HISTOCHEMICAL INVESTIGATION OF OXIDOREDUCTASES IN EXPERIMENTAL SILICOSIS

UDC 616.24-003.662-092.9-07:616.24-008.931:577.158)

N. T. Raikhlin and I. M. Shnaidman

Histochemistry Laboratory (Head—Candidate of Medical Sciences N. T. Raikhlin), Division of Pathological Anatomy of Human Tumors (Head—Active Member of the Academy of Medical Sciences of the USSR, Professor N. A. Kraevskii), Institute of Experimental and Clinical Oncology (Director—Active Member of the Academy of Medical Sciences of the USSR, Professor N. N. Blokhin), Academy of Medical Sciences of the USSR; Clinical and Roentgenological Division (Head—Docent A. I. Nesis), Rearch Institute of Work Hygiene and Occupational Diseases (Director—Candidate of Medical Sciences Z. K. Tulegenov), Karaganda
(Presented by Active Member of the Academy of Medical Sciences of the USSR, N. A. Kraevskii)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 10, pp. 112-117, October, 1965
Original article submitted April 10, 1964

The object of this investigation was to study some of the principles governing the formation of silicotic connective tissue, under the influence of silica. This approach to the problem was predetermined by investigations [4-6] conducted by one of the authors (N. T. Raikhlin) which revealed that silicotic connective tissue has certain special features. In the present investigation histochemical methods were used to study the activity of several oxidoreductases and the histochemical peculiarities of the ground substance of the connective tissue in various stages of the development of experimental silicosis.

EXPERIMENTAL

Silicosis was produced in albino rats by the intratracheal injection of a suspension of quartz dust in physiological saline. Altogether 66 animals were used in the experiments and sacrificed at different times (from 1 day to 12 months after injection).

Dehydrogenases and diaphorases were demonstrated in cryostatic sections by means of nitro-tetrazolium blue salt (nitro-BT), using the technique described in Pearse's textbook [2]. The dehydrogenases of lactic, malic, isocitric, glutamic, and succinic acids and of α-glycerophosphate and glucose-6-phosphate, nicotinamide-adenine dinucleotide (NAD) diaphorase, and nicotinamide-adenine dinucleotide phosphate (NADP) diaphorase were studied. Cytochrome oxidase was demonstrated by Burstone's method [7].

Paraffin-wax sections were stained with hematoxylin-eosin, picrofuchsin, resorcin-fuchsin, and impregnated by Gomori's method. Acid mucopolysaccharides were demonstrated by the Hale—Miller method and also by toluidine blue at different pH values (some sections received preliminary methylation, demethylation, and sulfatation), and neutral mucopolysaccharides by the PAS reaction. Glycogen was investigated by Shabadash's method, RNA by Brachet's method, and DNA by Feulgen's method. Neutral fat and phospholipids were demonstrated by Sudan red and black. The method of specific destruction of corresponding components of the tissue by amylase, bacterial and testicular hyaluronidase, ribonuclease, and collagenase, with subsequent counterstaining, was used in this investigation.

DISCUSSION OF RESULTS

On the 1st-3rd day after injection of silica dust, at the places where it was deposited (the interalveolar septa, the perivascular and peribronchial spaces) proliferation of young, undifferentiated connective-tissue cells took place. These cells were characterized by low dehydrogenase, diaphorase, and cytochrome oxidase activity. The histiocytes proliferating in the interalveolar septa gave a well marked reaction for the investigated enzymes.

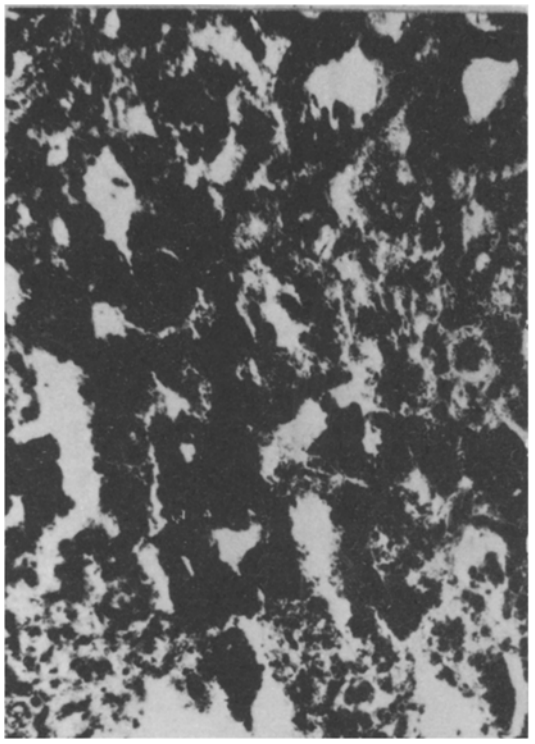

Fig. 1. High α-glycerophosphate dehydrogenase activity in the cells of the silicotic nodules 21 days after injection of silica dust into rats. Reaction with nitro-BT. 36 ×.

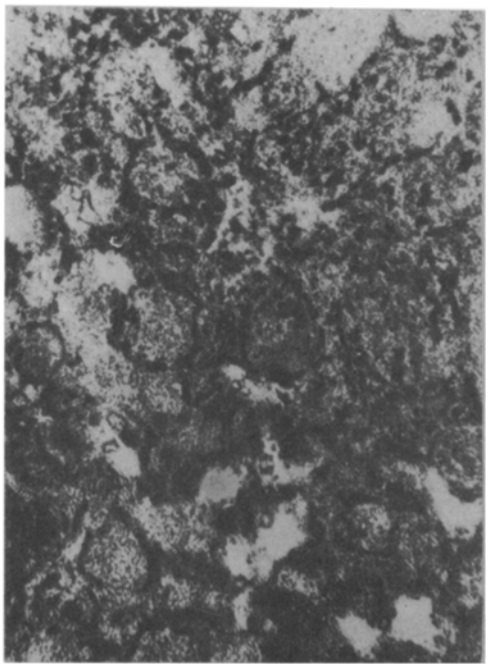

Fig. 2. Different degrees of α-glycerophosphate dehydrogenase activity in cells of different silicotic nodules 56 days after injection of silica dust into rats. Reaction with nitro-BT. 36 ×.

On the 7th day after injection of dust, large areas of cellular proliferation were seen to be developing in the lung tissue, consisting of immature cells and of young and adult fibroblasts. The young and mature fibroblasts were characterized by high NAD-diaphorase activity and also by high activity of the dehydrogenases of lactic acid, α-glycerophosphate, and malic acid, as revealed by the deposition of numerous diformazan granules in the cytoplasm of the cells. The activity of the dehydrogenases of isocitric, glutamic, and succinic acids in these cells was somewhat weaker, and that of the glucose-6-phosphate dehydrogenase and of NADP—diaphorase was much lower. Investigation of the cytochrome oxidase in the cytoplasm of the young and adult fibroblasts revealed numerous granules of indonaphthol violet.

The immature connective-tissue cells situated more at the periphery showed less enzymic activity. Their cytoplasm contained much RNA and many glycogen granules. As these cells were transformed into fibroblasts, their RNA content diminished, and hardly any was found in the fibrocytes. The number of glycogen granules in the fibrocytes also diminished. The DNA content in the connective-tissue cells remained constant during their differentiation.

At this time (7 days after administration of the dust) thin argyrophilic fibers began to appear in the ground substance of the newly formed silicotic connective tissue, and their number increased with time. Silver impregnation after oxidation with potassium periodate led, as a rule, to intensification of the argyrophilia on account of carbohydrate components.

On the 14th-21st days, numerous silicotic nodules had formed in the lungs as a result of the accumulation of proliferating cells. These nodules consisted mainly of adult cells, principally fibrocytes with hyperchromic nuclei, and fibroblasts. Younger cells were present at the periphery of the nodules. The activity of all the investigated oxidoreductases in the cells of the silicotic nodules was maximal at this period (Fig. 1). At the end of the 2nd week solitary collagen fibers, staining by the PAS reaction, appeared in the nodules. After incubation of the sections in a solution of collagenase for 24 h, the collagen fibers of the silicotic nodules were stained pink with picrofuchsin.

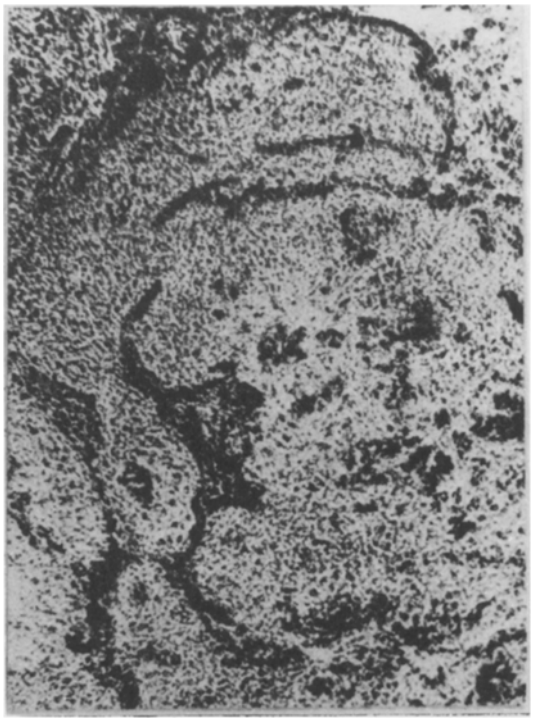

Fig. 3. Considerable lowering of α-glycerophosphate dehyrogenase activity in the cells of the silicotic nodules 7 months after injection of silica dust into rats. Reaction with nitro-BT. 36 ×.

On the 35th day after injection of dust the ordinary histological examination revealed numerous mature silicotic nodules in the lungs. The cells of most of the silicotic nodules continued to show high enzymic activity, although in some nodules its level had begun to fall. The number of collagen fibers in the silicotic nodules continued to increase, as also did the intensity of their staining by the PAS reaction. After incubation of the sections in a solution of collagenase the collagen fibers of the silicotic connective tissue stained pale pink and yellow with picrofuchsin. The action of collagenase on the collagen fibers of the lungs of the control animals did not alter their staining properties.

Staining the sections with toluidine blue at pH 7.6 and 6.0 revealed weak metachromasia of the ground substance of the silicotic nodules; at pH 4.0 this was rarely seen, and at pH 2.0 it was completely absent. After treatment of the sections with testicular and bacterial hyaluronidase the metachromasia of the silicotic nodules disappeared. Methylation of the sections led to disappearance of the metachromasia, and as a rule it did not return fully after demethylation. The metachromasia of the ground substance was not increased by preliminary sulfatation of the sections. The Hale–Muller reaction revealed no acid mucopolysaccharides in the ground substance and the fibrous structures of the silicotic connective tissue.

On the 56th day after administration of dust many silicotic nodules rich in cells were observed in the lungs. The activity of the oxidoreductases had fallen sharply in the cells of most of these nodules. Solitary polymorphic diformazan granules were seen in the cytoplasm of the fibrocytes and fibroblasts. As a rule the highly active cells were situated at the periphery. At the same period small silicotic nodules whose cells showed marked activity were seen, together with silicotic nodules with cells whose enzymic activity was intermediate between these two extreme forms (Fig. 2).

Impregnation revealed a dense network of argyrophilic fibers in the nodules. The collagen fibers reached a high degree of development and gave a strong PAS reaction, resistant to the action of α-amylase and of testicular and bacterial hyaluronidase.

After $3\frac{1}{2}$–9 months, separate and confluent silicotic nodes could be seen in the lungs, in which coarse argyrophilic fibers, brown in color, were revealed by impregnation. The concentrically arranged thick collagen fibers gave a strong PAS reaction and showed weak metachromasia. The cells of the silicotic nodes were fibrocytes in whose cytoplasm little or no RNA or glycogen could be detected. On staining with Sudan red and black, no lipids or phospholipids were seen in these cells.

The enzyme activity in the cells of the silicotic nodes continued to fall (Fig. 3); polymorphic diformazan granules appeared in the cells. As at the preceding time, a certain number of nodules of different degrees of maturity and, correspondingly, with different levels of activity, were seen in the sections.

One year after injection of dust, separate nodes and continuous airless zones formed by the confluence of large silicotic nodes could be seen in the lungs. The collagen fibers in the nodes showed hyalinosis in some places. The behavior of the collagen fibers towards collagenase and staining by the PAS reaction and toluidine blue remained visibly unchanged. The number of cells in the confluent fibrous foci was considerably smaller. In the cells which were left the enzyme activity fell sharply and many cells showed no enzyme activity whatever.

Analysis of the results shows that the activity of the investigated enzymes in the cells differed sharply at stages of development of the silicotic process, and consequently, so also did the functional activity of the cells. Four main stages of development of this process may be distinguished.

The first stage covers the first few days after administration of dust and is characterized by low enzymic activity in the immature connective-tissue cells. At this period, consequently, the conditions are unfavorable for the processes of glycolysis, respiration, synthesis, and so on.

The second stage (1-3 weeks) is characterized by a marked increase in the enzymic activity of the proliferating fibroblasts and fibrocytes forming the silicotic nodules. At this period the cells exhibit high functional activity and they present favorable conditions for energy metabolism and plastic processes.

The third stage (5 weeks) corresponds to the initial lowering of the enzymic activity in the cells of the separate nodules.

The fourth stage (8 weeks and later) is critical, for it is the time when an intensive fall in the activity of the dehydrogenases, diaphorases, and cytochrome oxidase begins to take place.

In the 3rd, and especially in the 4th stages, the functional activity of the cells falls progressively, and their ability to carry out respiration or glycolysis, and also their ability to perform plastic metabolism are sharply depressed.

It follows from these findings and from data reported in the literature [9, 10, 12] that the fall in the activity of the respiratory enzymes may be attributed to injury to the mitochondria. This hypothesis receives support, in particular, from the highly polymorphic character of the deposited formazan granules, which according to abundant evidence [2, 3, 8, 11, 14], is a highly sensitive indicator of changes in the mitochondria.

This histochemical investigation of the intercellular silicotic connective tissue shows that, as a result of inadequate accumulation of muco- and glycoproteins and of acid mucopolysaccharides, which are either present in very small amounts or are depolymerized, in the silicotic nodules a pathological connective-tissue is formed. Comparison of the histochemical features of the development of the intercellular connective tissue with the changes in the enzymic activity of the cells shows that the fall in the activity of the oxidoreductases coincides with the period of intensive collagen formation and, consequently, the maturation of the silicotic connective tissue takes place in conditions of abnormal metabolism of the connective-tissue cells. It is natural to suppose that the metabolic changes found in the cells to some extent may explain the special features of the intercellular silicotic connective tissue, previously described by several authors [1, 4-6, 13], and discussed in greater detail in the present paper, and comprising the formation of pathological, imperfect collagen fibers under the influence of silica.

LITERATURE CITED

1. P. P. Dvizhkov, M. S. Tolgskaya, and G. P. Sokolova, Abstracts of Proceedings of a Conference on the Pneumoconioses at the Institute of Work Hygiene and Occupational Diseases [in Russian], Moscow (1963), p. 8.
2. A. G. E. Pearse, Histochemistry [Russian translation], Moscow (1962),
3. V. V. Portugalov, In the book: Plastic and Repair Processes [in Russian], Moscow (1959), p. 202.
4. N. T. Raikhlin, In the book: Histochemical Methods in Normal and Pathological Morphology [in Russian], Moscow (1958), p. 145.
5. N. T. Raikhlin, In the book: Proceedings of a Symposium on Connective Tissue [in Russian], Moscow (1960), p. 99.
6. A. I. Strukov and N. T. Raikhlin, Arch. Path., 71 (1961), p. 142.
7. M. Burstone, J. Histochem. Cytochem., 7 (1959), p. 112.
8. M. Hannibal and M. Nachlas, J. Histochem. Cytochem., 9 (1961), p. 603.
9. W. Kikuth, In the book: Die Staublungenerkrankungen. Darmstadt, 3 (1958), p. 353.
10. W. Klosterköter and H. Themann, Die Staublungenerkrankungen. Darmstadt, p. 373.
11. D. Scarpelli and E. Pearse, J. Histochem. Cytochem., 6 (1958), p. 369.
12. H. Schlipköter, Cited by W. Kikuth.
13. H. Schmidt-Mattiesen, Arch. path. Anat., 327 (1955), p. 419.
14. D. Williams and H. Whiteley, J.Histochem. Cytochem., 11 (1963), p. 89.