the large pharmacological doses of testosterone administered here suggests that the animal may not be completely insensitive to androgens (*10*, *24*). The Tfm rat will not exhibit masculine behavior when treated with physiological levels of testosterone (*22*, *23*). However, our findings are consistent with reports that testosterone-activated sexual behavior is due to aromatization of the androgen to estrogen in the brain (*5*, *25*, *26*). Since the ejaculatory behavior of the Tfm rat was more responsive to a single dose of estradiol than to seven large doses of testosterone, it is possible that the testosterone-induced male behavior was mediated by estrogen metabolites of testosterone aromatization. In this context, it should be noted that the Tfm mouse has normal levels of brain aromatase (*27*).

The occurrence of at least a partially defeminized and masculinized brain in an otherwise phenotypically female Tfm animal suggests that perinatal androgens are not required to masculinize the developing brain. It seems reasonable to assume that the testes of the Tfm animals are the source of estrogens during the perinatal period as they are during adulthood (*15*), or that the testes of these animals are the source of testosterone (*10*) that is subsequently aromatized to estrogens (*27*) in the brain. The estrogens so formed then bind to neural cytosolic estrogen receptors (*14*) and masculinize the differentiating brain by directing genome readout.

BERNARD H. SHAPIRO
Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104

DAVID C. LEVINE
NORMAN T. ADLER
Department of Psychology, University of Pennsylvania

References and Notes

1. A. Jost, *Recent Prog. Horm. Res.* **8**, 379 (1953).
2. P. DeMoor and C. Denef, *Endocrinology* **82**, 480 (1968).
3. C. A. Pfeiffer, *Am. J. Anat.* **58**, 195 (1936).
4. R. E. Whalen and D. A. Edwards, *Anat. Rec.* **157**, 173 (1967).
5. F. Naftolin, K. J. Ryan, I. J. Davies, V. V. Reddy, F. Flores, Z. Petro, M. Kuhn, R. J. White, Y. Takaoka, L. Wolin, *Recent Prog. Horm. Res.* **31**, 295 (1975).
6. C. A. Barraclough and J. L. Turgeon, *Symp. Soc. Dev. Biol.* **33**, 255 (1975).
7. C. Dougherty and P. G. McDonald, *Differentiation* **2**, 275 (1974).
8. J. M. Morris, *Am. J. Obstet. Gynecol.* **65**, 1192 (1953).
9. N. Nes, *Nord. Veterinaermed.* **18**, 19 (1966).
10. C. W. Bardin, L. P. Bullock, R. J. Sherins, I. Mowszowicz, W. R. Blackburn, *Recent Prog. Horm. Res.* **29**, 65 (1973).
11. A. J. Stanley, L. G. Gumbreck, J. E. Allison, R. B. Easley, *ibid.* **29**, 43 (1973).
12. D. Bennett, E. A. Boyse, M. F. Lyon, B. J. Mathieson, M. Scheid, K. Yanagisawa, *Nature (London)* **257**, 236 (1975).
13. K. Einarsson, J-A. Gustafsson, A. S. Goldman, *Eur. J. Biochem.* **31**, 345 (1972).
14. B. Attardi, L. N. Geller, S. Ohno, *Endocrinology* **98**, 864 (1976).
15. B. H. Shapiro and A. S. Goldman, *ibid.* **96**, 1392 (1975).
16. ——, J-A. Gustafsson, *ibid.* **97**, 487 (1975).
17. P. F. A. Van Look, W. M. Hunter, C. S. Corker, D. T. Baird, *Clin. Endocrinol.* **7**, 353 (1977).
18. S. Ohno, *J. Steroid Biochem.* **8**, 585 (1977).
19. F. P. Zemlan and N. T. Adler, *Horm. Behav.* **9**, 345 (1977).
20. B. R. Komisaruk and C. Diakow, *Endocrinology* **93**, 548 (1973).
21. A. R. Caggiula and R. Eibergen, *Comp. Physiol. Psychol* **3**, 414 (1969).
22. B. H. Shapiro, A. S. Goldman, H. F. Steinbeck, F. Neumann, *Experientia* **32**, 650 (1976).
23. F. A. Beach and M. G. Buehler, *Endocrinology* **100**, 197 (1977).
24. A. S. Goldman and B. H. Shapiro, *J. Steroid Biochem.* **8**, 31 (1977).
25. H. H. Feder, F. Naftolin, K. J. Ryan, *Endocrinology* **94**, 136 (1974).
26. R. E. Whalen, C. Battie, W. G. Luttge, *Behav. Biol.* **7**, 311 (1972).
27. J. M. Rosenfeld, J. D. Daley, S. Ohno, E. V. YoungLai, *Experientia* **33**, 1392 (1977).
28. For an abstract of the preliminary results reported here, see D. C. Levine, N. T. Adler, B. H. Shapiro, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 618 (1978). B.H.S. is a recipient of a PHS Career Development Award (HD-00260). This research was partially supported by grants from the Biomedical Research Support Program (5 S07 RR 5464) and NIH grant HD 04522.

24 January 1980

Asbestos as a Cofactor in Carcinogenesis Among Nickel-Processing Workers

Abstract. *It has been widely assumed, especially in the absence of other explanations, that lung cancer and nasal sinus cancers observed among nickel smelter workers are the result of the carcinogenicity of nickel. Although there may be such influence, supplementary hypotheses are also possible. The nickeliferous ores from at least one major smelter in New Caledonia (excess numbers of cancers have been found in these smelter workers) are derived from serpentinized host rocks which contain large amounts of chrysotile asbestos. Analysis indicates that nickel ores from this area are heavily contaminated by these fibers. The deposits are mined for their nickel content, but workers may be exposed to the asbestos fibers contained in the deposits. Hygiene measures limited to the avoidance of nickel may be inadequate under such circumstances and should be reevaluated so as to prevent the inhalation of asbestos-containing dusts.*

A recent report concerning the incidence of carcinoma of the lung among nickel workers of New Caledonia (*1*) attracted the attention of investigators in the field of metal carcinogenesis to the effects of nickel compounds. These effects have been well documented and reported for both worker populations and laboratory animals (*2*).

Uncertainties exist concerning the exposure of workers to nickel and the appearance of malignant disease (*3*). The distribution of respiratory tract cancer in some instances has not followed a clear pattern of exposure to nickel compounds but was more closely correlated to "total dust" with specific carcinogens not identified (*4*). It has been difficult to explain varying observations in different mining areas, in different smelters, and in a number of countries. There have been other curious inconsistencies about nickel carcinogenesis. Perhaps most important, the excess numbers of lung and nasal sinus cancers have been almost exclusively found among employees of nickel smelters, and the hazard did not follow the metal from its refining to metalworking plants. General improvements in housekeeping and dust control in smelters, made before the risk was even suspected, without control of specific agents or processes, were found to sharply reduce or eliminate later incidence of cancer (*5*). Despite these measures, since there clearly has been increased cancer risk in nickel smelters, nickel has been considered the cause.

In August 1976 we were asked by the International Metalworkers Federation (*6*) for advice concerning what might be done about an increasing burden of cancer among workers belonging to its affiliated union in New Caledonia, employed at the large nickel-mining and -smelting operation at Nouméa. Some of our past studies have alerted us to the importance of the large geological literature on the complex mineralogical nature of metal ore deposits—for example, the contamination of Lake Superior with amphibole gangue minerals (*7*) and the identification of chrysotile asbestos in crushed stone used as road surfacings in Maryland (the materials were derived from serpentine rock formations) (*8*).

In March 1979 Dr. Julian Lee of Sydney, Australia, visited us and provided clinical evidence in support of the increased incidence of lung cancer in workers in the New Caledonia smelter (*1*); patients from Nouméa, flown to Sydney for treatment, had been operated on. Moreover, he heard reports from surgeons on Nouméa that pleural mesothelioma had occurred among some employees of the facility. This observation reminded us of the inclusion of a case of mesothelioma in a nickel worker in the review by McDonald and McDonald of

0036-8075/80/0718-0420$00.50/0

mesothelioma in Canada (9). The several lines of inquiry seemed to converge, with one of the possibilities being that the host rock of at least some of the nickel deposits might include one or another of the asbestos minerals (10). The occurrence of lung cancer, mesothelioma, and cancers of the upper respiratory tract would be consistent with this explanation (11) and with the fact that asbestos is virtually ubiquitous in serpentinized ultramafic rocks, one of the three common sites of nickel deposits.

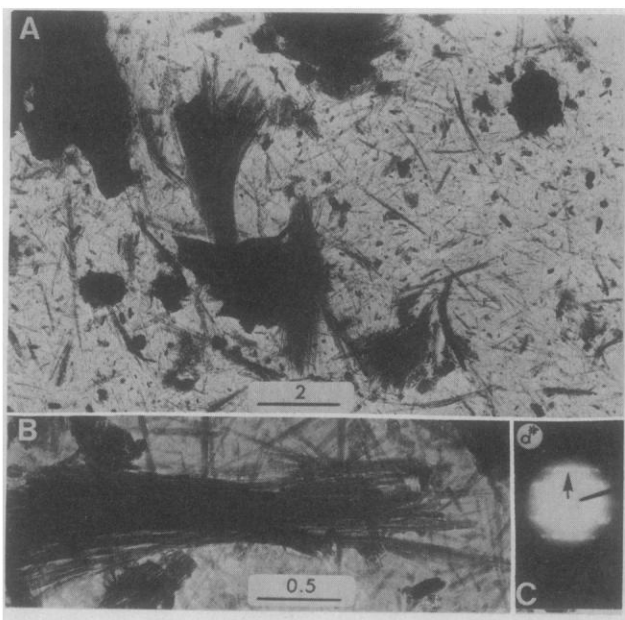
A review of the existing data suggested that the nickel deposits in New Caledonia were probably contaminated with asbestos. The primary ore here is mineralogically referred to as "garnierite" (12). Once a mineral name, this designation is now relegated to usage as a general field term for any green, fine-grained, alteration product of a nickel-containing silicate (13). Other nickel-bearing silicate phases occur admixed in lateritic ores containing "garnierites" (14). In New Caledonia, for example, serpentine and "garnierite" are considered to make up the ore body. These are admixed on every scale (10).

We have studied several "garnierite" specimens from the collection of the American Museum of Natural History (AMNH) in New York, one of which was from Nouméa (15). We were also able to obtain three specimens of ores that were being processed from worked pits on Nouméa (6). We examined the New Caledonian specimens and the pre-smelted ores by polarized light microscopy, continuous scan x-ray diffraction, transmission electron microscopy with selected area electron diffraction, and by means of an electron microprobe technique (an energy-dispersive x-ray spectrometry system as part of an analytical electron microscope).

Examination of the New Caledonian specimens by polarized light microscopy revealed that they consist of apple-green grains and cleavage fragments of varieties of pyroxene and olivine. The latter minerals were extensively altered, with atypical indices of refraction (IR). The "garnierite" grains never achieved extinction when examined between crossed Nicol prisms and possessed optical characteristics consistent with serpentine aggregates. The IR's measured on these grains ranged from 1.550 to about 1.570. It was not known if the aggregate grains contained fibers, plates on end, or other disoriented mineral particles. Examination of the New Caledonian ores revealed a number of other mineral phases, including plates with properties consistent with those of chlorite. No free fibers were observed in any specimens. The ore specimens contained similar particles and mineral fragments but fewer aggregate grains.

Specimens of the New Caledonian "garnierite" examined by continuous scan x-ray diffraction methods were found to consist primarily of two mineral phases. The strongest diffraction intensities came from a clay mineral with the major reflection centered near 14 Å. This peak was extremely broad, with its higher order reflections very difficult to accurately measure. The second major phase was consistent with a serpentine mixture. The major reflections occurred at 7.3, 4.5, 3.6, 2.5, and 1.5 Å. Because of marked line-broadening, it was not possible to distinguish the different serpentine minerals. Several very broad reflections were present at 7.5, 4.3, and 2.3 Å from the clay phase. The serpentine reflections were also observed in the three ore specimens. These tended to be better defined in form (sharper) with less pronounced line-broadening. The major phases in these materials appeared to be a serpentine mineral, with the clay content markedly decreased. A 9.4-Å reflection was resolved; this reflection suggests the presence of a talc-like mineral.

Examination of the AMNH specimen of New Caledonian "garnierite" and the Nouméan ores by transmission electron microscopy with a JEOL 100CX analytical electron microscope demonstrated the presence of chrysotile fiber in all the specimens examined (Figs. 1 and 2). The Nouméan "garnierite" was predomi-



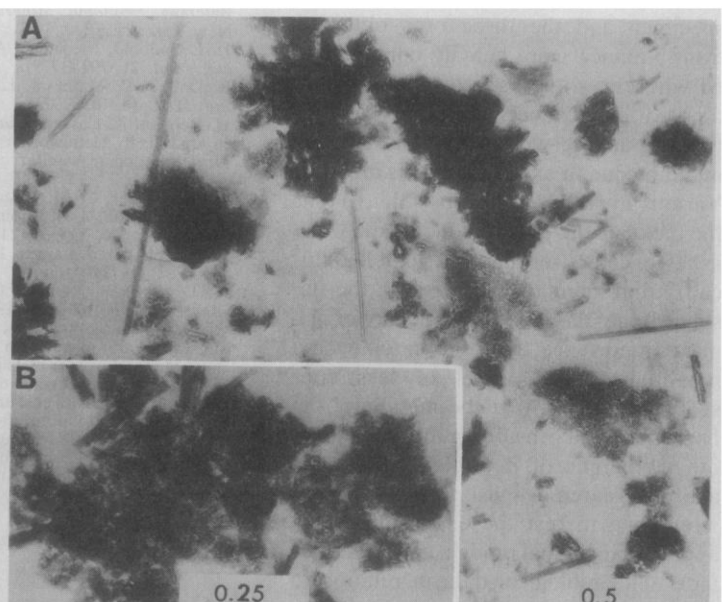


Fig. 1 (left). (A) Photomicrograph of a representative field of "garnierite" from Nouméa, New Caledonia. The specimen (AMNH 32584) is a mixture of serpentine minerals (predominantly nickeloan chrysotile), clay, and other silicates. Scales are in micrometers. (B) Photomicrograph showing the nature of the opened chrysotile asbestos bundles. Most of the chrysotile in the specimen may be easily separated into fibril units. (C) The SAED pattern obtained on the fiber bundle shown in (B). The arrow marks the a^* direction on the SAED pattern. Morphological characteristics and structure indicate that the mineral fiber is chrysotile. Fig. 2 (right). (A) Representative photomicrograph of a processed ore specimen from New Caledonia, prior to smelting. The ore is primarily serpentine minerals, including chrysotile, and other sheet silicates. Scales are in micrometers. (B) Photomicrograph showing the intimate admixed nature of the clays, sheet serpentine minerals, and chrysotile. The fiber content of the ore is less than in the "garnierite" standard. Unless examined with magnifications obtained with the transmission electron microscope, the fine chrysotile fibrils (about 350 Å in diameter) would not be seen.

nantly chrysotile fiber and sheet silicates (Fig. 1), whereas the Nouméan ores appeared to be mixtures of clay (montmorillonite), other sheet silicates including the serpentine phases, and especially large amounts of chrysotile fiber (Fig. 2). The sheet silicates consisted of aggregates, often intergrown and contiguous with chrysotile fibrils. Selected-area electron diffraction (SAED) patterns obtained on single clay grains displayed Debye-Scherrer rings, which clearly indicated the aggregate nature of these particles on the submicron-sized scale. Chrysotile in the ore specimens consisted of fibers and fibrils with shapes ranging from equant (clumps) to the more characteristic asbestiform fibers. The SAED patterns obtained on chrysotile tended to range from single, well-defined reflections to Debye-Scherrer rings. The most common pattern consisted of both forms (Fig. 1C). Chemical analyses of chrysotile fibers from "garnierite" indicate a marked increase in the integrated peak count ratio of silicon to magnesium from 1.73 ± 0.05 measured on non-nickeliferous chrysotile specimens from Globe, Arizona, to 5.41 ± 0.05 obtained on most Nouméan chrysotile fibers, to 7.84 ± 0.05 obtained on some low-magnesium chrysotile fibers. The increasing peak count ratio reflected magnesium depletion and was accompanied by a corresponding increase in the nickel x-ray emission.

The New Caledonian "garnierite" specimen and the three ores obtained from Nouméa were heavily contaminated with chrysotile asbestos. The occurrence of lung cancer, and possibly other tumors, among workers at this smelter might be due to exposure to this mineral. Various hypotheses are possible: (i) that the nickel is the essential factor, with the asbestos fibers serving as a vehicle to localize and maintain the metal in the tissue; (ii) that the asbestos fibers are of primary importance, with the carcinogenic potential of the nickel of less consequence; or (iii) that the metal and the fiber interact, both adding to the risk, another example of multiple factor interaction in carcinogenesis (*16*). It will be interesting to test these possibilities in appropriate animal models and to reevaluate epidemiological data in relation to exposures in various smelters. Also, mineralogical analysis of lung tissues from miners in these locations will provide needed information.

Examination of geological data from a number of "garnierite"-bearing nickel deposits around the world and of one in the United States (Riddle, Oregon), and preliminary analyses of other "garnierite" specimens, suggest that asbestos contamination by either chrysotile or amphibole fibers may be common. Asbestos contamination of chromite ores should also be studied in relation to lung cancers in chromate workers (*17*). Other ores, for example, platinum and related metals, are derived from or are associated with serpentinized host rocks. Here too a mineral-biological interface may be present.

ARTHUR M. LANGER
ARTHUR N. ROHL
IRVING J. SELIKOFF
Environmental Sciences Laboratory, Mount Sinai School of Medicine, City University of New York, New York 10029
GEORGE E. HARLOW
MARTIN PRINZ
Department of Mineral Sciences, American Museum of Natural History, New York 10024

References and Notes

1. R. Lessard, B. Reed, J. Maheux, *J. Occup. Med.* **20**, 815 (1978).
2. See extensive bibliographies in F. W. Sunderman, Jr., chairman, *Medical and Biological Effects on Environmental Pollutants: Nickel* (National Research Council, Washington, D.C., 1975) and in (*1*).
3. One of the striking features remaining unexplained is the lack of increased cancer risk in human populations employed in secondary industries which use these materials [F. W. Sunderman, Jr., *Fed. Proc.* **37**, 40 (1978)]. In the Soviet Union, lung and gastric cancers occur in excess of the anticipated numbers. The Soviet nickel deposit is located in the Ural Mountains, in the same geological area as the chrysotile operations. Both the chrysotile and nickel ores occur in serpentinized ultramafic bodies [see A. M. Bateman, *Economic Mineral Deposits* (Wiley, New York, 1959)].
4. J. H. Godbold, Jr., and E. A. Tompkins, *J. Occup. Med.* **21**, 799 (1979).
5. R. Doll, J. D. Mathews, L. G. Morgan, *Br. J. Ind. Med.* **34**, 102 (1977).
6. H. Rebhan, K. Casserini, and R. Ahlberg of the International Metalworkers Federation made New Caledonian smelter ore specimens available to us.
7. A. M. Langer, C. M. Maggiore, W. J. Nicholson, A. N. Rohl, I. B. Rubin, I. J. Selikoff, *Ann. N.Y. Acad. Sci.* **330**, 549 (1979).
8. A. N. Rohl, A. M. Langer, I. J. Selikoff, *Science* **198**, 1202 (1977).
9. A. D. McDonald and J. C. McDonald, *Can. Med. Assoc. J.* **109**, 359 (1973).
10. Based on charge and ionic size, nickel readily substitutes for magnesium and iron in silicate mineral structures. It is dispersed in the silicate minerals of the upper mantle. The New Caledonian nickel deposit, of Tertiary age, occurs in serpentinized peridotite, the host rock probably derived from mantle sources. Although the nickel concentration is only about some 2000 parts per million in the parent ultramafic rocks, the process of laterization, under tropical conditions, has produced an in situ concentration of iron and nickel oxide-hydroxide complexes. These pockets are numerous and grade as a continuum into the encapsulating serpentinized peridotite. Residues of weathered serpentinite occur throughout the laterite, with admixtures on the microscopic scale being common. The nickel concentration in the laterite may approach several percent. The serpentine in this deposit has not been studied in detail. The nickel ore is called "garnierite," which was considered a mineral species with the chemical formula $(MgNi)_3Si_2O_5(OH)_4$. A number of other hydrous nickel-magnesium oxides and silicates occur throughout this deposit [J. Avias, in *19th International Geological Congress* (Comptes Rendus, Algiers, 1954), vol. 12, p. 271]. The serpentine on New Caledonia is known to contain both chrysotile and traces of asbestiform tremolite [see the mineralogical description of the deposit in J. W. Montoya and G. S. Baur, *Am. Mineral.* **48**, 1227 (1963)]. This geological picture fits well the three modes of origin for nickel deposits: primary sulfides, nickeliferous silicates, and laterites-serpentines [see G. C. Ware, *U.S. Bur. Mines Bull.* **630**, 607 (1965)].
11. I. J. Selikoff and D. H. K. Lee, *Asbestos and Disease* (Academic Press, New York, 1978). For information on the incidence of such disease, see I. J. Selikoff, E. C. Hammond, H. Seidman, *Ann. N.Y. Acad. Sci.* **330**, 91 (1979).
12. The history of "garnierite" on New Caledonia is detailed in Montoya and Baur (*10*), with mineral descriptions from A. La Croix (1942), E. de Chetelat (1947), and S. Caillere and M. S. Henin (1960) cited.
13. The mineral "garnierite" was originally described as a nickel—magnesium silicate. Later it was observed to consist of many admixed mineral phases, so that it has become a generic term, like asbestos [see W. T. Pecora, S. W. Hobbs, K. J. Murata, *Econ. Geol.* **44**, 13 (1949); G. T. Faust, *Am. Mineral.* **51**, 279 (1966); H. Hayashi and T. Nishiyama, *J. Jpn. Assoc. Min. Pet. Econ. Geol.* **71**, 17 (1976)].
14. Past characterization of "garnierite" and nickeloan phases was based primarily on analytical measurements carried out on bulk materials. Therefore, mineral phases within mixtures escaped identification. Observed in "garnierite" deposits are the following: nickeloan talc, willemseite; nickeloan "serpentine," nepouite; nickeloan chlorite, schushardite, and nimite; nickeloan montmorillonite, pimelite; and nickeloan quartz, chrysoprase. Many other terms are in the mineralogical literature but have been discredited because the deposits have been found to be mixtures of nickel silicates; for example, "garnierite," noumeite, and nepouite, all found in New Caledonia, are observed to consist of mixtures of pimelite and serpentine phases [see references in (*8*), S. A. DeWaal, *Am. Mineral.* **55**, 31 (1970); *ibid.*, p. 18].
15. American Museum of Natural History specimen 31584, "garnierite," Nouméa, New Caledonia.
16. I. J. Selikoff and E. C. Hammond, in *Persons at High Risk of Cancer*, J. F. Fraumeni, Ed. (Academic Press, New York, 1975), pp. 467–483.
17. *Health of Workers in Chromate Producing Industry* (Publication 192, U.S. Public Health Service, Washington, D.C., 1953); Committee on Biological Effects of Atmospheric Pollutants, *Chromium* (National Academy of Sciences, Washington, D.C., 1974).
18. This investigation was supported at the Environmental Sciences Laboratory, Mount Sinai School of Medicine of the City University of New York, by the National Institute of Environmental Health Sciences under center grant ES00928 and American Cancer Society grant R-38.

4 April 1980

Pit Connections and Translocation in Red Algae

Wetherbee (*1*) described pit plugs between carposporophyte cells of the red alga *Polysiphonia novae-angliae* and suggested that the pit plugs are structurally specialized for nutrient translocation. His hypothesis is based on interpretations of plug ultrastructure; no direct evidence of translocation was presented. The structural features Wetherbee described involve plug shape, plug core density, and most importantly, the presence or absence of a plug cap membrane (membrane between plug core and cytoplasm of adjacent cells).

0036-8075/80/0718-0422$00.50/0