INTERACTION OF MINERAL FIBRES WITH EXTRACELLULAR MATRIX AND MESOTHELIUM AFTER INTRAPERITONEAL INJECTION IN RATS

J. FRIEMANN* • S. Gonzalez† • F. Pott‡ • K. Junker* • B. Voss* • K.-M. MÜLLER*

*Institut für Pathologie an den Berufsgenossen-schaftlichen Krankenanstalten "Bergmannsheil Bochum," FRG
†Pontificia Universidad Catolica De Chile. Dep. De Anat. Pathol. Santiago
‡Med. Inst. für Umwelthygiene an der Universität Düsseldorf, FRG

INTRODUCTION

Serosal tests have proven as appropriate methods for detecting the neoplastic potency and fibrogenicity of asbestos fibres and man-made mineral fibres.[4,7,15,24,28,29] The results have strongly supported the hypothesis, that besides the elongated shape of the fibrous particles (lengths and diameter) their dose, durability and possibly also their surface properties may be the cause of their pathogenic effects.[5,6,16,22] The correlation between fibre-induced fibrosis and the development of mesotheliomas had been discussed contradictory and not yet been fully understood.[8,9,14,18,19,23,25,26] The mainly commercially used kinds of asbestos crocidolite and chrysotile obviously possess different fibrotic and neoplastic potency in man.[1,2,3,12,13,17,20,21,27] Because of this fact our studies on rat omentum aimed first for the presentation of differences in the composition of the extracellular matrix components collagen types I and III, laminin and fibronectin in crocidolite and chrysotile-induced granulomatous lesions. In addition we have examined in which way these fibrous natural dusts with very different physico-chemical properties lead to malignant transformation of the mesothelium. We have wondered, if these mechanisms were the same, which account for the tumour-inducing effect of some man-made mineral fibres.

Material and Methods

Our investigations were carried out on omentums of altogether 64 female Sprague-Dawley rats which had been sacrificed under narcotization 8 hours to 15 months after intraperitoneal injection of 1.5 or 15 mg crocidolite (South Africa, like UICC reference sample but fibre lengths greater) and chrysotile B (UICC reference sample) either. The omentums were divided into several parts and their preparation and fixation in formaldehyde, cold phosphate-buffered (0,5 M, ph 7,4) 2,25% glutaraldehyde and 1,3% osmium tetroxide for the light microscopical and the electron microscopical examination were done in normal manner. Sections were stained with HE chromo-trope-aniline blue, Prussian blue or Toluidin blue and Uranyl acetate. Cryostat sections for the immunofluorescence microscopical investigations were incubated with specific antibodies against collagens types I and III, the multifunctional glycoprotein fibronectin and the basement membrane glycoprotein laminin or with non-immune-serum as described elsewhere.[11] We further have examined specimens of the omentums from long term carcinogenicity studies on natural and man-made mineral fibres which were already referred in detail.[24] In these intraperitoneal tests very low doses between 0.05 and 0.5 mg asbestos, for example, have led to tumour incidences of about 20 to 80%. Only those animals have been chosen for our light microscopical investigations, however, which had been sacrificed in a bad health condition not earlier than two years after the intraperitoneal injection of different fibrous dusts. They have macroscopically shown no tumour growth. Doses of all intraperitoneally applied dusts and the life-spans of the animals after injection are listed in Table I.

Results

Focal granulomatous lesions of the omentum have been found only in those animals, which had been intraperitoneally injected with more than 1 mg of either natural or man-made mineral fibres in 1 ml physiological saline solution. Already 3 days after crocidolite administration the fibres have accumulated within the area of the "milk spots" of the omentum. These are circumscribed deposits of cells belonging to the monocyte macrophage system especially in the area of vascular branching. The granulomatous foreign body reactions characteristically have had a large number of mononuclear macrophages and multinucleated giant cells not only within the first week but also in the end of the investigation period. The crocidolite fibres are spread over the entire area of the granulomatous lesions and they are deposited especially at the surface of multinucleated giant cells in form of larger accumulations (Figure 1B). Shorter fibres can also be seen under the electron microscope within the cytoplasm of macrophages. New collagen, especially type III, can be demonstrated with the immunofluorescence microscope already after 3 days. At that time you can electron microscopically identify mainly macrophages and some lymphocytes in perivascular and submesothelial position. Fibroblasts and myofibroblasts are nearly absent (Figure 2A). There was a steady increase of fibrillogenesis throughout the 6 months studied immunofluorescence microscopically and during this time the collagen fibres were distributed symmetrically between the macrophages all over the lesions (Figure 1A).

On the other hand in chrysotile-induced lesions collagen types I and III synthesis was limited on the periphery of the granulomas (Figure 1C). Different to earlier reports chrysotile fibre bundles could be shown in the center using light microscopy (phase contrast or differential interference contrast) even up to six months (Figure 1D).[28] They seem to possess collagenolytic activity. The cellular debris lying between them can only be detected with the transmission electron microscope (Figure 2B).

Table I
Intraperitoneally Applied Natural Mineral Fibres (a) and Man-made Mineral Fibres (b)

	Dosis i.p. (mg)	Microscopically investigated omentums (n)	Life-span after i.p. injection (months)
a)			
Crocidolite (S.Africa)	1-15	32	<1-15
Chrysotile	1-15	32	<1-15
	0,05	7	24-27
Actinolite	0,01	6	24-28
	0,05	8	25-28
	0,25	4	25-28
	0,25 + PVNO	7	24-26
Erionite, Oregon	2,00	3	28
Wollastonite	100	5	26-28
b)			
Glass fibres JM 104/475	5	6	27-28
Polypropylene fibres	50	3	28
Kevlar fibres	20	2	27

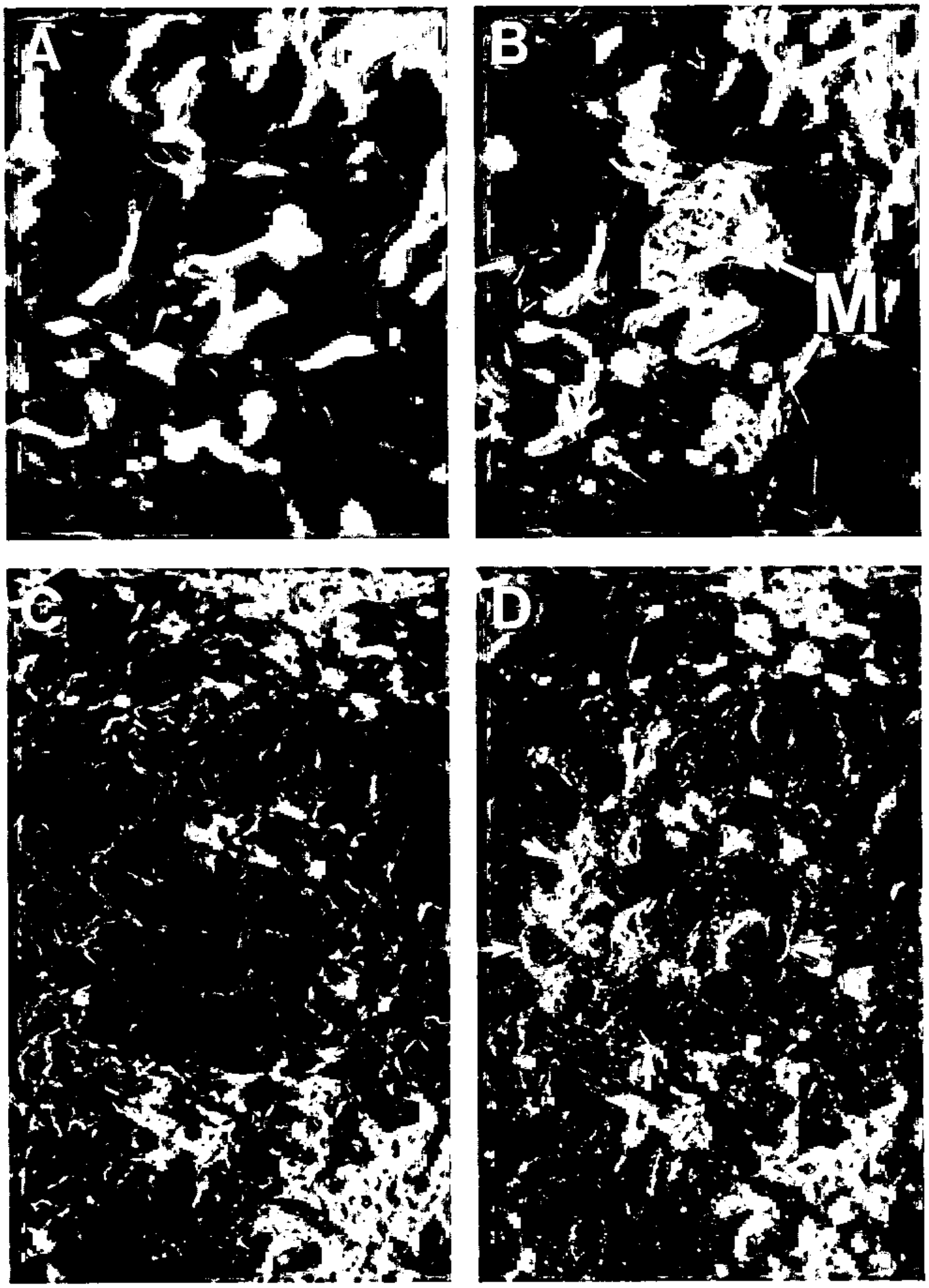

Figure 1. Asbestos induced fibrosis in the rat omentum 1–2 weeks after the i.p. injection of the dusts.
(A) and (B) Intact pericellular network of collagen type III in crocidolite containing granulation tissue.
((A) Immunofluorenscence microscopy 460 X;
(B) The same section as in (A) with light from the bottom and phase-contrast 460 X)
M: Multinuclear giant cells containing crocidolite fibres.
(C) and (D) Dissolution of collagen fibres (type III) and necrotic macrophages in the center of chrysotile containing granulomas.
((C) Immunofluorescence microscopy 290 X;
(D) the same section as in (C) with light from the bottom and phase-contrast 290 X)
➡ Chrysotile fibres

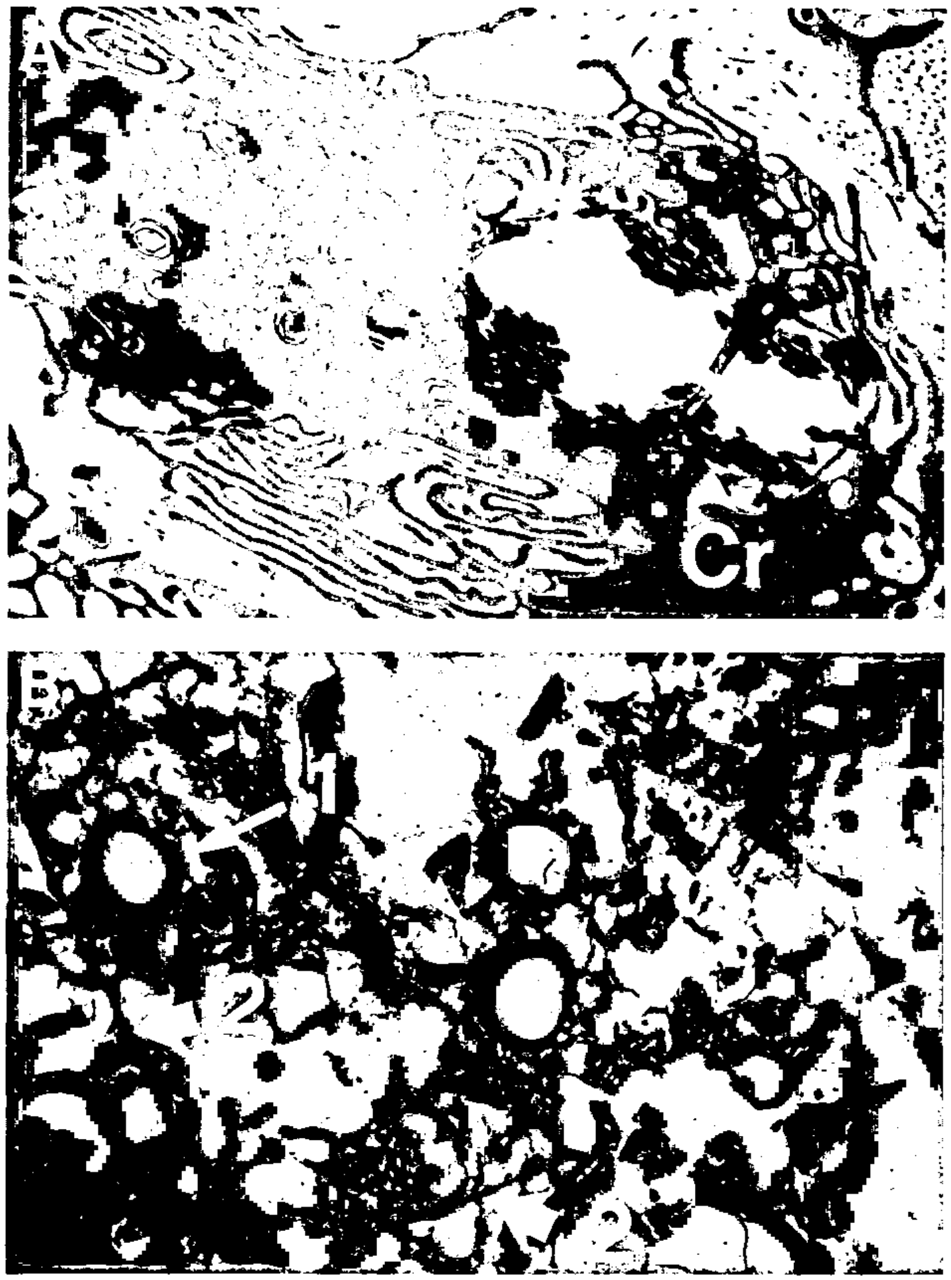

Figure 2. (A) 3–7 days after i.p. injection of crocidolite the granulomatous lesions in the rat omentum are dominated by macrophages. Fibroblasts and myofibroblasts are nearly absent. (EM 55.500X) Cr: Crocidolite.
(B) Cellular debris and chrysotile fibres in the center of granulomas six months after i.p. injection of 15 mg/ml saline solution. (EM 7.000X).
1. Fatvacuoles
2. Chrysotile fibres

As we have reported elsewhere the glycoprotein fibronectin can be found between and at the surface of macrophages in crocidolite induced inflammatory infiltrates and especially accumulates at the surface of very long crocidolite fibre bundles.[10] Therefore we have discussed the importance of its opsonic activity in case of asbestos induced fibrosis. Also, chrysotile fibres and clustered cellular debris were coated with fibronectin (Figure 3). In this way delayed scarring of the inflammatory reactions induced by chrysotile depositions in the fat tissue of the omentum might be promoted too. The scarring has not been finished 6 months after chrysotile application. Until this moment also the remarkable differences in the vascularization of chrysotile and crocidolite induced lesions continue (Figure 4). The surface properties of chryso-

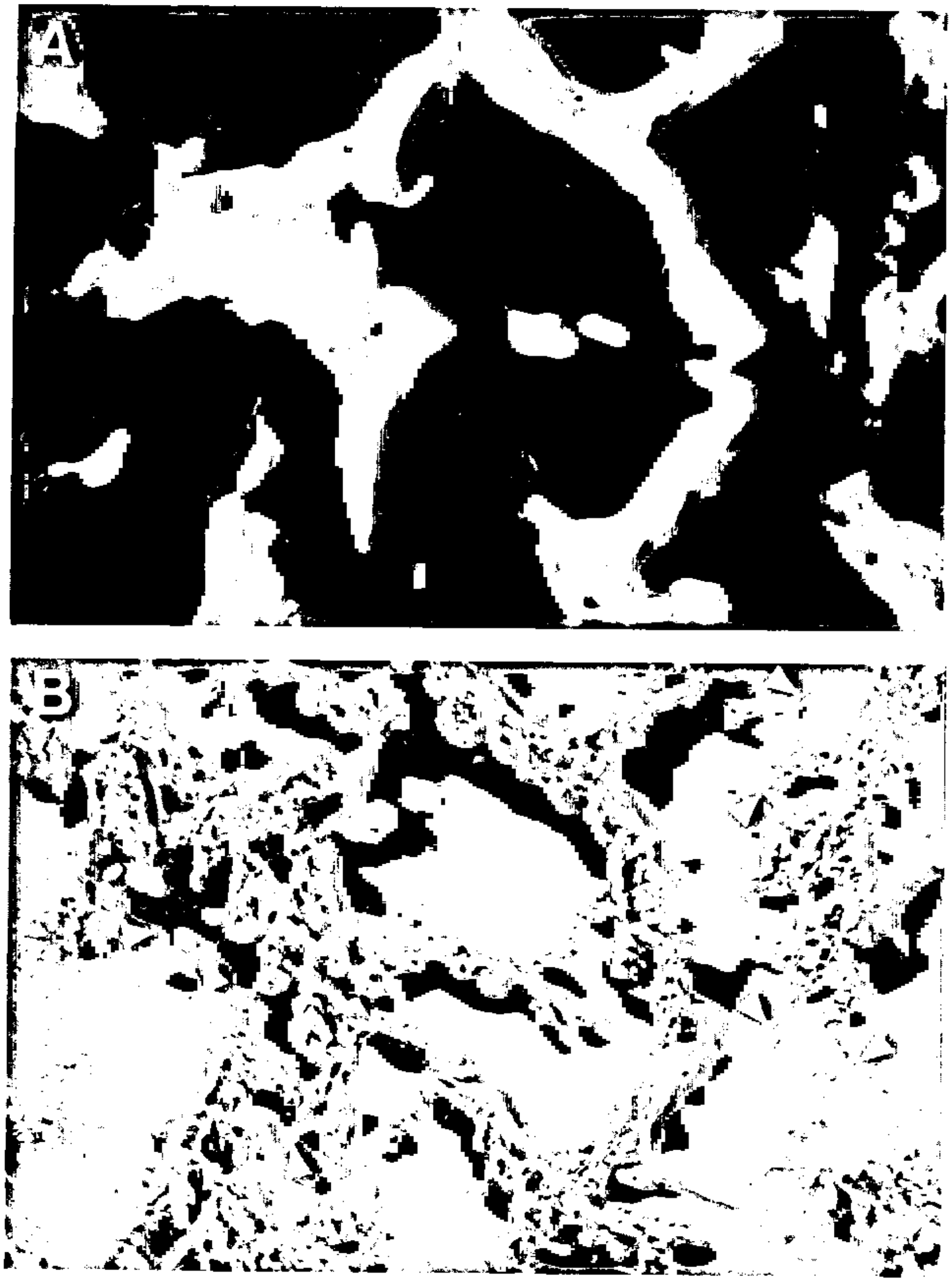

Figure 3. Immunofluoroscence microscopical demonstration of fibronectin (A) at the surface of chrysotile fibres and clustered cell detritus (B) in the center of a foreign-body granuloma in the rat omentum 2 months after i.p. injection of the fibrous dust.
((A) Immunofluorescence microscopy 460X;
(B) The same section as in (A) with light from the bottom, polarization and phase-contrast 460X)
➡ Chrysotile fibres

tile fibres seem not only to counteract the chemotactic and opsonic activity of fibronectin but also a vascular sprouting.

In combination the reaction patterns of extracellular matrix components, crocidolite induces a well vasculated granulation tissue and after 6 months much more fibrosis than chrysotile (Figure 5 left on top). Around the latter foreign-body-granulomas were formed with central necrosis and collagenous connective tissue only in the periphery (Figure 5 at the bottom). Fibres containing granulation tissue and granulomas, both however, are no obligatory conditions for mesothelial proliferation (Figure 5, right).

On the contrary even without contact to the focal dust containing lesions we have found narrow connective tissue

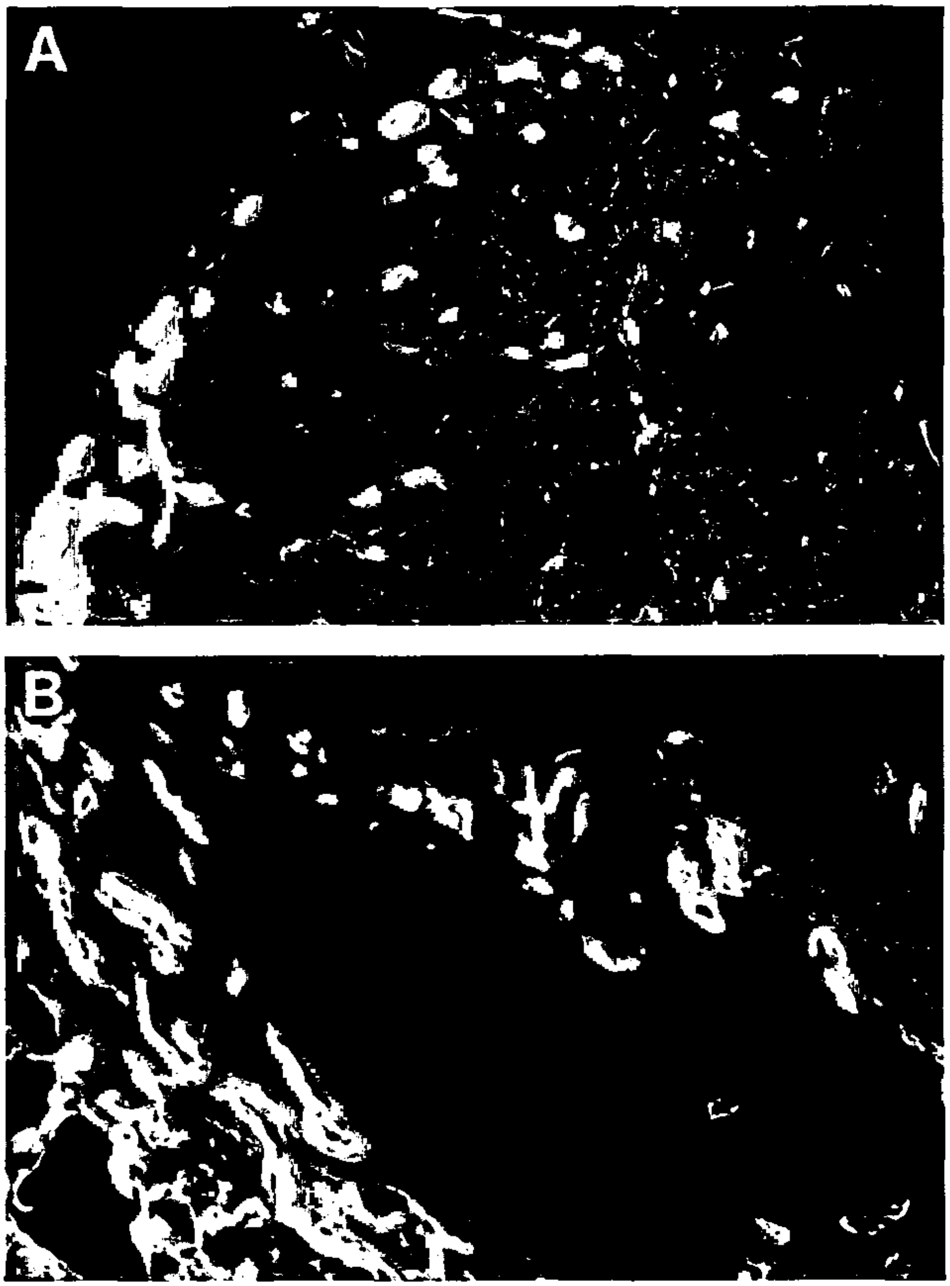

Figure 4. Dense vascularization of the granulation tissue induced by crocidolite fibres (A) and only in the periphery of a chrysotile containing granuloma (B).
((A) and (B) Immunofluorescence microscopy with an antibody against the basement-membrane glycoprotein laminin; (A) 190X, (B) 300X)

strands directly beneath the mesothelium already 7 days after intraperitoneal injection of 15 mg crocidolite, for example (Figure 6A). The covering cells were rounded, enlarged and often multinuclear. They have never stored asbestos fibres. In our opinion these changes can be conceived as a repairing process of the submesothelial mesenchyme. They are not only present in the fat areas of the omentum but also in the normally very thin mesothelial duplicatures spread between them (Figure 6B). The latter are the preferred localization of the generally less intensive chrysotile induced submesothelial fibrosis which also last for months and years after fibre administration. Even 28 months following the intraperitoneal injection of not more than 0.05 mg chrysotile/ml fibrotic thickening of the mesothelial duplicatures of the omentum could be observed.

Although macroscopically the omentums from long term carcinogenicity studies showed no tumour growth we microscopically have often found focal mesothelial proliferations associated with submesothelial fibrosis. In some ani-

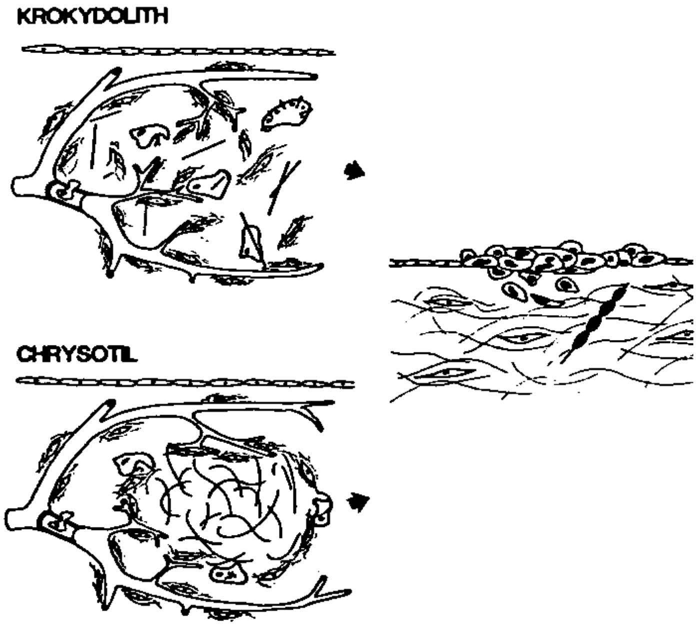

Figure 5. Crocidolite containing granulation tissue (left side on top) and chrysotile-induced granulomas (left side on the bottom) in the rat omentum as non obligatory precondition of mesothelial proliferation (right side).

mals mesothelial proliferation has reached the intensity of early mesotheliomas infiltrating the underlying connective tissue (Figure 7B). The activation of the submesothelial mesenchyme and the mesothelial proliferation, both proved to be a rather unspecific answer to injuries of the mesothelium by fibrous dusts provided that the single particles were only long and fine enough. These changes could be observed in a similar way 28 months after intraperitoneal deposition of the natural mineral fibres, type actinolite, and of man-made mineral fibres as glass microfibres and Kevlar (Figure 7A). They seem to depend in the quantity on fibre type only.

CONCLUSIONS

1. Phagocytosis of asbestos fibres is obviously mediated by fibronectin and in early fibrosis macrophages may be one place of collagen synthesis.
2. There are remarkable differences in the intensity of collagen synthesis in crocidolite induced granulation tissue with a symmetrical fibrillogenesis and in chrysotile induced granulomas with central necrosis and newly formed collagen fibres only in the periphery.
3. Natural mineral fibres and man-made mineral fibres tested intraperitoneally induce lesions of the serosal surfaces which are followed by submesothelial fibrosis and mesothelial proliferations up to the development of malignant mesotheliomas. These changes seem to depend in the quantity on fibre type only.

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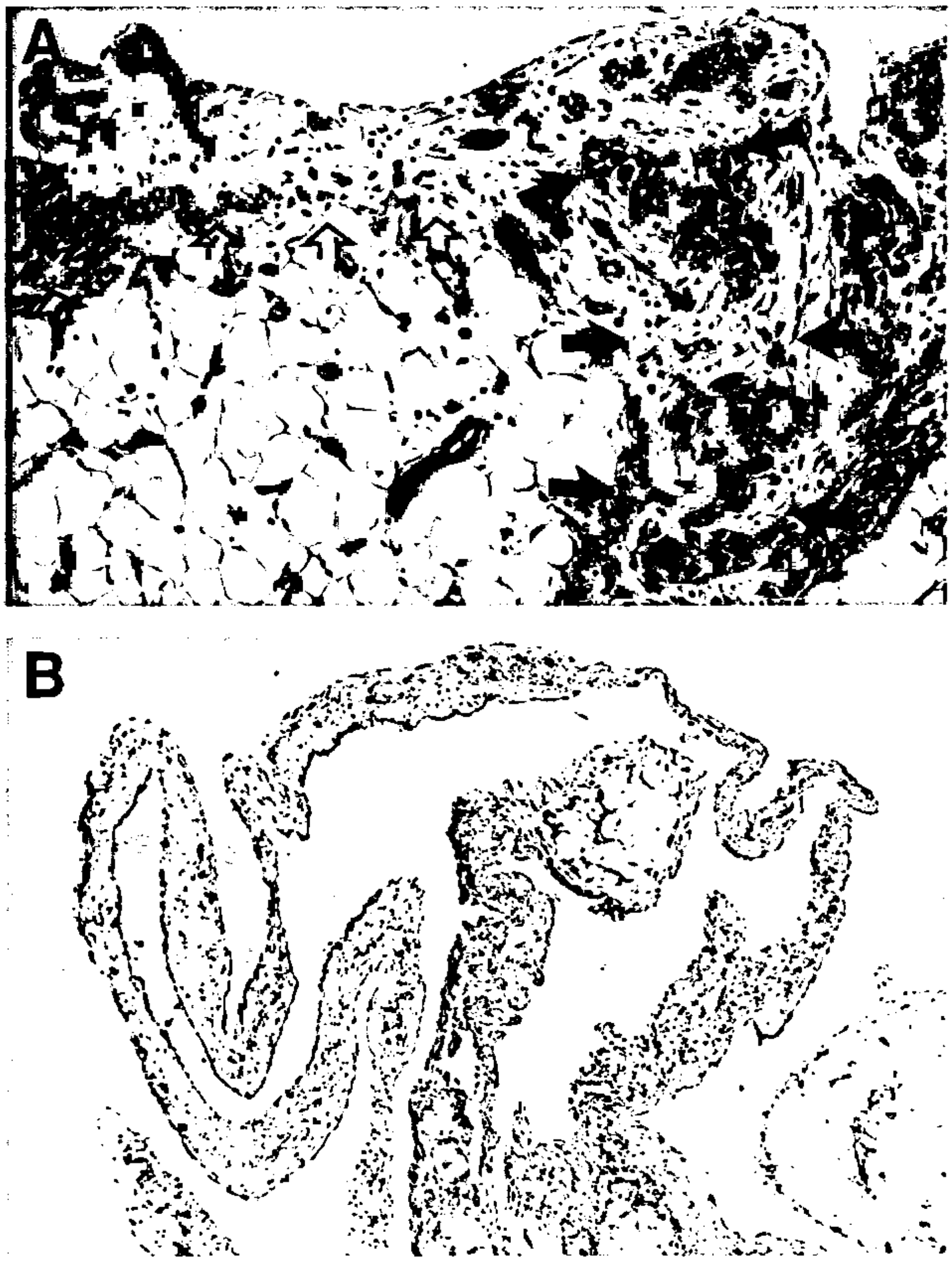

Figure 6. (A) Submesothelial fibrosis without crocidolite fibres visible with the light microscope (➡) and adjacent proliferating mesothelial cells without close connection to the foreign-body granuloma (➡) on the right side of the figure, 7 days following crocidolite administration.

(B) Cross section of mesothelial duplicatures of rat omentum. 3 weeks after crocidolite application the fibrotic thickening is remarkable.

((A) HE 74 X; (B): HE 60 X)

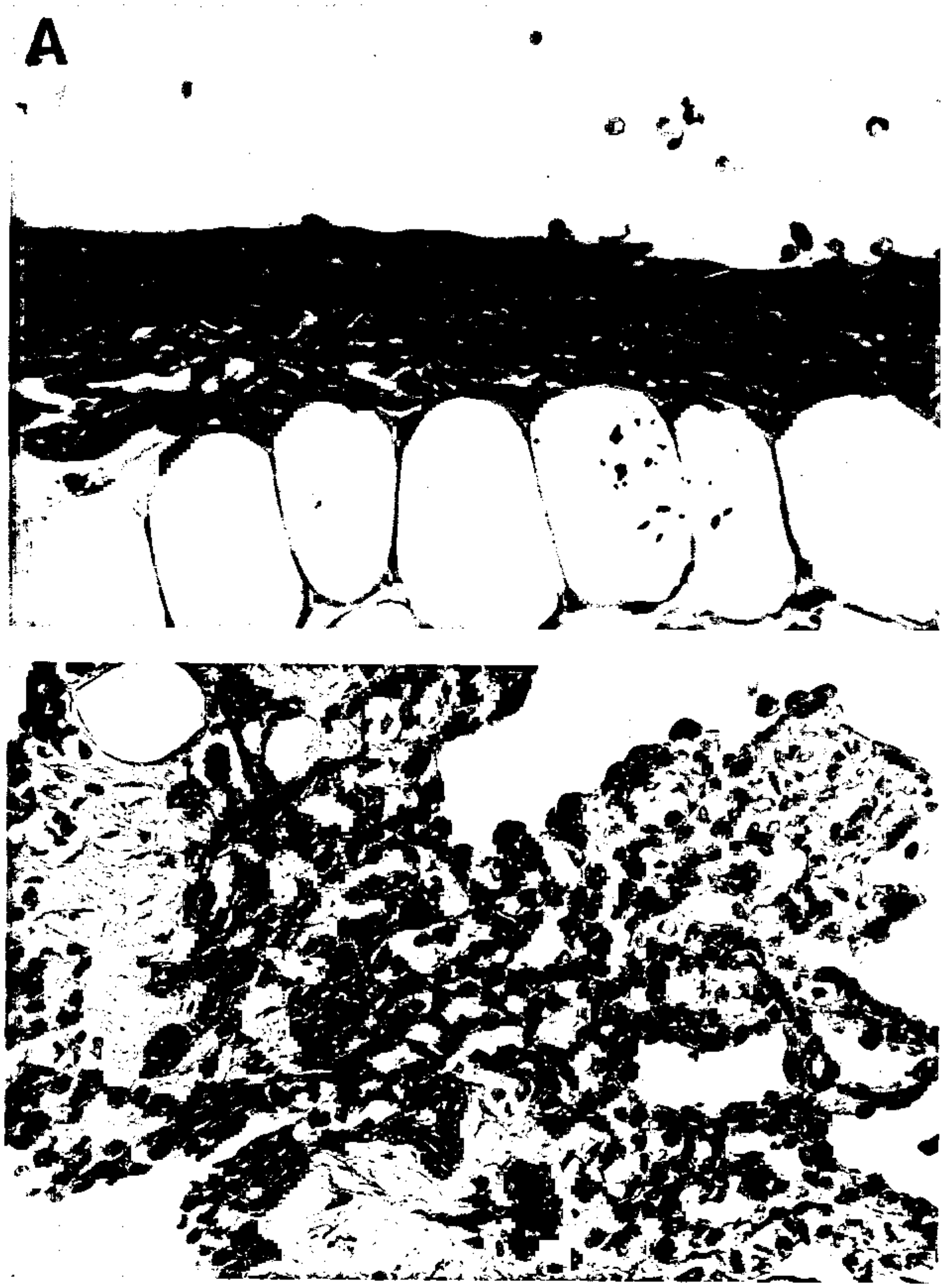

Figure 7. (A) Proliferation of probably preneoplastic cells in the submesothelial tissue 28 months after glass micro-fibre-induced injury of the mesothelial lining. (HE 400 X)
(B) Early mesothelioma of the mesothelial duplicature of rat omentum 21 months after exposure to actinolite. (HE 360 X)

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