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Effect of Inhaled Commercial Hydrous Calcium Silicate Dust on Animal Tissues

An Experimental Study

G. W. H. SCHEPERS, M.D., D.Sc.
T. M. DURKAN, M.E.
and
A. B. DELAHANT, Saranac Lake, N. Y.

Commercial hydrous calcium silicate is one of the products that have been studied by long-term inhalation experiments at The Saranac Laboratory. These studies were commenced in 1943 under the direction of Dr. L. U. Gardner and with the assistance of two of us (T. M. D. and A. B. D.) who carried the work to its completion after Dr. Gardner's death. Guinea pigs, rats, and hamsters were used, and the experiments were designed to reveal the nature of the pulmonary tissue reaction to inhaled dust of the material in normal animals and in those harboring an experimentally induced tuberculous infection.

From experience with other siliceous materials one would expect to find that a product composed only of calcium silicate would be relatively inert in its effect on tissue. Extensive clinical studies of industrial workers exposed to cement dust in high concentration have, for instance, shown that the effect of the inhaled dust of that material on the lungs is insignificant. Portland cement is composed principally of two calcium silicates, namely, dicalcium silicate and tricalcium silicate. Recognition must be given, however, to the possibility that any potentially hazardous raw materials used to make a product might not be entirely converted to a nonhazardous form during the manufacturing process and, therefore, might appear to a greater or less extent in an unchanged condition in the final product. The clay and shale used in making cement often have a quartz content of 20% to 30% or more, but chemical reactions, occurring when the raw mixture is heated, convert practically all the hazardous free-silica mineral to a relatively harmless silicate. In most samples of cement the amount of quartz that has come through the manufacturing process unchanged is less than 0.1%.

The hydrous calcium silicate product used in The Saranac Laboratory studies was made from calcium hydroxide and silica plus a moderate amount of asbestos, which was incorporated in the mixture to impart certain desirable physical properties to the finished product. Analysis of one sample of this finished product disclosed that approximately 80% of the raw mixture had been converted to hydrous calcium silicate and that about 15% was magnesium silicate (Table 1). About 1% quartz persisted in the final product. Probably the greater part of the magnesium silicate was present as the fibrous mineral chrysotile, although a portion may have been in the form of serpentine, a mineral similar to chrysotile in chemical composition but one which is nonfibrous.

EXPERIMENTAL METHOD

To determine the effect of inhaled dust of the product on pulmonary tissue, an inhalation experiment was conducted. In that experiment groups of guinea pigs, rats, and hamsters were exposed in a cubical dust room, 8 ft. in dimension, in which

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Director, (Dr. Schepers), Associate Director (Mr. Durkan), and Research Associate (Mr. Delahant), The Saranac Laboratory.

TABLE 3.—*Pulmonary Reaction to Inhaled Commercial Hydrous Calcium Silicate Dust*
Guinea Pigs Were Exposed by Inhalation Continuously to the Dust Until Death

		Microscopic Evidence of Pneumoconiosis										Reaction in Pulmonary Lymph Nodes				
Guinea Pig, No.	Exposure to Dust, Mo.	Pigmentation	Lymphoid Hyperplasia	Bronchiolitis and Bronchial Obliteration	Bronchial Dilatation	Adenomatoid Hemeplasia	Phagocytosis	Cellular Proliferation	Fibrosis	Adsorption Bodies	Emphysema	Pleural Reaction	Enlargement	Pigmentation	Macrophage Infiltration	Fibrosis	Periadenitis
1, 2, 4, 6	2	..	+	+	..	..	+	+	..	..	..	..	+	..	+	..	..
39, 41, 42, 44	4	..	+	+	..	..	+	..	..	..	..	..	+	..	+	..	..
46, 48, 49, 50	6	..	+	+	..	..	+	..	..	..	..	..	+	..	+	+	..
26, 29, 31, 37	8	..	+	+	..	..	+	..	..	..	..	+	+	..	..	..	..
40, 42, 43, 50	10	..	+	+	+	..	+	+	..	..	..	..	+	..	+	..	+
	12	+	+	+	+	..	+	+	..	..	..	..	+	..	+	+	+
	18	+	+	+	+	..	+	+	..	+	..	..	+	+	+	+	+
	21	+	+	+	+	..	+	+	..	+	..	+	+	+	+	..	+
	24	+	+	+	+	..	+	+	+	+	..	+	+	+	+	..	+
	27	+	+	+	+	+	+	+	+	+	+	..	+	+	+	..	+
	30	+	+	+	+	+	+	+	+	+	+	+	+	..	+	..	+
	33	+	+	+	+	+	+	+	+	+	+	+	+	..	+	+	+
	36	+	+	+	+	+	+	+	+	+	+	+	+	..	+	..	+

Symbols: ±, slight or incipient reaction; +, moderate reaction; +++, marked reaction.

TABLE 4.—*Pulmonary Reaction to Inhaled Commercial Hydrous Calcium Silicate Dust*
Rats and Hamsters Were Exposed by Inhalation Continuously to the Dust Until Death

		Microscopic Evidence of Pneumoconiosis								Reaction in Pulmonary Lymph Nodes			
Animal No.	Exposure to Dust, Mo.	Lymphoid Hyperplasia	Bronchiolitis and Bronchial Obliteration	Bronchial Dilatation and Dilatation	Phagocytosis	Cellular Proliferation	Fibrosis	Adsorption Bodies	Emphysema	Pleural Reaction	Enlargement	Macrophage Infiltration	Fibrosis	Periadenitis
Rats Exposed to the Dust														
201, 202, 203, 204	6	+	..	..	+	..	..	..	+	..	+	+	..	..
205, 206, 207, 208	9	+	..	..	+	+	..	..	+	..	+	+	..	..
209, 210, 211, 212	12	+	+	+	+	+	..	..	+	+	+	+	..	+
213, 220, 223, 224	15	+	+	+	+	+	+	+	+	+	+	+	+	+
225, 231, 235, 236	18	..	+	+	+	+	+	+	+	+	+	+	..	+
Rats Exposed to the Dust														
1, 2	3	..	+	..	+	..	..	..	+	..	+	..	..	..
11, 12	9	..	+	+	+	+	..	..	+	..	+	+	..	+
4, 6	12	..	+	+	+	+	..	+	+	+	+	+	..	+
14, 16	18	..	+	+	+	+	+	+	+	+	+	+	+	+

Symbols: ±, [illegible] change; +, slight or incipient reaction; ++, moderate reaction; +++, marked reaction.

marked tendency to peribronchiolar atelectasis, with epithelialization of the walls of the shrinking alveoli and cellular infiltration among these minute cystic spaces. This change produced a microscopic sectional effect resembling multiple adenomatosis, but as there is no lack of differentiation of the cellular components, no neoplastic change could be postulated. This adenomatoid pattern was seen only in the guinea pigs, but as its evolution was in the nature of a delayed phenomenon, it is possible that it would also have appeared in the rats and hamsters had exposure been continued in their cases beyond the 18th month.

Phagocytosis of the inhaled dust could be demonstrated in all animals from the commencement of the experiment. It was most marked in the case of the guinea pigs and least prominent as a feature in the case of the hamsters. The phagocytes were dominantly mononuclear macrophages until about the end of the first year of the exposure when multinucleated giant cells commenced to be substituted in progressively greater numbers. At the same time these giant cells grew in size so that they often filled the alveoli completely, and toward the end of the third year of exposure the giant cells replaced the mononuclear cells almost completely, especially around the distorted bronchioles. As time passed, their cytoplasm became increasingly more acidophilic and their centers more charged with ingested particles. There was no evidence of necrosis of these cells, such as occurs in experiments with finely divided quartz dust and silica fume, which provoke similar giant cells.

Interstitial cellular proliferation and infiltration are a late sign in all three species. It commences first toward the end of the ninth month of exposure, and for the succeeding six months it is almost entirely limited to macrophage accumulations around smaller blood vessels and bronchioles, at the angles between adjacent alveoli, and in the interlobular septa. Among these macrophages may be found isolated eosinophiles and plasmacytes. Fibrocytes first make an appearance between the 15th and the 18th month of dust inhalation, and soon after this strands of collagen may be perceived among the cells. Fibrosis proceeds slowly, however, before the end of the 30th month of dust exposure. Thereafter it is detectable in increasing amounts, particularly in relation to the adenomatoid areas around the bronchioles. Fibrosis also appears prominently in local areas of consolidation, which become progressively commoner toward the end of the third year of exposure. In animals killed, respectively, at 33 and 36 months from the start of the dusting, extensive areas of lobular consolidation could be found, and similar consolidation associated with cystic distention of trapped bronchi could be seen in several of the rats killed at the 15- and 18-month periods. No acute inflammatory process was demonstrable to account for these results.

Asbestos bodies of an elongated, slender, tapering, minute variety were demonstable by oil-immersion microscopy from about the 15th month onward in rats and hamsters and from the 18th month onward in the guinea pig. Occasionally unique clubbed [illegible] could be found. [illegible] in most instances the bodies were difficult to bring into focus and tended to be curved and spindle-shaped. Often they were intracellular, being partly engulfed by one or more macrophages. None were found lying free within alveoli. They were so constantly found in association with the areas of fibrosis that the conclusion of a causal relationship between these phenomena is almost inescapable.

Emphysema of the hypertrophic variety appeared as an early sign in the hamsters, in which it was present to a quite marked and widely distributed degree at the end of the 18 months of dust exposure. In the rats the lesions were also consistently present from an early stage but to a lower degree than in the hamsters. In the guinea pigs, on the contrary, hypertrophic emphysema was contrastingly absent during the first two years of the experiment. In the final 12 months, compensatory emphysema was manifested as a prominent lesion silhouetted against the foci of cellular proliferation and peribronchiolar atelectasis.

TABLE 5.—*Causes of Death in Guinea Pigs Exposed by Inhalation to Commercial Hydrous Calcium Silicate Dust and Also Infected with Tubercle Bacilli of the R_1 Strain*

Type of Experiment	Exposed Animals, No.	Cause of Death: Pneumonia Deaths, No.	Pneumonia Per Cent	Pericarditis Deaths, No.	Pericarditis Per Cent	Peritonitis Deaths, No.	Peritonitis Per Cent	Other Causes Deaths, No.	Other Causes Per Cent	Total Deaths, No.	Total Per Cent
Simultaneous phase	30	5	28.6	3	10.0	1	3.3	2	6.6	11	36.6
Reactivation phase	25	2	8.0	1	4.0	..	....	1	4.0	4	16.0
Preinposition phase	30	8	26.6	..	....	..	....	..	....	8	26.6
Control: Group A	34	4	11.7	..	....	..	....	..	....	4	11.7
Group B	25	3	12.0	1	4.0	2	8.0	..	....	6	24.0

animal, which was killed at 15 months, the lesions were widespread throughout the lung but fibrosis had been produced. In an attempt to develop a strain of guinea pig resistant to the infection that had caused the pneumonia, some of the animals were used for breeding during this experiment. The two animals that died from progressive tuberculosis (at 14 months) and the single animal (killed at 15 months) that exhibited widespread lesions were all used as breede during the experiment, as were also fo other animals in the experiment, which h failed to show extensive disease. Owing the element of uncertainty introduced by t factor of breeding, and also because of t high mortality from pneumonia and to ver the suggestive evidence of stimulating acti of the inhaled dust upon tuberculosis, t experiment was repeated.

TABLE 6.—*Course of Tuberculosis Induced in Guinea Pigs by Infection with Tubercle Bacilli of the R_1 Strain*

Control Study: Infection Only; No Dust Exposure

Guinea Pig, No.	Survival After Infection, Days	Fate	Pulmonary Lesions: Arrested Tubercles, No.	Spreading Tuberculosis	Caseation	Calcification	Pleuritis	Reaction in Pulmonary Lymph Nodes: Enlargement	Tubercle Formation	Dissemination: Liver	Spl
42.....	48	Died	3	..	..	..	..	+	..	..	.
57 }	60	Killed	6	..	+	..	..	——	+	T+	T
58 }		Killed	15	..	+	..	..	——	+	T—	T
59 }	150	Killed	7	..	..	..	..	——	..	..	.
60 }		Killed	4	..	..	..	..	——	—	..	T
63 }	180	Killed	3	..	..	..	..	——	+	..	.
64 }		Killed	5	..	..	..	+	——	..	..	.
72.....	195	Died	..	——	——	..	..	——	——	T——	T+
97.....	200	Died	2	..	..	..	——	+	..	..	.
65 }	240	Killed	4	..	..	+	..	——	+	..	.
66 }		Killed	11	..	..	+	..	——	..	..	.
88.....	255	Died	2	..	..	..	..	——	..	..	.
81 }	300	Killed	0	..	..	..	..	——	..	..	
74 }		Killed	4	..	..	..	..	——	+	..	
98.....	315	Died	1	..	..	——	..	+	..	..	
89 }	365	Killed	7	..	..	..	..	——	..	..	
90 }		Killed	10	..	..	+	..	——	..	..	
67 }	420	Killed	4	..	..	..	..	+	..	..	
96 }		Killed	5	..	..	..	..	+	..	..	
69 }	450	Killed	3	..	..	..	..	——	..	..	
70 }		Killed	1	..	..	..	..	——	..	..	
71 }	480	Killed	3	..	..	..	..	——	..	..	
72 }		Killed	3	..	..	..	..	——	..	..	
75 }	540	Killed	2	..	..	..	..	+	..	..	
76 }		Killed	2	..	..	..	..	+	..	..	
77		Killed	3	..	..	..	..	——	..	..	
78		Killed	5	..	..	..	..	——	..	..	
79		Killed	2	..	..	..	..	——	..	..	
80		Killed	6	..	..	..	..	——	..	..	
91 }	730	Killed	6	..	..	..	..	——	..	..	
92		Killed	2	..	..	..	..	——	..	..	
94		Killed	4	..	..	..	..	——	..	..	
95		Killed	3	..	..	..	..	——	..	..	
96		Killed	0	..	..	..	..	——	..	..	

Symbols: —, slight reaction; ——, moderate reaction; ———, advanced reaction; T, tubercle formation.

pig. Massive caseating lesions were found in the lungs, the liver, and the spleen. It was suspected that this animal may have been accidentally infected with virulent human tubercle bacilli. Consequently, bacilli were recovered, cultured, and subinoculated into the groins of four healthy guinea pigs. These animals developed local abortive lesions characteristic of the R_1 bacillus, and no systemic tuberculosis ensued. By this study it was fairly satisfactorily demonstrated that Guinea Pig 73 was unduly susceptible to tuberculosis.

In the guinea pigs which were caused to breathe the hydrous calcium silicate dust after infection, localized spreads of the tuberculosis occurred in 13 out of the 30 cases. In four of these the tuberculous process was quite advanced, and in four others it was moderately marked at the time of autopsy. It should be noted too that five of these spreads occurred during the second year of the experiment, i. e., long after the tubercles should have been arrested and healed. Attention should also be drawn to the presence of cavitation within two tuberculous pneumonic areas in Guinea Pig 25.

Pleural adhesions of a chronic tough variety were also quite common in the animals receiving the dust exposure. The pulmonary lymph nodes showed a greater prevalence of late active tuberculous foci, such lesions in the control animals having usually disappeared from the nodes before the end of the first year.

From this simultaneous phase experiment it would seem, therefore, that the hydrous calcium silicate had a mild to moderately adverse effect on the course of the R_1 tubercle infection.

REACTIVATION PHASE: COURSE OF TUBERCULOSIS IN GUINEA PIGS WHICH WERE EXPOSED TO HYDROUS CALCIUM SILICATE DUST SEVERAL MONTHS AFTER INFECTION WITH R_1 TUBERCLE BACILLI

Infected guinea pigs were placed in the dust chambers at intervals of two months, i. e., 10 at two months, 8 at four months, and 8 more at six months after infection. In order to follow the course of the tissue reaction, a pair of animals of the first subgroup (two months in normal air) was killed after only 2 months of dust exposure, and, in addition, animals from all groups were killed in pairs after 4, 8, and 12 months of exposure to the dust. Examination of the tissue of the dusted animals failed to reveal a significant reactivation of the tuberculous disease by the inhaled dust (Table 8). Only five animals showed evidence of spreading pulmonary tuberculosis. In three the disease was minimal in extent, and in two others there was moderately extensive local spread. Caseation persisted in one animal to the end of a year, and slight to moderate foci of fibrosis could be discerned in a few animals, suggesting that not all the tubercles healed by resolution. Chronic pleuritis and pleural adhesions were present in 10 of the guinea pigs, which was considerably in excess of what is customarily found in a typical reaction to the introduction of R_1 tubercle bacilli. Dissemination of the tuberculous process to abdominal organs and to the pulmonary lymph nodes did not occur in any but exceptional cases. Guinea Pig 52 was probably one of these "sports." The term "sport" is given to a guinea pig in which the inhalation infection with the attenuated bacilli is not confined principally to the lungs and pulmonary lymph nodes but extends also to other organs and produces in them tuberculous changes of sufficient extent to be recognized macroscopically. Since "sports" represent a departure from the normal pattern of tissue reaction to attenuated tubercle bacilli, whether the infection is combined with dust exposure or not, such animals must be excluded in assaying the effect of an inhaled dust upon a tuberculous infection. It is believed that "sports" are animals whose native resistance to the attenuated R_1 organism is unusually low. Support for this belief is given by experience with quartz dust, a definitely hazardous material which reactivates an inhalation infection produced by attenuated R_1 bacilli but ordinarily is not associated with tuberculous extension to organs other than the lungs and pulmonary lymph nodes.

TABLE 9.—*Influence of Inhaled Commercial Hydrous Calcium Silicate Dust on the Course of Experimentally Induced Tuberculosis in Guinea Pigs*

Predisposition Phase: Animals, After Being Exposed to the Dust for Three Months, Were Injected with Tubercle Bacilli of the R_v Strain and Then Were Immediately Returned to the Dust Room Where Their Dust Exposure Was Continued Until Death

Guinea Pig. No.	Further Exposure to Dust After Infection, Days	Fate	Pulmonary Lesions				Dissemination			
			Arrested Tubercles, No.	Spreading Tuberculosis	Cavitation	Pleuritis	Pulmonary Lymph Nodes: Enlargement	Pulmonary Lymph Nodes: Tuberculosis	Liver	Spleen
107	0	Killed	..	..	..	..	..	..	..	..
5	0	Killed	..	..	..	..	..	..	..	..
9	0	Killed	..	..	..	..	..	..	..	..
10	0	Killed	..	..	..	..	..	..	..	..
11	0	Killed	..	..	..	..	..	..	..	..
91	30	Died	..	..	..	..	++	+	..	..
92	60	Killed	17	+	..	..	..	..	..	..
12	60	Killed	21	+	..	..	++	+	..	..
94	120	Killed	16	+	..	..	++	+	..	..
96	120	Killed	14	+	..	..	++	+	..	..
98	180	Killed	7	++	..	..	++	+	..	..
97	180	Killed	11	+	..	..	++	+	..	..
114	220	Died	6	+++	+++	++	+	..	..	..
108	220	Died	3	++	..	+++	+	..	..	..
112	240	Killed	0	..	..	..	+	+	..	..
113	240	Killed	20	+	..	..	++	+	..	..
99	300	Killed	2	++	..	++	++	+	..	..
106	300	Killed	3	++	..	++	++	+	..	T+
115	345	Died	..	+++	..	++	+	..	..	..
95	365	Killed	2	++	..	..	++	+	..	..
100	365	Killed	4	..	...	..	++	+	..	..
119	406	Died	..	+++	++	+	+	+	..	..
117	425	Died	..	+++	..	+	++	+	..	..
105	460	Died	..	++	..	..	+	..	..	..
104	465	Died	..	+++	..	+	++	+	..	..
116	480	Killed	..	+++	..	..	+	+	..	..
120	480	Killed	..	++	..	++	+	+	..	..
101	630	Killed	4	+	..	..	+	..	..	..
102	630	Killed	3	+	..	..	++	+	..	..
110	630	Killed	3	+	..	++	+	+	..	..

Symbols: +, slight or incipient reaction; ++, moderate reaction; +++, marked reaction; T, tuberculosis.

It is of interest to note that the extension of the disease was almost entirely confined to the pulmonary tissues, tuberculous foci being detected in the spleen of one animal only. The reaction in the pulmonary lymph nodes was not of a significant nature or degree.

These findings indicate that a tuberculous infection which originates in guinea pigs several months after a prolonged exposure of the animals to dust of the hydrous calcium silicate product was initiated may be unfavorably influenced by the inhaled dust. A separate control experiment was set up, using 23 guinea pigs, as the predisposition-phase experiment was started at a later stage than the preceding studies so that a fresh R_1 culture had to be employed. The results obtained were so similar to those given in Table 6 that the latter may suffice for the purpose of this paper.

ANALYSIS OF TISSUE OF EXPOSED ANIMALS

Chemical analysis of lung tissue of uninfected guinea pigs that had inhaled the hydrous calcium silicate dust for periods up to 36 months yielded the data reported in Table 10. It will be noted that as the period of exposure became longer the values for the tissue ash gradually increased, thus showing that mineral matter was accumulating in the lungs. There was a pronounced increase in the silica component up to about 30 months and then a slight decrease. This phenomenon is illusory, as it is due to the relatively rapid rate at which inorganic matter was deposited

Why the alveolar ducts and bronchioles should have dilated at an early phase in the rats was not clearly revealed. No proximal obstruction could be displayed which could account for the distention on mechanical principles. Possibly the cause may be related to the tendency to peribronchiolar atelectasis. Perhaps a neurogenic mechanism was at the root of it all. To be true, the larger cystic distentions were usually found within areas of chronic diffuse consolidation, and it is possible that in such instances the distention of the trapped air passages resulted mechanically from cicatricial contraction.

The origin of two types of emphysema which were discovered may have similar explanations. While that which developed in the guinea pigs was definitely compensatory to the foci of atelectasis and fibrosis which it surrounded and the emphysema found in the rats and hamsters was of the hypertrophic variety, both may have had a common origin in the damage to the bronchioles.

The mild to moderate tuberculogenic effect of the hydrous calcium silicate dust manifested itself in local or even diffuse spreads, delayed healing, and dissemination to other organs. It is not yet possible at this stage to attribute this phenomenon definitely to either the chrysotile or hydrous calcium silicate components. The fact that the tuberculous process did not persist in the pulmonary lymph nodes, whereas it was activated in the lungs, parallels the observation that only in the lungs were asbestos bodies found. It must be presumed that the hydrous calcium silicate dust was transported from the lungs to the lymph nodes, to judge by macrophage accumulation at the latter sites, and it is probable that the dust was more densely concentrated in the lymph nodes than in the pulmonary tissues. This suggests that the stimulation to perpetuation and spread of the tuberculous process may have derived from the chrysotile fibers rather than from the hydrous calcium silicate. Such a conclusion naturally requires further confirmation through experimental investigation.

SUMMARY

Inhalation studies have been conducted using a commercial product composed of hydrous calcium silicate and chrysotile on normal guinea pigs, rats, and hamsters and on guinea pigs infected with tubercle bacilli of the R_t strain.

The dust caused marked chronic bronchiolitis, with terminal peribronchiolar focal fibrosis, bronchiectasia, and epithelialization of atelectatic alveoli. The final lesions closely resembled those found in experimental asbestosis and included asbestos bodies.

The course of experimentally induced tuberculosis was mildly to moderately adversely affected by the prolonged inhalation of the dust.

It seems likely that the chrysotile component of the commercial product, rather than the hydrous calcium silicate, was the cause of the deleterious effects on the tissues of the experimental animals.

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