exposed, all survived)	Malignant lymphomas * ..... 4 Fibrosarcoma (intra-abdominal) ..... 1 Adenocarcinoma of thyroid 1
V	$Ni(CO)_4$, chronic, multiple exposures of 4 ppm for 30 minutes 3 times weekly until death (of 64 rats exposed, all were alive 3 weeks after exposures)	Malignant lymphomas * ..... 5 Squamous carcinoma in salivary gland and an adrenal carcinoma ..... 1 Adenocarcinoma of the lung with metastases 1
VI (control)	Alcohol-ether, chronic, multiple exposures in concentrations similar to group V (of 32 rats exposed, all were alive 3 weeks after exposures)	Malignant lymphoma * ..... 1 Myelogenous leukemia ..... 1 Fibrosarcoma (intra-abdominal) ..... 1 Adenocarcinoma of pancreas (probably ductal) 1

* Nearly all of the malignant lymphomas in all groups can be classified as histiocytic (reticulum cell) sarcomas and the most common major involvement was in the lungs or mediastinum.

and test animals. Other types of tumors included fibrosarcoma, myelogenous leukemia and squamous cell carcinoma of the skin.

Three rats exposed to nickel carbonyl developed pulmonary carcinoma with metastases. The incidence of three pulmonary carcinomas among the 80 rats that lived to or beyond the 2 year latent period after exposure to nickel carbonyl is considered highly significant. This significance is based not only on the fact that no pulmonary carcinomas had developed in the control rats with comparable longevity but also on a number of reports in the literature [26,30,31] dealing with the incidences of spontaneous tumors in rats. These reports indicate that the lungs in rats are peculiarly resistant to primary pulmonary carcinoma.

TABLE II

PULMONARY CANCER WITH METASTASES IN RATS EXPOSED TO NICKEL CARBONYL

Type of exposure	Concentration of $Ni(CO)_4$ in parts per million	Death of rats months after initial exposure	Type of tumor
3 times weekly for 1 year	4	24	Squamous cell carcinoma
3 times weekly for 1 year	4	24	Squamous cell carcinoma
3 times weekly for 26 months	4	26	Adenocarcinoma
Single exposure	35	27	Anaplastic carcinoma
Single exposure	80	24	Adenocarcinoma
Single exposure	80	26	Anaplastic carcinoma

In Table II are listed 6 rats that developed pulmonary carcinomas with metastases following exposure to nickel carbonyl in this study and in an earlier one.[17] These neoplasms occurred among 89 rats that lived 2 years or more after the initial exposure to nickel carbonyl. The lesions were classified as squamous cell carcinoma (2); adenocarcinoma (2); and anaplastic carcinoma (2). All of the lesions were found between 24 and 27 months after the initial exposure to nickel carbonyl. Three of the rats had been subjected to mild chronic exposures and 3 had been subjected to a heavy single exposure.

DISCUSSION

Pulmonary carcinoma occurs only rarely as a spontaneous lesion in rats. In our series of experiments over a period of 12 years, carcinoma of the lung had never been encountered in an untreated control rat. On the other hand, 6 rats exposed to nickel carbonyl were found to have

pulmonary carcinomas with metastases. With the exception of Gillman, Gilbert and Spence,[32] many investigators have emphasized the rarity of spontaneous pulmonary carcinoma in experimental rats.[17,18,26,30,31,33] The spontaneous pulmonary tumors in a group of 327 rats reported by Gillman and co-workers were described as being similar histologically to the urethane-induced lesions observed in mice by Mostofi and Larsen.[27] Mostofi classified these lesions as "adenomatoid alveolar cell tumors in mice" and did not indicate that they were malignant. Gillman and co-workers,[32] however, suggested that the 3 tumors they found in the lungs were carcinomas, indistinguishable from the adenomatous tumors reported by Mostofi. We, too, have found occasional papillary adenomas in rat lungs but have considered such lesions to be benign. One such tumor was found in a control rat VI-1 which lived 33 months. In this tumor, the histologic sections did not suggest malignancy (Fig. 9) and no evidence of metastasis was found in this rat. Mostofi observed no metastases in his studies and pointed out that he would not refer to such urethane-induced lung tumors in mice or rats as carcinomas.[34] The incidence of tumors in rats reported in the studies of Gillman and co-workers is strikingly different from that in the series of 31,868 rats reported by Curtis, Bullock, and Dunning,[30] and from that in the series of 498 rats reported by Saxton, Sperling, Barnes and McCay[31] in which neither reported the finding of any carcinomas in the lungs. In view of these reports, the interpretations of Gillman and associates are open to uncertainty.

Owing to the histologic character of some of the multicentric lesions observed in the lungs in our rats we are led to suggest that small circumscribed lesions characterized by moderate hyperchromatism in nuclei and papillary infolding of alveolar walls may represent relatively early morphologic evidence of neoplastic change. Such changes could possibly eventuate in malignant neoplasm if carcinogenic influences continue to be active.

The question of a possible effect of nickel carbonyl on the incidence of other types of tumors seems far less certain. The most common type of neoplastic disease in all our groups of animals was malignant lymphoma, frequently a histiocytic or reticulum cell sarcoma. The anatomic sites most frequently involved were the peribronchial regions and the mediastinal lymphoid tissues. The occurrence of lymphomas among older rats has been previously noted. Crain[33] found a total of 41 malignant lymphomas among 786 rats of which 189 had 200 tumors. Approximately one-third of the tumors were malignant and these included the 41 lymphomas. Saxton, and co-workers,[31] reported lymphosarcomas of the lung among 234 tumors in rats. In our present series of 265 rats

living more than 3 weeks after exposure, 30 developed lymphoma. A relationship to nickel exposure appears doubtful in view of the high spontaneous incidence of lymphoma in rats reported in the literature and found among our control animals.

Our present investigations involved a limited test of the protective action of N,N′ sodium diethyldithiocarbamate-trihydrate against the carcinogenic action of inhaled nickel carbonyl in rats. Although no positive protective action was apparent in these studies, it should be emphasized that "Dithiocarb" was not given prophylactically and therefore, the results are inconclusive.

In earlier studies,[17,19] it had been shown that carcinoma of the lung developed in rats that inhaled nickel carbonyl thrice weekly for one year in an estimated total dosage of 1930 μgm of nickel. The amount of nickel contained in the main stream smoke of a single cigaret was shown to be 0.37 μgm. The amount of nickel inhaled by the experimental rats is comparable to that contained in the main stream smoke of approximately 260 packs of cigarets.

Summary

Our studies indicate that inhaled nickel carbonyl is carcinogenic to the lungs of rats, a species generally considered to be peculiarly resistant to pulmonary cancer. In a combined series of studies, 6 rats exposed to nickel carbonyl developed pulmonary carcinoma with metastases. The lesions included the common types of pulmonary cancer, squamous cell carcinoma, adenocarcinoma and anaplastic carcinoma. All of the pulmonary lesions were found between 24 and 27 months after the initial exposure to nickel carbonyl.

The amount of nickel capable of inducing lung cancer in the rat is comparable to the amount of nickel inhaled by persons smoking less than 15 cigarets per day for a period of a year.

The mean weight of rats chronically exposed to nickel carbonyl was found to be consistently less than that of the control rats throughout the entire 3-year period of study.

After 2 years of chronic exposure to nickel carbonyl, the percentage of deaths in the exposed group of rats was approximately 3 times greater than in the control groups.

References

1. Baader, E. W. Berufskrebs. In: Neuere Ergebnisse auf dem Gebiete der Krebskrankheiten. Adam, C., and Auler, D. S. Hirzel Verlag, Leipzig, c. 1937, pp. 116–117.
2. Barnett, G. P. Annual Report of the Chief Inspector of Factories for the Year 1948. H. M. Stationery Office, London, c. 1949.

3. CAMPBELL, J. A. Lung tumours in mice and man. *Brit. M. J.*, 1943, **1**, 179–183.
4. DOLL, R. Cancer of the lung and nose in nickel workers. *Brit. J. Indust. Med.*, 1958, **15**, 217–223.
5. DOLL, R. Specific Industrial Causes. In: Carcinoma of the Lung, BIGNALL, J. R. The Williams & Wilkins Company, Baltimore, c. 1958, pp. 45–59.
6. GILMAN, J. P. W. Metal carcinogenesis. II. A study of the carcinogenic activity of cobalt, copper, iron and nickel compounds. *Cancer Res.*, 1962, **22**, 158–162.
7. GILMAN, J. P. W., and RUCKERBAUER, G. M. Metal carcinogenesis. I. Observations on the carcinogenicity of a refinery dust, cobalt, oxide, and colloidal thorium dioxide. *Cancer Res.*, 1962, **22**, 152–157.
8. GOLDBLATT, M. W. Occupational carcinogenesis. *Brit. M. Bull.*, 1958, **14**, 136–140.
9. HATEM-CHAMPY, S. Affinity of folic acid for nickel and nickel-induced cancer. *C. R. Acad. Sc.*, 1962, **254**, 1177–1179.
10. HATEM-CHAMPY, S. Cancers caused by nickel and by a nickel-imidazole complex. *C. R. Acad. Sc.*, 1961, **253**, 2791–2792.
11. HUEPER, W. C. Experimental studies in metal cancerigenesis. IX. Pulmonary lesions in guinea pigs and rats exposed to prolonged inhalation of powdered nickel. *Arch. Path.*, 1958, **65**, 600–607.
12. HUEPER, W. C.; KOTIN, P.; TABOR, E. C.; PAYNE, W. W.; FALK, H., and SAWICKI, E. Carcinogenic bioassays on air pollutants. *Arch. Path.*, 1962, **74**, 89–116.
13. LØKEN, A. C. Carcinoma of the lung in nickel workers. *Tidsskr. norske laegefor.*, 1950, **70**, 376–378.
14. MORGAN, J. G. Some observations on the incidence of respiratory cancer in nickel workers. *Brit. J. Indust. Med.*, 1958, **15**, 224–234.
15. PASSEY, R. D. Some problems of lung cancer. *Lancet.*, 1962, **2**, 107–112.
16. SUNDERMAN, F. W., JR. Studies of nickel carcinogenesis: Alterations of ribonucleic acid following inhalation of nickel carbonyl. *Am. J. Clin. Path.*, 1963, **39**, 549–561.
17. SUNDERMAN, F. W.; DONNELLY, A. J.; WEST, B., and KINCAID, J. F. Nickel poisoning. IX. Carcinogenesis in rats exposed to nickel carbonyl. *Arch. Indust. Hyg.*, 1959, **20**, 36–41.
18. SUNDERMAN, F. W.; KINCAID, J. F.; DONNELLY, A. J., and WEST, B. Nickel poisoning. IV. Chronic exposure of rats to nickel carbonyl; a report after one year of observation. *Arch. Indust. Hyg.*, 1957, **16**, 480–485.
19. SUNDERMAN, F. W., and SUNDERMAN, F. W., JR. Nickel poisoning. XI. Implication of nickel as a pulmonary carcinogen in tobacco smoke. *Am. J. Clin. Path.*, 1961, **35**, 203–209.
20. SUNDERMAN, F. W., JR., and SUNDERMAN, F. W. Studies of nickel carcinogenesis. The subcellular partition of nickel in lung and liver following inhalation of nickel carbonyl. *Am. J. Clin. Path.*, 1963, **40**, 563–575.
21. WILLIAMS, W. J. The pathology of the lungs in five nickel workers. *Brit. J. Indust. Med.*, 1958, **15**, 235–242.
22. SUNDERMAN, F. W., JR.; HORN, R. S., and SUNDERMAN, F. W. Binding of nickel and other trace metals by ribonucleic acid. *Fed. Proc.*, 1962, **21**, 311.
23. WEST, B., and SUNDERMAN, F. W. Nickel poisoning. VII. The therapeutic effectiveness of aklyl dithiocarbamates in experimental animals exposed to nickel carbonyl. *Am. J. M. Sc.*, 1958, **236**, 15–25.

24. SUNDERMAN, F. W., and SUNDERMAN, F. W., JR. Nickel poisoning. VIII. Dithiocarb: a new therapeutic agent for persons exposed to nickel carbonyl. *Am. J. M. Sc.*, 1958, **236**, 26–31.

25. SUNDERMAN, F. W.; KINCAID, J. F.; KOOCH, W., and BIRMELIN, E. A. A constant flow chamber for exposure of experimental animals to gases and volatile liquids. *Am. J. Clin. Path.*, 1956, **26**, 1211–1218.

26. HORN, H. A., and STEWART, H. L. Spontaneous pulmonary tumor in the rat: report of a lesion. *J. Nat. Cancer Inst.*, 1952, **12**, 743–749.

27. MOSTOFI, F. K., and LARSEN, C. D. The histopathogenesis of pulmonary tumors induced in Strain A mice by urethan. *J. Nat. Cancer Inst.*, 1951, **11**, 1187–1221.

28. SUNDERMAN, F. W.; RANGE, C. L.; SUNDERMAN, F. W., JR.; DONNELLY, A. J., and LUCYSZYN, G. W. Nickel poisoning. XII. Metabolic and pathologic changes in acute pneumonitis from nickel carbonyl. *Am. J. Clin. Path.*, 1961, **36**, 477–491.

29. SUNDERMAN, F. W., and KINCAID, J. F. Nickel poisoning. II. Studies on patients suffering from acute exposure to vapors of nickel carbonyl. *J.A.M.A.*, 1954, **155**, 889–894.

30. CURTIS, M. R.; BULLOCK, F. D., and DUNNING, W. F. A statistical study of the occurrence of spontaneous tumors in a large colony of rats. *Am. J. Cancer*, 1931, **15**, 67–121.

31. SAXTON, J. A., JR.; SPERLING, G. A.; BARNES, L. L., and MCCAY, C. M. The influence of nutrition upon the incidence of spontaneous tumors of the albino rat. *Acta Unio internat. contra cancrum*, 1948, **6**, 423–431.

32. GILLMAN, J.; GILBERT, C., and SPENCE, I. Phaeochromocytoma in the rat. Pathogenesis and collateral reactions and its relation to comparable tumours in man. *Cancer*, 1953, **6**, 494–511.

33. CRAIN, R. C. Spontaneous tumors in the Rochester strain of the Wistar rat. *Am. J. Path.* 1958, **34**, 311–336.

34. MOSTOFI, F. K. Personal communication.

LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

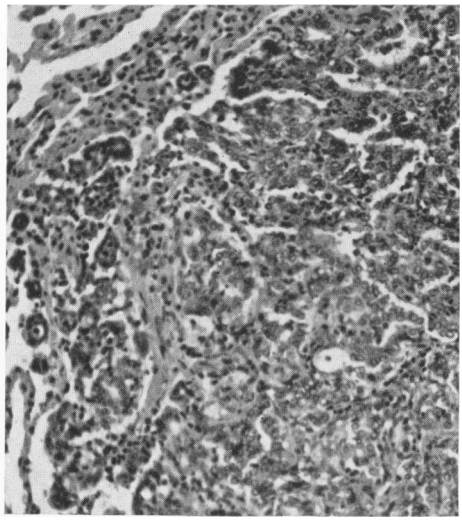
FIG. 1. Adenocarcinoma of lung, rat V-82 sacrificed after 28 months of tri-weekly exposures to nickel carbonyl. × 160.

FIG. 2. Hyperplastic folds of alveolar lining cells with hyperchromatic nuclei. These lesions, considered to be "early" neoplastic transitions, were found scattered through the same lung pictured in Figure 1. × 160.

FIG. 3. Metastatic adenocarcinoma invading heart muscle in rat V-82. There is more fibrotic stroma in this lesion than in the primary tumor shown in Figure 1 or in the other secondary tumors. × 160.

FIG. 4. Adenocarcinoma of lung in rat I-462 which died 24 months after a single exposure to nickel carbonyl. The tissue is moderately autolyzed but the papillary character of the neoplasm is easily recognized. × 160.

FIG. 5. Metastatic adenocarcinoma in liver secondary to the pulmonary lesion shown in Figure 4. × 160.



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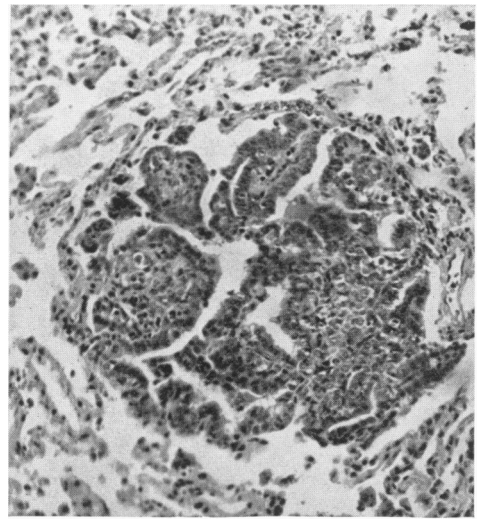

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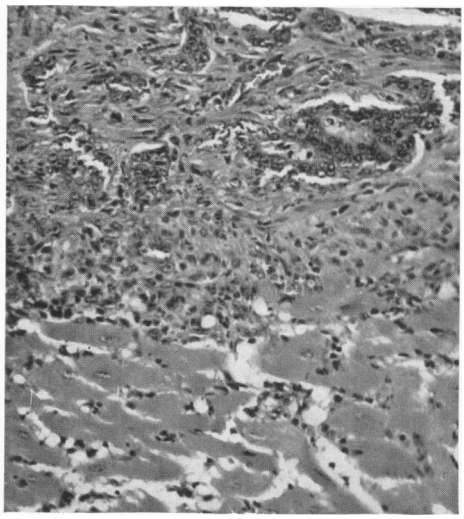

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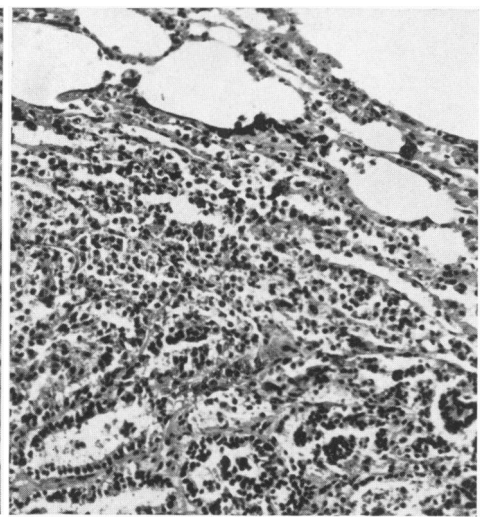

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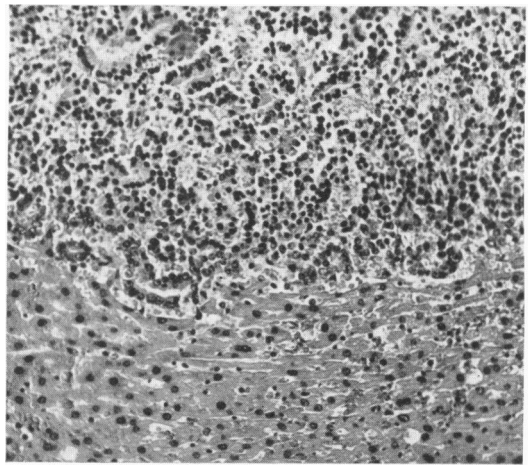

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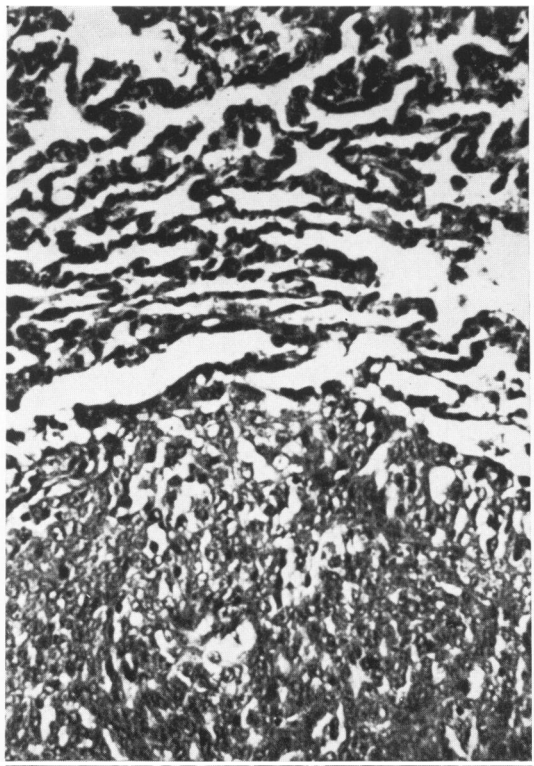

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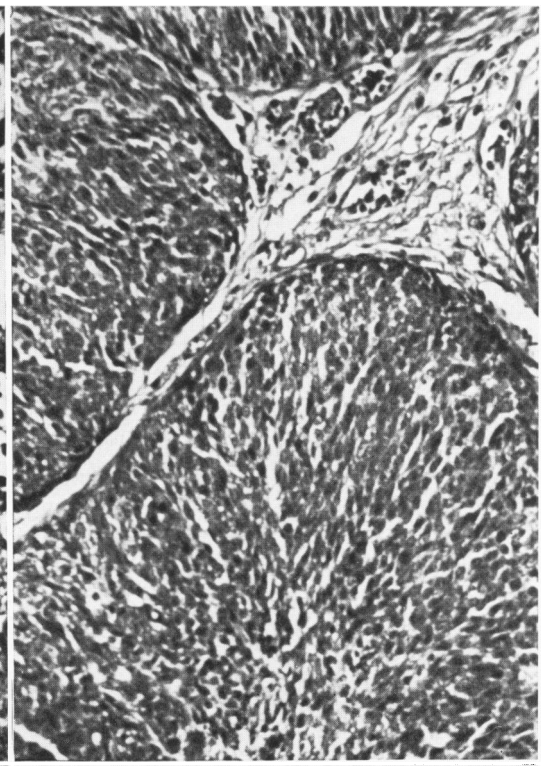

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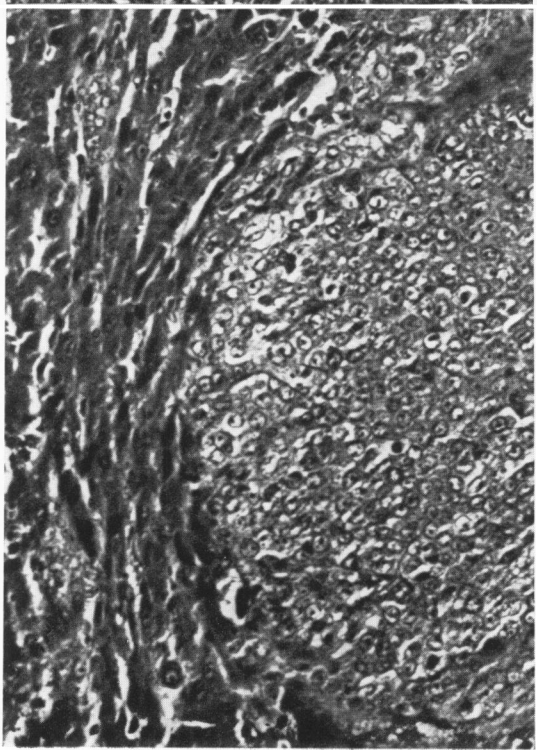

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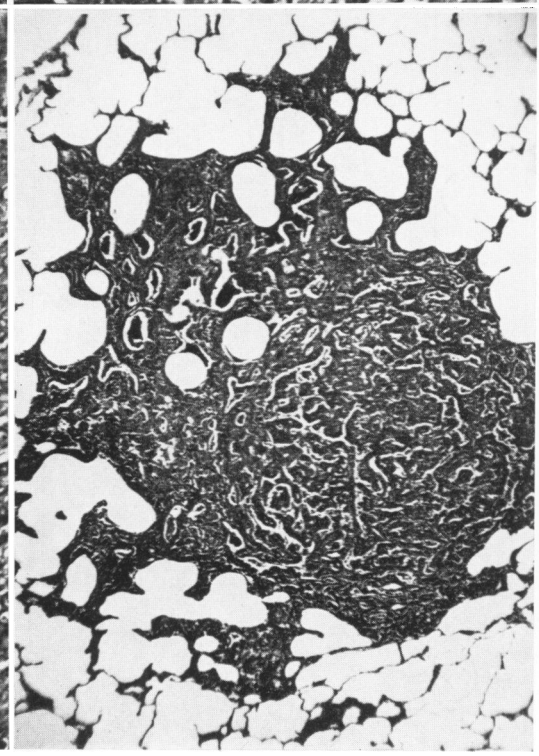

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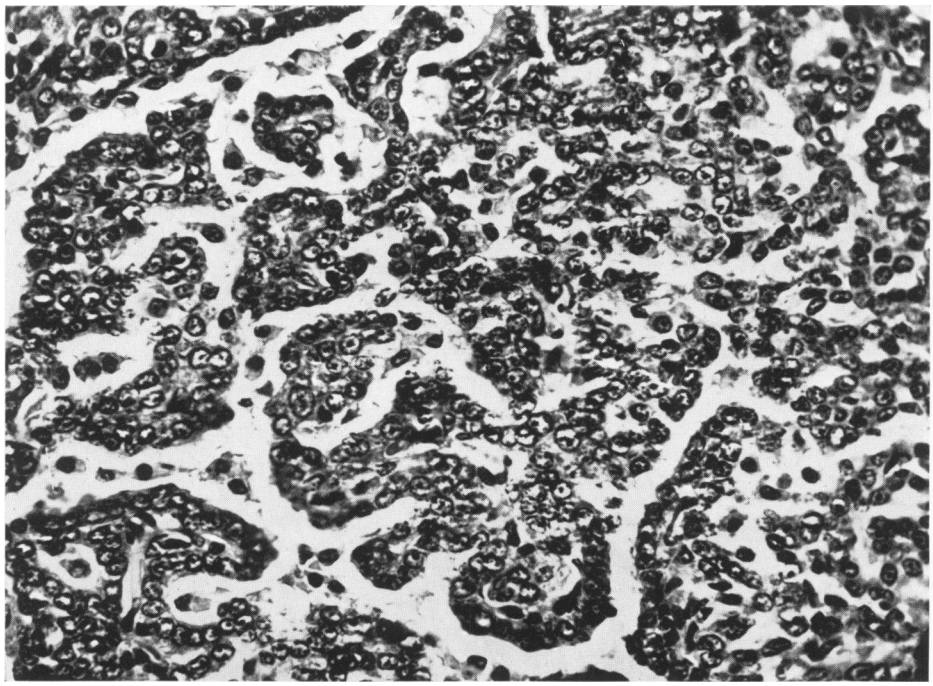

FIG. 6. Relatively solid and apparently expansile anaplastic carcinoma in the lung of rat II-479 also exposed only once to nickel carbonyl. Death occurred 26 months after exposure. × 225.

FIG. 7. Section of a representative area in one of many secondary anaplastic tumor masses found in extrapulmonary regional sites in rat II-479. These masses exhibit the same expansile character as the primary tumor shown in Figure 6. × 225.

FIG. 8. Liver with partially necrotic anaplastic carcinoma secondary to that portrayed as primary pulmonary carcinoma in Figure 6. × 225.

FIG. 9. Well circumscribed papillary adenomatoid alveolar cell tumor in the lung of control rat VI-1. This is considered to be a benign lesion. × 52.5.

FIG. 10. Higher magnification of the central area in the tumor shown in Figure 9. The papillary and alveolar character of the benign lesion is evident at this magnification. × 445.