THE DIFFERENT BIOLOGICAL EFFECTS OF DUSTS APPLICATED INTRATRACHEALLY SEPARATELY OR IN MIXTURES IN RATS

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An intraperitoneal injection of pure natural anhydrite has been shown to cause a mild fibrosis, which has been attributed to the content of quartz.[8] We postulated that anhydrite enhances the effect of the quartz. Cadmium sulfide also induces pulmonary fibrosis in our rat intratracheal test.[2] It is known, that polyvinylpyridine-N-oxide (PVNO) possesses an inhibitory action on quartz. However, we were able to show that CdS behaves differently in a mixture with PbS than with PVNO.[3]

In order to investigate the behaviour of dusts in mixtures, we used the rat intratracheal test to study anhydrite, cadmium sulfide and titanium dioxide dusts separately or in mixtures with Döorentruper quartz DQ 12 or polyvinylpyridine-N-oxide. The individual dusts and the artificial dust mixtures were initially made into suspensions, which were then physically characterized.[5] The results of this characterization are presented in the poster session.

A variety of dust samples were instilled in suspension in 0.5 ml of physiological saline solution into the trachea of female SPF Wistar rats (weight approximately 200 gr). The animals were sacrificed 3 months after instillation. The half of each left lung was hydrolyzed in sealed reagent tubes with 6 N.HCl for 16 to 18 hrs at 104°C. Following cooling and filtration the corresponding hydrolysate (0.1 to 0.5 ml) was neutralized with NaCl and the total hydroxyproline content determined.[7] The other half of each left lung was weighted, homogenized and extracted with chloroform-methanol (2:1). The total lipid content was then determined.[1] The corresponding right lungs of these animals and their regional lymph nodes were fixed in 4% formalin, dehydrated in graded alcohols and embedded in paraffin. Sections were stained with HE and EvG.

RESULTS

Those animals treated with anhydrite showed no increase in total lipid or hydroxyproline content in the lungs when compared with controls. After titanium dioxide these parameters were slightly raised. Treatment with PVNO or PVNO with CdS elicited only a mild increase in total lipid content compared with controls. Following separate instillation of quartz we found a dose-dependent increase in both total lipids and total hydroxyproline. Separate instillation of cadmium sulfide also caused significant elevation of both parameters (Table I).

Both successive and simultaneous instillation of anhydrite and quartz led to marked reduction in total lipid and hydroxyprolin content, compared with values obtained after separate quartz treatment. However, with decreasing quartz concentration or increasing anhydrite concentration an increase in lipid and hydroxyproline content was observed. Total lipids and hydroxyproline content in the lungs were also significantly elevated following a combination of titanium dioxide and quartz compared with values obtained with separate application of titanium dioxide.

The highest increase of total hydroxyproline content was established in animals which had been treated with quartz. The lowest level of hydroxyproline was achieved after simultaneous instillation of anhydrite with quartz. PVNO combined with quartz led to a clear reduction in hydroxyproline content, but significantly increased it when combined with CdS compared with that obtained after separate application of CdS.

Inflammatory changes in control lungs were not found. Following separate quartz treatment numerous disseminated histiocytic nodules with marked synthesis of collagenous connective tissue were seen both in the pulmonary tissue (Figure 1) and in the lymph nodes (Figure 2). The content of these changes was dose-dependent. In addition, in the lungs alveolar proteinosis was also observed.

After successive instillation of 35 mg anhydrite and 11 mg quartz a marked nodular histiocytic reaction without fibrosis was observed (Figure 3). In addition, simultaneous intratracheal instillation of 2 mg quartz with 35 mg anhydrite elicited in lymph nodes a minimal reaction with suggestion of nodule formation without fibrosis (Figure 4).

With increasing anhydrite dose the number of foam cells and foreign body giant cells decreased, whilst histiocytic nodules increased. In all anhydrite-quartz groups, independent of the anhydrite dose, merely numerous birefringent crystals were observed, without any increase in connective tissue component or alveolar proteinosis. In the swollen lymph nodes numerous partially confluent histiocytic nodules with a minimal collagenous reaction were found (Figure 5).

In the rat lung the separate intratracheal instillation of titanium dioxide failed to elicit a fibrous reaction. The foam

Table I

Means (x) and Standard Deviations (s) of Total Lipids and Total Hydroxyproline in Rat Lung 3 Months After Intratracheal Instillation

group (dust sample)	Total lipids/ lung mg		hydroxiproline/lung mg	
	x	s	x	s
35 mg anhydrite	43,47	5,06	3,40	0,52
2 mg DQ 12	122,80	49,19	4,94	1,12
5 mg DQ 12	191,95	49,93	5,85	0,67
7 mg DQ 12	202,94	45,48	8,20	1,33
11 mg DQ 12	207,13	58,46	8,22	1,29
20 mg PVNO	48,11	4,55	3,66	0,31
20 mg CdS	66,25	3,46	5,18	2,24
5 mg anhydrite + 11 mg DQ 12	156,96	13,37	3,81	0,70
20 mg anhydrite + 11 mg DQ 12	78,76	25,60	4,12	1,20
50 mg anhydrite + 11 mg DQ 12	78,83	13,04	3,73	0,92
35 mg anhydrite + 2 mg DQ 12	37,99	2,52	3,10	0,26
35 mg anhydrite + 5 mg DQ 12	40,34	3,42	3,22	0,14
35 mg anhydrite + 11 mg DQ 12	66,34	9,39	3,15	0,17
30 mg TiO_2	53,14	12,65	3,30	0,37
30 mg TiO_2 + 5 mg DQ 12	101,78	20,80	4,81	0,65
2 mg PVNO+11 mg DQ	55,53	5,97	3,47	1,21
11 mg PVNO+11 mg DQ	55,52	6,09	4,14	0,51
2 mg PVNO+20 mg CdS	58,55	3,87	5,72	0,70
11 mg PVNO+20 mg CdS	58,46	4,65	6,24	0,81
20 mg PVNO+20 mg CdS	59,76	5,22	6,50	0,84
control	44,17	5,00	3,26	1,03

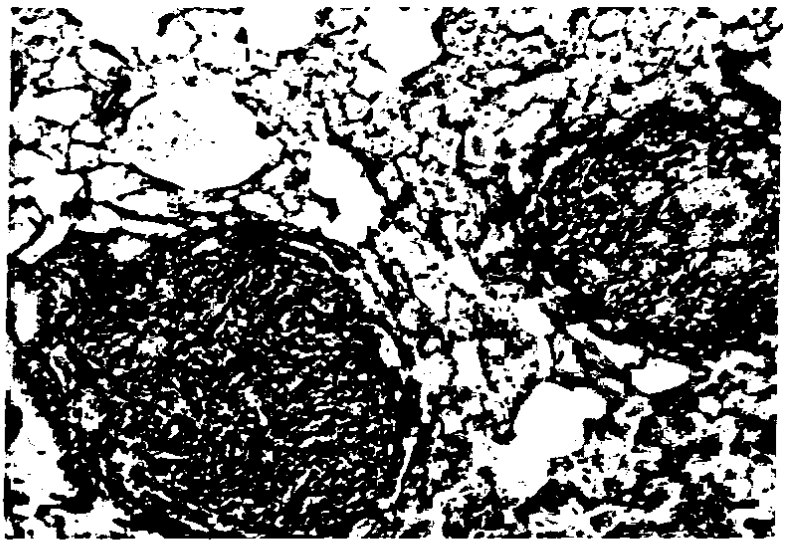

Figure 1. Rat lung with histiocytic nodules, a fresh formed collagen tissue and alveolar proteinosis 3 months after intratracheal instillation of 5 mg Dörentruper quartz.

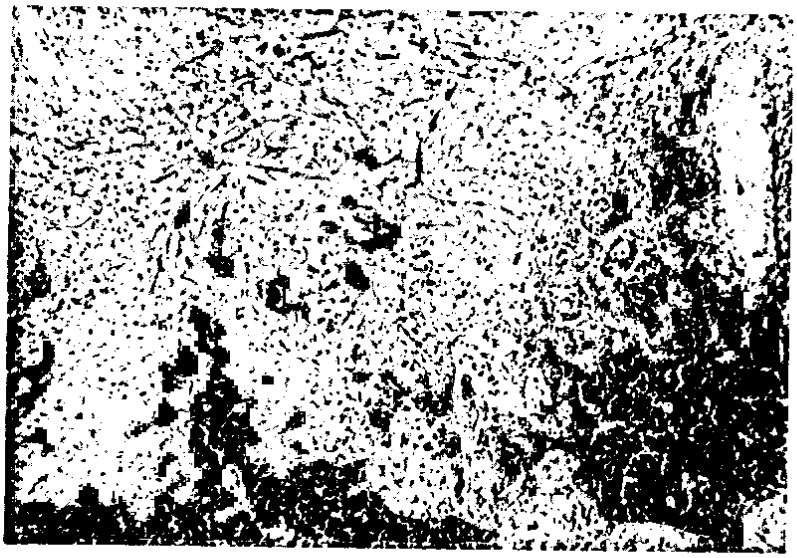

Figure 2. Partial compact fibrosis in the lymph nodes after intratracheal instillation of 5 mg Dörentruper quartz. EvG stain, magnification 50 fold.

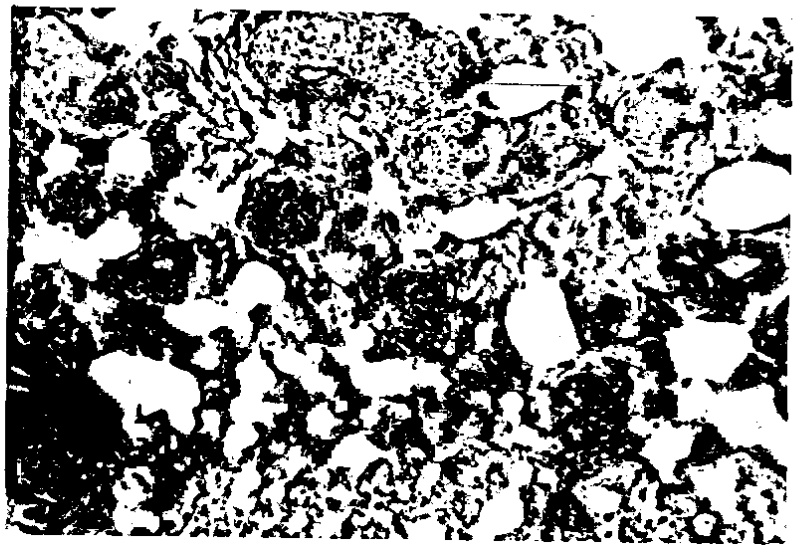

Figure 3. Rat lung with small histiocytic nodules without fibrosis after successive intratracheal instillation of 35 mg anhydrite and 11 mg Dörentruper quartz DQ 12. EvG stain, magnification 50 fold.

cell reaction in the lung after separate titanium dioxide instillation (Figure 6) was markedly enhanced following a combination of titanium dioxide and quartz (Figure 7). The alveolar septae beiing markedly thin and the alveolar epithelia much flattened. After combined treatment with titanium dioxide and quartz, the lymph nodes showed dense deposits of brown pigment and a focal histiocytosis.

After instillation of 20 mg PVNO there was no evidence of inflammation. Nodules of fibrosis did not occur.

After intratracheal instillation of 20 mg CdS, in the lungs, inflammation, moderate fibrosis within the nodular granulomata and single groups of foam cells were seen (Figure 8). Lymph nodes contained dense deposits of finally granular pigment but did not give evidence of fibrosis.

Figure 4. Rat lymph node with focuslike histiocytic reaction without fibrosis after simultaneous intratracheal instillation of 2 mg Dörentruper quartz with 35 mg anhydrite. EvG stain, magnification 50 fold.

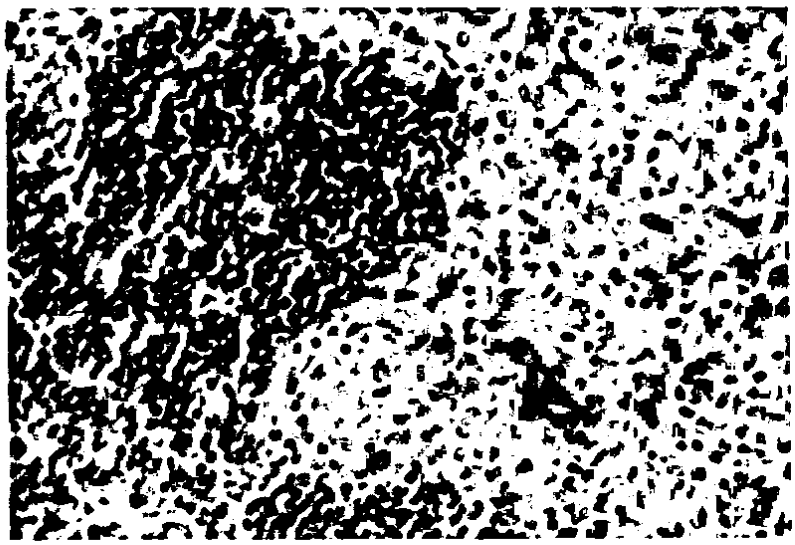

Figure 5. Rat lymph node with a slight fibrosis after simultaneous intratracheal instillation of 11 mg Dörentruper quartz with 35 mg anhydrite. EvG stain, magnification 50 fold.

With increasing PVNO dose at a constant level of CdS, the lungs revealed abundant granular material, which was not birefringent. An inflammatory reaction without nodule formation or foam cells were present. The inflammatory component in the lungs and the dust deposits in both lungs and lymph nodes increased with the total dose.

After the simultaneous instillation of 20 mg PVNO and 20 mg CdS a focal increase in connective tissue in the lung in the form of fibrosis (Figure 9) was observed. In the lymph nodes a focal fibrosis, occasionally, in the form of nodules was seen.

The simultaneous instillation of PVNO and quartz, neither in the lungs nor in the lymph nodes was there any fibrous reaction. Dust deposits could not be found in the lymph nodes.

Numerous nodular granulomata, pricipally histiocytosis with

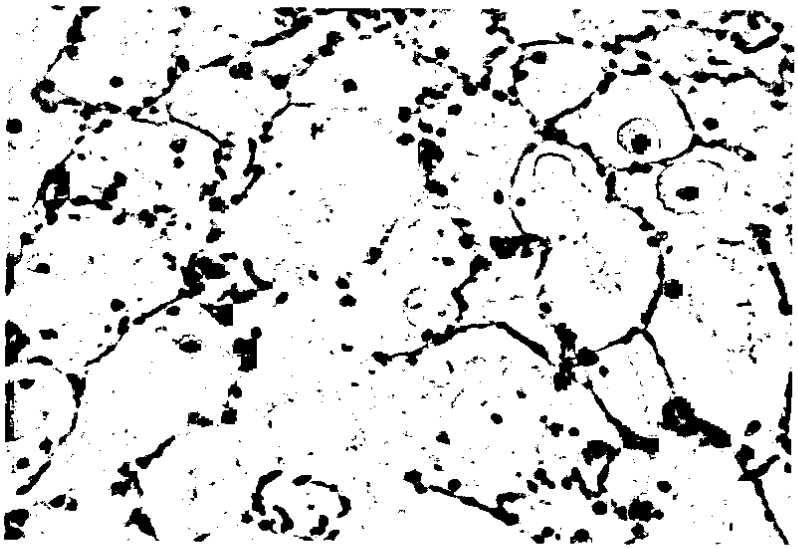

Figure 6. Rat lung, a slight foam cell reaction after intratracheal instillation of 30 mg titanium dioxide. EvG stain, mgnification 125 fold.

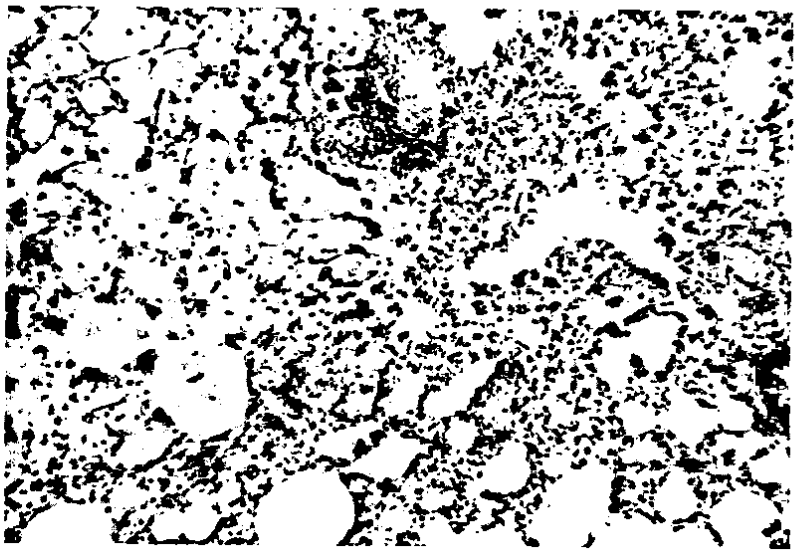

Figure 7. Rat lung, a intensifier foam cell reaction after intratracheal instillation of Dörentruper quartz with titanium dioxide. EvG stain, magnification 63 fold.

a tendency toward confluence, were not in evidence in the lung until 6 months following combined instillation of 2 mg PVNO and 11 mg Dörentruper quartz.

DISCUSSION

In the various mixtures PVNO showed variable behaviour, i.e. with quartz inhibitory, with CdS stimulatory. A similar behaviour was found with anhydrite: It acted inhibitory on quartz, whereas with the bounding catalyser, a mixture of iron and potassium sulfide, no inhibition could be found.[8]

Furthermore, the physical characteristics of the dust mixtures employed[6] indicates that the act of mixing imparts to the dusts properties which cannot be calculated on an additive basis. The mixture will thus react totally differently from the individual dusts applied separately. The observations lead us to the suggest that we may be dealing with a general principle, namely that each dust can demonstrate different behaviour in different dust mixtures.

Qualitative differences in histological appearance were estab-

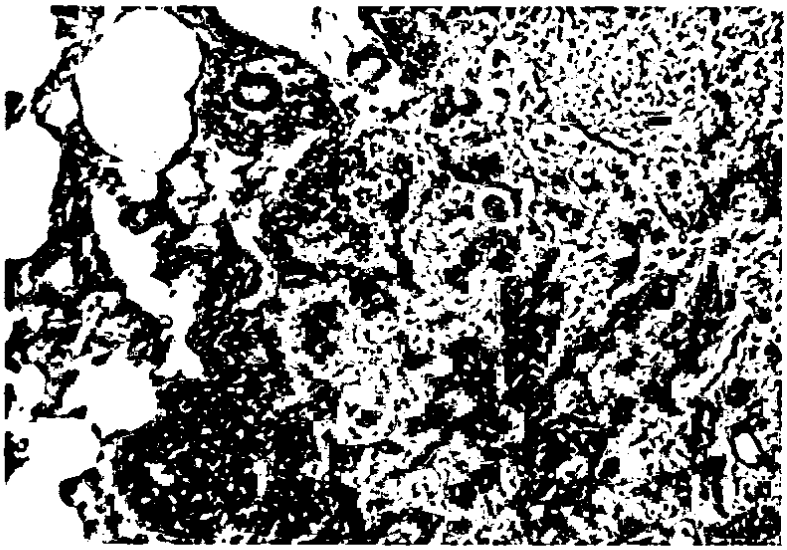

Figure 8. Rat lung, occasional foci of inflammation, moderate fibrosis within nodular granulomata after intratracheal instillation of 20 mg CdS. EvG stain.

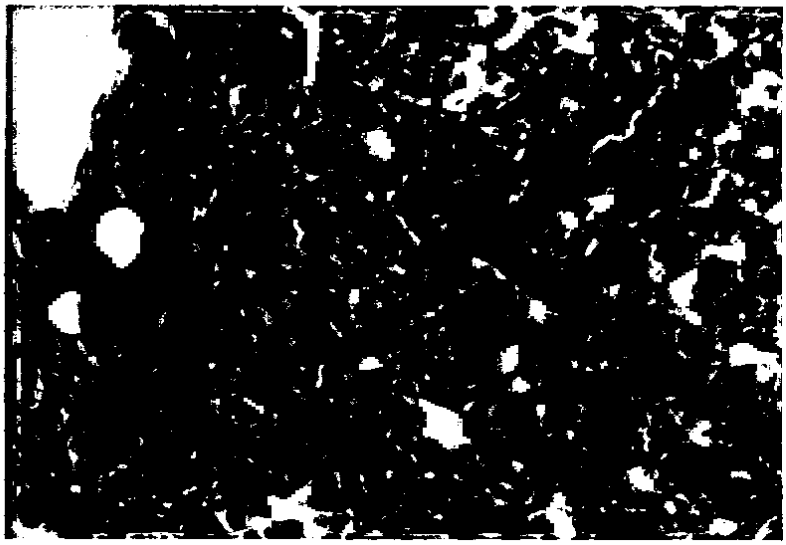

Figure 9. Rat lung, focal fibrosis after simultaneous intratracheal instillation of 20 mg Cds and 20 mg PVNO. EvG stain.

lished between the various experimental groups. The intratracheal instillation of CdS elicited a marked inflammatory reaction in the lungs. However, in contrast with the quartz effect, no sinus histiocytosis could be found in the lymph nodes. The addition of PVNO enhanced the effect of CdS by increasing the degree of penetration of the dust, resulting in fibrosis in the lymph nodes, too.

Increasing anhydrite dose in the dust mixture with quartz led to a decrease in form cell content but an increase in the number of histiocytotic nodules. By contrast, the combination of titanium dioxide with quartz not only enhanced the foam cell reaction but also caused flattering of the alveolar epithelium with the formation of thin alveolar septae. After the combined application of titanium dioxide and quartz the lymph nodes exhibited sinus histiocytosis with no evidence of fibrosis. Combining 35 mg anhydrite with 11 mg quartz elicited a minimal connective tissue reaction in the lymph nodes, but not in the lung.[4] The morphological differences correlated well with the alterations in the hydrogen concentration in suspensions of saturated solutions.[6]

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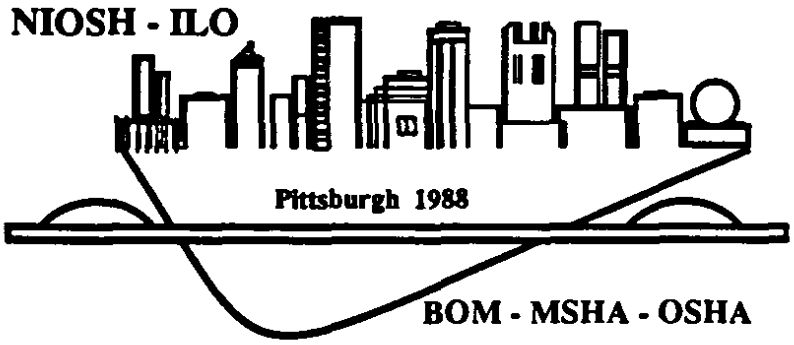

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