

Silica and the Immune System

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Abstract. This article collects the evidence that shows that the biological reactions to Silica are due to the stimulation of the Immune System. Both Innate and Adaptive Immunity are involved. The following sets of events take place sequentially: (1) Silica is recognized as a PAMP (pathogen-associated molecular pattern) by the Receptors of Innate Immunity; (2) This causes the stimulation first and then the death of the key cells of Innate Immunity (the macrophages); (3) While stimulated, macrophages produce cytokines (IL-1 and TNF) that stimulate fibroblasts; (4) The same and possibly other cytokines produced by silica-activated macrophages induce the maturation of dendritic cells, which are the connecting elements between the Innate and the Adaptive (lymphoid) Immune Systems; (5) It follows a polyclonal activation of the Adaptive Immunity; (6) The end result is the formation of fibro-hyaline tissue. In view of the double involvement of the Innate and the Adaptive Immune Systems and their cooperation in the stimulation of fibrosis, Silicosis can be considered as a “Collagen” Disease, related to other diseases of that group like Rheumatoid Arthritis, Lupus erythematosus and Scleroderma. Not surprisingly the incidence of these Diseases has been shown to be significantly increased in human exposed to Silica.

Key words: silica, innate immunity, collagen diseases

Introduction

More than 40 years ago Vigliani and Pernis reported extensive serological and cellular evidence of activation of the Immune System in human and in experimental Silicosis (1). They also underscored the reciprocal potentiation of Silicosis and some “Collagen” diseases like Scleroderma and Rheumatoid Arthritis. Their conclusion was that, in absence of any evidence of specific immune reactions to Silica, the immunological reactions that appeared to play a role in the pathogenesis of Silicosis, were non specific and rather akin to the generalized stimulation of the Immune System as observed under the influence of some bacterial products like the endotoxins of Gram-negative microorganisms. In the meantime, and particularly in the last two decades, it has become clear that the Immune System consists of two parts, which are interconnected. One part is responsible for the specific

cellular and humoral responses to antigens (the adaptive immune responses) and another, more ancient in phylogeny, that is mainly involved in the immediate resistance to invasion by infectious agents, which has been called the “Innate” Immune System.

On the basis of this new knowledge, it is now possible to state that the pathogenesis of Silicosis is entirely immunological and that it involves initially a stimulation by Silica of the Innate immune System, followed later by a polyclonal activation of the lymphoid, adaptive, moiety of Immunity.

Receptors and Cells of the Innate Immune System

The Receptors of Innate Immunity are very different in origin, structure, and function, from those of the Adaptive Immune System. They are relatively limited in diversity, they have been shaped by natural selection and are ubiquitously expressed in the cells of

the Innate Immune System. On the other hand, the Receptors of Adaptive Immunity have a diversity of several millions, generated by random re-arrangements in the genome, they are selected by somatic selection and are clonally expressed by the cells of the adaptive System (T and B lymphocytes).

The basic function of discriminating between self and non-self ligands is performed by the lymphocyte receptors by selection in the body (Tolerance versus Immunity), whereas the receptors of Innate Immunity have been selected by phylogeny to react with general molecular patterns associated with pathogens (PAMP) (1) and to avoid reaction with self.

It appears from what we shall discuss henceforth, that Silica is recognized as a PAMP by the Innate Immune System. This is likely to be the consequence of the fact that the surface of crystalline Silica is covered by Hydroxyl groups attached to Silicon atoms (3). These groups (Si-OH or silanol groups), together with firmly bound water may mimic the PAMP atomic arrangements of carbohydrates of bacterial or fungal cell walls. Table 1 gives a summary list of the Innate Immunity Receptors in Vertebrates.

Table 1. Innate immunity receptors in vertebrates

Soluble Molecules

Collectins: Mannose-binding Protein (MBL)

Surfactant Protein-A

Surfactant Protein-B

Conglutinin

CL-43

Complement: Recognition Components: C1, C2, C3, C4

Attack Components: C5, C6, C7, C8, C9

Membrane-bound Molecules

Mannose Receptor

Scavenger Receptors (SR-AI, SR-AII, MARCO, CD-36, SR-CI)

LPS Receptor and CD14

Toll-like Receptors (TLR-2, TLR-3, TLR-4, TLR-5, TLR-7, TLR-9)

Cytosol Molecules

NOD1, NOD2

Silica and the Soluble Molecules of Innate Immunity

In 1959 Pernis, Gambini and Levis (4) reported the presence in the serum of rabbits treated with Silica of a heat-labile Zymosan-agglutinating Factor. The properties of this factor (provisionally called Quartz Factor) were described in detail and they appear to be identical to those of the Mannose-binding Lectin (MBL) that was discovered much later and shown to be a "pluripotent molecule of the innate immune System" (5). It is reasonable to assume that the drastic increase of the bactericidal property of the serum of Silica-treated rabbits observed in separate experiments (6) was due to an increase of MBL.

In research still going on to day, S. Kuipers, H. Van Dijk, A. Cattaneo and B. Pernis (unpublished) have shown that Silica is a potent activator of MBL, actually more potent than many bacteria and viruses. Silica can, therefore, activate Complement through the lectin-dependent pathway. Activation of Complement by Silica through the alternate pathway has been shown by Callis et al. (7) and the binding of C'3 on Silica particles present in thin sections of human silicotic nodules exposed to fresh guinea-pig serum was detected by immunofluorescence by B. Pernis in 1963 (8).

The capacity of Silica to react with different soluble molecules of the Innate Immune System is therefore proven. However, it is not proven that these reactions are essential in the pathogenesis of the fibrotic process leading to the production of silicotic tissue. Callis e.a. (see n.7) found no difference between the fibrosis induced by Silica in the lungs of mice lacking C'5 (B10.D2/Sn mice) and that produced by the same treatment in the lungs of control C5-sufficient animals. It is therefore likely that the interactions between Silica and the Innate Immune System that lead to the silicotic fibrosis involve the Cells of this System.

Silica and Macrophages

Macrophages are the central cells of the Innate Immune System. They perform both effector functions, killing and clearing bacteria, fungi and viruses, and regulatory functions through the production of various cytokines (TNF, IL-1, Il-6, Il-8, Il-12) that

regulate the proliferation and differentiation of the cells of the Innate Immune System as well as those of the Adaptive System and the fibroblasts. Silica has a double effect on the macrophages that actively phagocytose the particles: first it stimulates them, then, after some hours, it causes the death of an increasing portion of the cells.

We do not know the molecular details of the process that leads to stimulation, but it is likely that it involves both binding Receptors, like the scavenger receptor SR-A1 (9) or the mannose Receptor, and then Receptors that contribute to the transmission of the signal, like the TLR-2. A cooperation between different receptors at the level of the phagosome has been shown to be operative in the case of Macrophages that have ingested Zymosan (10) and we have noted in the preceding Section the possible analogies between Zymosan (particles of insoluble carbohydrates derived from the yeast cell wall) and Silica for what concerns the reactivity with the soluble receptors of the Innate Immune System. The signal pathway would then lead to the activation of NF- κ B and to the transcription and translation of the genes of the inflammatory cytokines (11). Amongst these most important is TNF since a very relevant publication by Piguet et al. (12) has shown that TNF is essential for the development of silica-induced pulmonary fibrosis. TNF would thus explain the link between the stimulation of the Innate Immune System by Silica and fibrosis, a process common to other "collagen" diseases, as we shall discuss later. But other cytokines are also released by the macrophages stimulated by Silica, among these is Interleukin-1 (13, 14), which would also stimulate T lymphocytes, thus establishing a link with the Adaptive Immune System. Furthermore, there is the possibility that cytokines secreted by the macrophages stimulated by Silica may feed back positively on the proliferation of the precursors of macrophages in the bone-marrow, precursors that are common to other cells of the Innate Immune System, like the Mast-cells and the immature Dendritic cells. After some hours of stimulation (6h-24h) the macrophages that have phagocytosed Silica particles die through a process of cytoplasmic swelling that has been classified as "oncosis," (15) and is quite different from the more common process of apoptosis.

The percentage of Macrophages that die after exposure to Silica *in vitro* vary with the concentration of Silica and, interestingly, also depend from the crystalline state being most intensive with Tridymite than with Quartz and being absent with amorphous Silica (16). The phenomenon of Silica-induced death appears to be specific for macrophages since it does not occur with other phagocytes like granulocytes (17) or phagocytic Amoeba (B. Pernis and C. Pericone, unpublished). We do not know the receptors and the signal pathways that lead to the process of Silica-induced death of macrophages and how this may differ from those involved in the stimulation, but I like to point out that similar events of macrophage death by "necrotic" oncosis also follow 6-24 hours after the phagocytosis of some bacteria (18, 19).

Cell death of macrophages that have phagocytosed Silica also occurs *in vivo*, so that with a relatively limited number of Silica particles multiple cycles of macrophage stimulation and death can occur, with progressive stimulation of the Innate Immune System, until ultimately the mineral particles are embedded in the thick hyaline collagen tissue of the nodules and so excluded from the contact with other macrophages.

Silica and Mast-cells

Mast-cells are tissue-dwelling immune effector cells that derive from bone-marrow precursors common with macrophages and dendritic cells. The role of Mast-cells in Innate Immunity has been well documented (20). Likewise there is evidence for a role of Mast-cells in the development of experimental (21) and human (22) "collagen" diseases.

In 1958, Pernis, Saffiotti and Tommasini (23) published a paper showing that Mast-cells were abundant in the pulmonary lesions of rats treated with intra-tracheal injections of Silica, and that many of these cells were degranulating. This participation of Mast-cells to the silicotic process started 1-2 months after the administration of Silica, and became progressively more intense together with the development of fibrosis.

Various cytokines are presumably involved in this interaction between Mast-cells and Silicosis, in particular TNF, which is known to be synthesized and secreted by activated Mast-cells (24). In any event the

presence of numerous Mast-cells in the tissues of experimental silicosis, and the morphological evidence of their activation, constitute yet another fact that shows participation of the Innate Immune System to the biological reactions to Silica.

Silica and Dendritic Cells

In Vertebrates, Dendritic Cells are at the hinge between the Innate and the Adaptive Immune Systems. Dendritic cells express all the cell-bound Receptors of Innate Immunity and they respond to pathogen-associated molecular patterns (PAMP) quite so well as macrophages. Upon stimulation the immature Dendritic cells become mature and become efficient antigen-presenting cells for T lymphocytes through the membrane expression of antigenic peptides bound to histocompatibility carriers and the simultaneous expression of co-stimulatory molecules. T-lymphocytes that have not encountered antigen before ("naive" T cells) cannot be stimulated except by antigens presented by mature Dendritic cells. In this way Dendritic Cells (DC) screen potential foreign molecules through reactivity with their receptors for PAMP, and hand over that information to T lymphocytes indicating the presence of potential antigens worthy of recognition by the clonal receptors (and the cells) of the Adaptive Immune System. Dendritic cells are derived from progenitors in the bone-marrow common to Macrophages and Mast-cells.

In vitro experiments to study the effect of Silica on DC have been performed by Bower et al. (25, 26). They have shown that immature DC (although they are actively phagocytic) are neither killed nor stimulated by Silica alone. On the other hand immature DC incubated overnight with Silica in the presence of Macrophages are induced to mature to cells that can efficiently cooperate with T lymphocytes. The molecules that are released by the Macrophages that have ingested Silica and that act to stimulate the DC maturation have not been completely identified, but they appear to include Il-1, GM-csF (granulocyte/macrophage colony stimulating Factor) and possibly other molecules in the range of 50 kD.

Perhaps the best, although indirect, evidence that supports a stimulatory action of Silica on DC *in vivo*,

comes from a work (1962) of Pernis and Paronetto (27), who observed a large (more than ten fold) increase in the production of antibodies to a T-dependent antigen (ovalbumin) in rabbits that had received Silica intravenously. The increase in antibody production developed slowly in these animals, and reached the higher levels three months after the administration of Silica. Comparable results were obtained in Silica-treated rats that received horse serum as a mixture of antigens. In rats an histological study was also performed that showed that the increase in antibody reactivity was parallel to the progressive enlargement of what was called at that time the reticulo-endothelial System (Macrophages and Dendritic cells) and of the areas that included T and B lymphocytes as well as immunoglobulin-containing Plasmacells.

The present re-interpretation of those findings is that repeated cycles of Silica ingestion by macrophages produced factors that induced the maturation of more and more DC so that the response of CD4 T lymphocytes to the antigen presented by these cells was correspondingly enhanced.

Silica and Lymphocytes

Lymphocytes, T and B, are the cells of the Adaptive Immune System. The original observation of Vigliani and Pernis (see n.1) showed evidence of proliferation of lymphocytes and maturation to plasma-cells in human and experimental Silicosis. Furthermore accumulations of immunoglobulines were observed in human silicotic nodules (28). However, studies on the effects of Silica on lymphocytes *in vitro* have reported either divergent effects (29) on B lymphocytes, or no effects at all (30) on T lymphocytes.

The apparent discrepancy is easily resolved by assuming that Silica particles have no direct effect on non-phagocytic cells like lymphocytes, but that the widespread stimulation of the Adaptive Immune System observed in human and in experimental Silicosis is the indirect consequence of the stimulation of the cells of the Innate Immune System (notably the Macrophages) and the consequent activation of the cells that connect the Innate with the Adaptive arms of Immunity (notably the Dendritic cells).

Silica and "Collagen" Diseases

It has long been realized that abnormalities of Collagen are not involved in the genesis of the so-called "Collagen" Diseases. Rather it has become increasingly apparent that various disturbances of the Immune System are behind the process of fibrosis that marks the diseases. Recently, attention has been given to the possibility that some of these immunological abnormalities may involve the System of Innate Immunity. Two alternative possibilities have emerged: one that stimulation of the Innate Immune System may be the first step to be followed by an involvement of the lymphoid, Adaptive Immunity, and the other that in some cases the reverse may happen.

As an example of the first possibility I shall quote the case of the activation by chromatin immune complexes of Toll-like receptor 9, which might then activate the DC and subsequently drive the adaptive immune response in systemic Lupus (SLE) (31, 32). In this instances the connection between the Innate and the Adaptive immune responses would be provided by the physiological link of the stimulation of the Dendritic cells. As an example of the second possibility, I may indicate the experimental rheumatoid arthritis in mice after transfer of an autoantibody to GPI (glucose-6-phosphate isomerase) (see n. 21). In this second example, the connection between the Innate and the Adaptive Immune System is reversed, and the link is provided by Complement.

It appears to me that Silicosis can be included in the Collagen Diseases of the first group, with a primary involvement of Innate Immunity followed, through the link of Dendritic cells, by the polyclonal activation of the system of Adaptive Immunity. At least in the case of Silicosis we may understand what might be some beneficial effects of the fibrotic reaction since the embedding of the stimulating Silica particle in the collagenous tissue may be the only way to stop (33) the continuing cycles of phagocytosis of the particles, followed by stimulation, and the death of the engulfing macrophages, which is the driving force of the whole process.

The identification of Silicosis as a Collagen Disease, with a clear immunological pathogenesis, is in good agreement with the fact that there is a significant

increase of the incidence of different Collagen Diseases in humans exposed to Silica.

Here are some recent reports of this association:

a) with Rheumatoid Arthritis: Klockars et al. (34), Steenland and Brown (35); b) with Systemic Lupus: Sanchez-Roman (36), Melhorn et al. (37), Parks, Conrad and Cooper (38); c) with Scleroderma: Hausteiner and Anderegg (39), Sluis-Cremer et al. (40), Gabay and Kahn (41), Koeger et al. (42). In the case of Scleroderma there is not only the frequency of association, but also the fact that the hyaline fibrous tissue of this disease is similar, in ultrastructure, to the hyaline of Silicosis (43).

Overview

Although much experimental work remains to be done, a comprehensive view of the biological reactions of vertebrates to Silica particles, and the pathogenesis of Silicosis in humans, is emerging. This is of an entirely immunological process that begins with the stimulation of the Innate Immune System that senses the surface of the Silica particles as if it were the surface of bacteria or fungi. This happens because somehow the chemical properties of this surface mimic those pathogen-associated molecular patterns (PAMP) that can react with one, or many, of the Receptors of the Innate Immune System. Subsequently, the activation of the Innate System is followed by a polyclonal hyperactivity of the lymphoid cells (Adaptive Immune System). The whole process reflects the effort of the vertebrate Immunity to eliminate what is sensed as a potential pathogen, but of course the Silica particles are indestructible and the only final solution is to bury them inside collagenous tissue. The immunological pathogenesis of Silicosis has much in common with that of the so-called "Collagen" Diseases, and it is not surprising that these are detected with increased frequency in humans exposed to Silica.

References

1. Vigliani EC, Pernis B. Immunological Aspects of Silicosis. *Adv Tuberc Res* 1963; 12: 230-79.
2. Janeway CA, Medzhitov R. Innate Immune Recognition. *Annu Rev Immunol* 2002; 20: 197-216.

3. Iler RK. The chemistry of silica: solubility, polymerization, colloid and surface properties, and biochemistry. Wiley, New York, 1979.
4. Pernis B, Gambini G, Levis F. A Heat-Labile Zymosan Agglutinating Factor Observed in Rabbits after Intravenous Injections of Quartz. *Proc Soc Exp Biol Med* 1959; 100: 49-53.
5. Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunology Today* 1996; 17: 532-40.
6. Pernis B, Vigliani EC. Recenti acquisizioni sulla patogenesi della Silicosi. *Med Lavoro* 1960; 51: 427-41.
7. Callis AH, Sohnle PG, Mandel GS, Mandel NS. The Role of Complement in Experimental Silicosis. *Environ Res* 1986; 40: 301-12.
8. Pernis B. Immunohistochemical Observations on the human silicotic Nodule. *Med Lavoro* 1963; 54: 354-63.
9. Platt N, Haworth R, da Silva R, Gordon S. Scavenger Receptors and Phagocytosis of Bacteria and Apoptotic Cells. *Advances in Cell and Molecular Biology of Membranes and Organelles* 1999; 5: 71-85.
10. Underhill DM, Ozinsky A, Hajjar A, et al. The Toll-like Receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* 1999; 401: 811-15.
11. Takeda K, Akira S. Toll-like Receptors in innate Immunity. *International Immunology* 2005; 17: 1-14.
12. Piguet PP, Collart MA, Grau GE, Sappino OP, Vassalli P. Requirement of tumor necrosis factor for the development of silica-induced pulmonary fibrosis. *Nature* 1990; 344: 245-7.
13. Schmidt JA, Oliver CN, Lepe-Zuniga J, Green I, Gery I. Silica-stimulated Monocytes Release Fibroblast Proliferation Factors Identical to Interleukin 1. *J Clin Invest* 1984; 73: 1462-72.
14. Pernis B, Vigliani EC. The Role of Macrophages and Immunocytes in the Pathogenesis of Pulmonary Diseases due to Mineral Dusts. *Ann J Indust Med* 1982; 3: 133-7.
15. Majno G, Ioris I. Apoptosis, Oncosis and Necrosis, an Overview of Cell Death. *Am J Pathol* 1995; 146: 3-15.
16. Marks J, Mason MA, Nagelschidt G. A Study of Dust Toxicity using a quantitative Tissue Culture Technique. *Brit J Industr Med* 1956; 13: 187-91.
17. Kessel RWI, Monaco L, Marchisio MA. The Specificity of the Cytotoxic Action of Silica - A Study in Vitro. *Brit J Exp Path* 1963; 44: 351-64.
18. Lührmann A, Sydor T, Fernandez-Mora E, Schulze-Luehrmann J, Takai S, Haas A. Necrotic Death of Rhodococcus equi-Infected Macrophages is Regulated by Virus-Associated Plasmids. *Infect Immun* 2004; 72: 853-62.
19. Guilloteau LA, Wallis TS, Gautier AV, MacIntyre S, Platt DJ, Lax AJ. The Salmonella Virulence Plasmid Enhances Salmonella-induced Lysis of Macrophages and Influences Inflammatory Responses. *Infect Immun* 1996; 64: 3385-93.
20. Boyce JA, Austen KF. The Role of Mast Cells in Innate Immunity. In *Innate Immunity*, eds. Ezekowitz R.A.B. and Hoffmann J.A. Humana Press, Totowa NJ. 2003.
21. Hong JI, Ohmura K, Mahmood A, et al. Arthritis Critically Dependent on Innate Immune System Players. *Immunity* 2002; 16: 157-68.
22. Olsson N, Ulfgren AK, Nilsson G. Demonstration of mast cell chemotactic activity in synovial fluid from rheumatoid patients. *Ann Rheum Dis* 2001; 60: 187-93.
23. Pernis B, Saffiotti U, Tommasini-Degna A. Il comportamento delle mastcellule polmonari nel corso della silicosi del ratto. *Med Lavoro* 1958; 49: 405-18.
24. Gordon JR, Galli SJ. Mast cells as a source of both preformed and inducible TNF/cachectin. *Nature* 1990; 346: 274-6.
25. Bowers WE, Ruhoff MS, Goodell EM, Stoltenborg JK. The Effect of Silica Treatment on Accessory Cell-Dependent Rat T Lymphocyte Proliferation. *Immunobiol* 1988; 176: 179-94.
26. Bowers WE, Ruhoff MS, Goodell EM. Conditioned Medium from Activated Rat Macrophages and Recombinant Factors, IL-1 beta and GM-CSF, Enhance the Accessory Activity of Dendritic Cells. *Immunobiol* 1990; 180: 362-84.
27. Pernis B, Paronetto F. Adjuvant Effect of Silica (Tridymite) on Antibody Production. *Proc Soc Exp Biol Med* 1962; 110: 390-2.
28. Ceppellini R, Pernis B. Presence of Plasma Globulins in the Hyaline Tissue in Cases of Silicosis. *Nature* 1958; 181: 55-6.
29. Moseley PL, Monick M, Hunninghake GW. Divergent effects of silica on lymphocyte proliferation and immunoglobulin production. *J Appl Physiol* 1988; 65: 350-7.
30. Hubbard AK. Role for T Lymphocytes in Silica-Induced Pulmonary Inflammation. *Lab Invest* 1989; 61: 46-52.
31. Boulè MW, Broughton C, Mackay F, Akira S, Marshak-Rothstein A, Rifkin IR. Toll-like Receptor 9-Dependent and-Independent Dendritic Cell Activation by Chromatin-Immunoglobulin G Complexes. *J Exp Med* 2004; 199: 1631-40.
32. Leadbetter EA, Rifkin IR, Holbaum AM, Beaudette BC, Schlomachik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002; 416: 603-7.
33. Vigliani EC, Pernis B. An Immunological Approach to Silicosis; in *Proceedings of Pneumoconiosis Conference Johannesburg 1959*. Edited by Orenstein AJ Churchill Ltd. London 1959.
34. Klockars M, Koskela RS, Jarvinen E, Kolari PJ, Rossi A. Silica exposure and rheumatoid arthritis: a follow up study of granite workers, 1940-81. *Br Med J* 1987; 294: 997-1000.
35. Steenland K, Brown D. Mortality Study of Gold Miners Exposed to Silica and Nonasbestiform Amphibole Minerals. An Update with 14 More Years of Follow-up. *Am J Ind Med* 1995; 27: 217-29.
36. Sanchez-Roman J, Wichmann I, Salaberri J, Vasela JM, Nunez-Roldan A. Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. *Ann Rheum Dis* 1993; 52: 534-8.
37. Melhorn CK, Dorner LK, Frank KH. Systemic lupus

- erithematosus after heavy exposure to quartz dust in uranium miners: clinical and serological characteristics. *Lupus* 1996; 5: 62-9.
38. Parks CG, Conrad K, Cooper GS. Occupational Exposure to Crystalline Silica and Autoimmune Disease. *Environ Health Perspect* 1999; 107: 793-802.
39. Hausteiner VF, Andereggs U. Silica Induced Scleroderma-Clinical and Experimental Aspects. *J Rheumatol* 1998; 25: 1917-26.
40. Sluis-Cremer GK, Hessel PA, Hnizdo E, Churchill AR, Zeiss EA. Silica, silicosis and progressive systemic sclerosis. *Br J Ind Med* 1985; 42: 838-43.
41. Gabay C, Kahn MF. les sclerodermies masculines: role de l'exposition professionnelle. *Schweiz Med Wschr* 1992; 118: 98-102.
42. Koeger AC, Lang T, Alcaix D, et al. Silica-associated connective tissue diseases. A study of 24 cases. *Medicine* 1995; 74: 221-37.
43. Pernis B, Bairati A, Frigerio G. La Ultrastruttura del tessuto fibrojalino della silicosi. *Med Lavoro* 1956; 47: 439-59.